Stress, vulnerability and resilience: a developmental approach

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A Developmental Approach

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A Developmental Approach

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STRESS, VULNERABILITY AND RESILIENCE

A DEVELOPMENTAL APPROACH

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Faculteit der Geneeskunde

To Lotte, Linde and Lucas

Contents

List of abbreviations

A	adenosine
A&ATE	adenotomy or adenotonsillectomy
ACTH	adrenocorticotrophic hormone
ADHD	attention deficit hyperactivity disorder
AGFI	adjusted for degrees of freedom (statistics)
ANOVA	analysis of variance (statistics)
ANS	autonomic nervous system
APOE	apolipoprotein E
ARS	Adolescent Resilience Scale
β	standardized coefficient (statistics)
BL	birth length
BMI	body mass index
BW	birth weight
BW/GA	birth weight adjusted for gestational age
BDNF	brain derived neurotrophic factor
C	cytosine
CAPS	Clinician-Administered Post-Traumatic Stress Disorder Scale
CBCL	Child Behavior Checklist
CD-RISC	Connor-Davidson Resilience Scale
Chr	chromosome
CI	confidence interval (statistics)
CIA	Central Intelligence Agency
cm	centimeter
CRH	corticotrophin releasing hormone
CRIES-13	Children's Impact of Event Scale (13 items)
CSHQ	Children's Sleep Habits Questionnaire
dbSNP	single nucleotide polymorphism database
DAT	dopamine transporter gene
DevOS	Developmental Origins: Singapore
Df	variance (statistics)
DNA	deoxyribonucleic acid
DRD2	dopamine receptor D2
DRD4	dopamine receptor D4
DSM-IV	diagnostic and statistical manual of mental disorders, 4th edition
DVVA	dermal vasoconstrictor assay
E	environment
EAS	Emotionality, Adaptability, Sociability
ECG	electrocardiography
EFA	exploratory factor analysis (statistics)
Ensembl gene	gene ensemble
EMR	early morning rise in amount of cortisol
ENT	ear nose and throat
ERP	event related potential
EU	European Union
F	F test, Fisher test (statistics)
g	grams
G	guanine
GA	gestational age
GABA	gamma aminobutyric acid
GABRB	GABA A receptor beta
GABRB3	GABA A receptor beta 3 subunit gene
GAS	general adaptation syndrome
GDP	gross domestic product
GHQ-28	General Health Questionnaire (28 items)
GFI	goodness of fit index (statistics)
Gt	genotype
Gti	genotype infant
Gtf	genotype father
Gtm	genotype mother
G x E interaction	gene - environment interaction
GR	glucocorticoid receptor
GR gene	glucocorticoid receptor gene
GUSTO	Growing up in Singapore Towards Healthy Outcomes
HC	head circumference
HPA	hypothalamic pituitary adrenal axis
11 beta HSD-2	11 beta-hydroxysteroid dehydrogenase type 2
5-HTT	serotonin transporter (also SERT)
5-HTTLPR	serotonin transporter promoter region
HTR	5-hydroxytryptamine
HTR2A	5-hydroxytryptamine 2A receptor

5-HTTPR	serotonin transporter polymorphism
HWE	Hardy-Weinberg equilibrium
HWE UNAFF	Hardy-Weinberg equilibrium number of unaffected individuals
IQ	intelligence quotient
kg	kilograms
KNO	keel-neus-oorheelkunde
L allele	long allele
LD	linkage disequilibrium
LD-DST	low-dose dexamethasone suppression test
LDL	low density lipoprotein
LS	heterozygous, long and short allele
LL	homozygous for two long alleles
M	mean (statistics)
MA	master of arts
MAF	minor allele frequency
MAOA	monoamine oxidase A
MD	Doctor of Medicine
Met	methionine
MR	mineralocorticoid receptor
MRI	magnetic resonance imaging
mRNA	messenger RNA
MSc	master of science
n	sample number/ number of observations (statistics)
NAA	N-acetylaspartate
NE	norepinephrine
NPY	neuropeptide Y
P	P value
PBMC	peripheral blood mononuclear cell
PNS	parasympathetic nervous system
Pr	probability (statistics)
PTSD	posttraumatic stress disorder
PTSS	posttraumatische stress stoornis
r	correlation coefficient (statistics)
RNA	ribonucleic acid
RMSEA	root mean square error of approximation (statistics)
Rs(SNP)	officially registered SNP, which has given an (rs) identifier number by dbSNP
RS	Resilience Scale
RSA	respiratory sinus arrhythmia
RSCA	Resiliency Scales for Children and Adolescents
RPM	Raven's Progressive Matrices
S allele	short allele
SAM	sympathetic adrenergic medullary axis
SCID	Structural Clinical Interview for DSM-IV diagnosis
SCL	solute carrier family neurotransmitter transporter
SCL6A4	solute carrier family 6 neurotransmitter transporter, serotonin, member 4
SCORM	Singapore Cohort Study of Risk Factors in Myopia
SD	standard deviation (statistics)
SEM	structural equation modelling (statistics)
SERT	serotonin transporter (also 5-HTT)
SNS	sympathetic nervous system
SNP	single nucleotide polymorphisms
SPSS	statistical package for the social sciences
SS	homozygous for two short alleles
STAI	Spielberger State-Trait Anxiety Inventory
SYRESS	Singapore Youth Resilience Scale
t	t-test score (statistics)
T	thymine
T-scale (CBCL)	normalized scores (on CBCL)
T102C	5-hydroxytryptamine 2A receptor polymorphism (rs6313)
TPH	tryptophan hydroxylase
TPH2	trytophan hydroxylase 2
TRF	Teacher Rating Form
USA	United States of America
US	United States
Val	valine
VCL	Vrijzinnig Christelijk Lyceum
VNTR	variable number tandem repeat
VS	Verenigde Staten
WHOQOL-BREF	World Health Organization Quality of Life Assessment-Bref
WISC	Wechsler Intelligence Scale for Children
x	'in interaction with'

1

Introduction

"Children of the world" by Sam van Druten, 9 years old

Stress, Vulnerability and Resilience, a Developmental Approach

1.1. Scope

This thesis is devoted to the field of individual differences in behaviour and emotions, and started out with the study of multiple effects of genetic, neurophysiologic and environmental influences during life on the outcome in young children. In this thesis we are interested in the interactive influence of genes and environmental factors in child populations across different cultures. Understanding their influence will stimulate better outcomes in children.

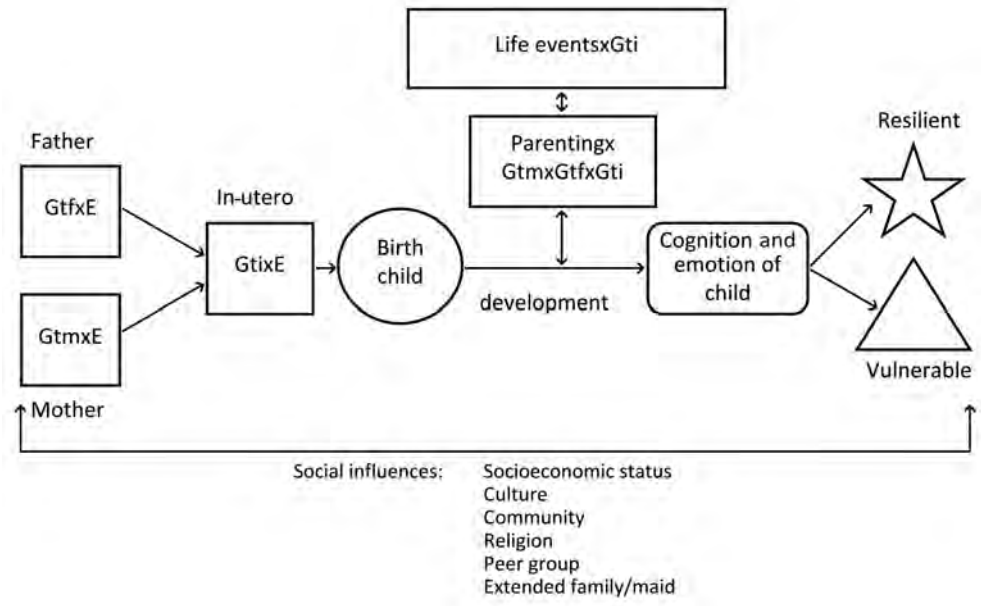
1.2. Aims and outlines

The aim of this thesis is to investigate influences of genes and environments on behavioural, emotional and cognitive outcomes in different samples, most derived from the general population. A developmental approach will be used to understand how nature (genotypes) and nurture (e.g. environmental risk or protective factors) interact to determine developmental outcomes. A biopsychosocial model is used to describe the interactions between genotypes, stress in-utero and stress later in life, as well as protective factors.

1.3. Biopsychosocial model

The biopsychosocial model used in this thesis covers a timeline from preconception until adolescence. This model describes the influences of the history of parents and the environment in-utero in interaction with genotypes on the outcome of a child at birth. After birth many other environmental factors in interaction with genotypes, most of all parenting and life events, have a further influence on socio-emotional and cognitive development of the child. These environmental influences are heterogeneous, meaning they can be protective or a potential risk. For example 'good' parenting is a protective factor, while 'bad' parenting (for example parents who neglect their children) is a risk factor for development of psychopathology in the child. Essential is how children appraise and cope with these events in life. The child's appraisal influences the impact of the stressor on the child (Lazarus and Folkman, 1984). To illustrate this, if a child considers a new school as an opportunity to meet new children the events will be less stressful than when a child is anxious to meet new children.
Our model describes the interactions between environment and genotypes of the parents, the fetus and child. Genotypes moderate environmental effects (Rutter, 2002), through effect on susceptibility to risk environments. In other words, an adverse environment has little effect if the genetic susceptibility is absent, while it may have a large effect when the genetic susceptibility is present. For example, Caspi et al. (2002, 2003) found that allelic variation in the monoamine oxidase A gene (MAOA) interacts with maltreatment on antisocial behaviour in young people and the allelic variation in the serotonin transporter gene interacts with traumatic experiences on the occurrence of depressive disorder.
However, social circumstances also influence the effects of environmental stress. For example, social support is a key regulator of stress. As a consequence, the interactions between environment and genotypes must be placed against the background of social factors such as socioeconomic status, culture, community, religion, peer group, extended family etc. See model 1. The social factors mentioned here are broadly defined and most of all influence other risk factors. For example the effect of low socioeconomic status is not an effect directly of the economic pressures themselves, but is mediated by family functioning and

parent-child relationships (Conger et al., 1994; Costello et al., 2003a). This underscores the importance of parenting on the development of the child.
The final outcome of cognition and socio-emotional development of the child will determine its vulnerability or – in absence of psychopathology although exposed to adversities – its resilience.



Model 1.
Gt = genotype, Gtf = genotype father, Gtm = genotype mother, Gti = genotype infant, E = environment, 'x' = interaction effect, for example: GtxE = interaction between genotype and environment

Although our biopsychosocial model describes a timeline until adolescence, risks and protective effects are important during the whole life cycles and not only confined to early life.

2. Background

2.1. Why a developmental approach?

During the last 50 years there have been dramatic changes in our understanding of mental disorders (Rutter, 2010; Rutter and Smith, 1995). One such change is the awareness of the prevalence and impact of psychiatric disorders in childhood (Costello et al., 2003b). Research findings reveal that most of the psychiatric disorders arise before adulthood (Costello et al., 2006). In recent years, studies have focused on the complex interaction between biologic and environmental factors and their influence on brain function, behaviour, emotion and cognition, and in the end: mental disorders. This approach is called 'developmental

psychopathology'. Developmental studies seek to explain the principals underlying changes in behaviour and cognition during development. From a developmental point of view mental health is not a state, but an outcome of dynamic processes over years, in which the individual is shaped by genotype in interaction with different environmental experiences during life (Rutter et al., 1997). An important aim in developmental studies is to understand the relation between genes and environmental influences, also in the past referred to as 'nature versus nurture'. In the past the difference between nature and nurture was thought to be stricter, with philosophers like Descartes (1596-1650), Rousseau (1712-1778), Darwin (1809-1882) and Gesell (1880-1961) being nativists, who believed that behaviour is strongly influenced by genes, while environmentalists emphasized the importance of experience in life. For example John Locke (1632-1704) thought the mind was a 'tabula rasa', a blank slate written on by experience. Locke, as well as Freud (1856-1939), are known for their emphasis on early life experience. Freud saw the infant as vulnerable, with parenting as the most important influence on development. However, like many more modern psychologists and psychiatrists, Freud already believed in an interactive model between nature and nurture, and he rejected the perspectives of nativists (nature) and environmentalists (nurture). He thought that nature produces the unconscious functioning of the mind, and that this functioning interacts with experiences in early life (mainly parents) (Eng, 1980).

Recently it has become clear that nature and nurture indeed should not be considered separate influences, but rather two parts of a constant interaction that contribute to the unique development of an individual (Rice et al., 2010; Rutter et al., 1997). The influence of both genotypes as well as environment starts from conception, although some may argue that it even starts prior to conception. For example, the way in which nutrition and health status of the mother and father influence the quality of the eggs and sperm (Vujkovic et al., 2009; Westphal et al., 2004). The interaction between genotypes and the environment is a clearly dynamic process. This is called gene x environment interaction (G x E interaction). G x E interaction is present when the effect of genotype on the development of psychopathology depends on the level of exposure to an environmental factor (Rutter et al., 1997). Environmental factors have a direct influence on the activity of genes, meaning that the same individual will develop differently under different circumstances (Belsky and Pluess, 2009; Meaney, 2010; Rutter et al., 1997). For example children carrying the S allele of the SERT gene will only exhibit suicidal behaviour later in life if they experienced traumatic experiences in early life. This implies that traumatic experiences, as an environmental factor, interact with the genotype of the child (Caspi et al., 2003).

Thus, the challenge does not concern the independent influence of genes or environment on a certain outcome, but rather the understanding how they interact with each other.

For studying these effects you can use different study designs. One way to study genetic and environmental effects and interactions between them is by using cohort studies. Cohort studies offer the advantage of collecting time-dependent exposure information before the onset of psychopathology (i.e., a prospective approach). This approach provides opportunities to identify individuals with a higher risk at an early stage, which is critical for developing interventions and understanding pathways leading to psychopathology. However, causal relationships cannot be clearly identified as confounders may complicate the associations between exposure and psychopathology. For example, is an individual depressed because of bullying in the past (causal relation), or is an individual, who is prone to bullying, also more

prone for developing depression (for example because of internalizing traits)? This problem is not an issue for G x E interactions as it is for pure environmental effects.
Another useful design is a case-control study, especially in comparing environments (Hardt and Rutter, 2004; Thomas and Greenland, 1985). Although both cohort and case-control studies are at risk for measurement errors, in case-control studies environments can differ systematically between cases and controls. For example, children with depressive symptoms might recall their history as more negative than children without these symptoms. However, this problem does not exist in measurement of genotype, and that makes the case-control design a good choice for studying associations between genotypes and risks for psychopathology (Clayton and McKeigue, 2001). Furthermore, for G x E studies it is important to identify the effect of functional genetic polymorphisms that might affect this risk factor (Clayton and McKeigue, 2001).

2.2. Stress

Environmental influences most typically include demanding, stressful events ('stressors') from the in-utero period through to adulthood. The result of stressors is called stress. The term stress is difficult to define and quantify (Hobfoll, 1988; Lazarus, 1993), but most researchers use some variant of Selye's definition that "stress is the non-specific response of the body to any demand made upon it". In this sense stress can be considered as a metabolic concept. Selye considered favorable events (e.g. taking a new job) as well as unpleasant events as stressors. He proposed that stress can be viewed in a positive or neutral way and referred to this as 'eustress'. He stated that 'eustress' is the amount of stress needed for an individual to live an active, healthy life, while he used 'distress' when levels of stress exceed an individual's ability to adapt to stress (Selye, 1975). Distress is considered to have a negative effect on health (Bicanic et al., 2008; Langeland and Olff, 2008; Selye, 1982).
Selye developed a model of stress, which he called the General Adaptation Syndrome (GAS). The GAS has three stages; first the 'alarm' stage, where the brain detects a threat or stressor triggering physiological arousal. The second stage is 'resistance' where the body adapts to the stressors and homeostasis is achieved. The last stage is 'exhaustion', which occurs after prolonged resistance, a reaction to the constant high metabolic demands of an extended alarm stage. During exhaustion the body's energy stores and hormone reserves are being depleted, which may upset homeostasis and leave the individual vulnerable to disease. This condition could lead to stress-related conditions such as atherosclerosis, hypertension or depression, as the stress response systems become exhausted (Selye, 1982).
A number of physiological processes change in response to environmental stress, including the production of stress-related hormones. There are two different important physiological stress systems; the sympathetic adrenergic medullary axis (SAM) and the hypothalamic pituitary adrenal axis (HPA). The SAM axis is a quick response system, with immediate sympathetic activation and release of catecholamines such as adrenaline and noradrenaline from the medulla of the adrenal glands (de Vente et al., 2003). Norepinepherine is also released within multiple brain circuits. Adrenaline and noradrenaline are both known as 'arousal hormones' and activate the cardiovascular and neuroendocrine functions. The SAM axis mobilizes and diverts energy to muscles, heart, and brain while at the same time it reduces blood flow to the internal organs and the gastro-intestinal system. During the stress response only the essential organs function and energy is conserved through the suspension

of the non-essential systems, such as the digestive system (Henry, 1992; Porges, 1991; Ursin and Olff, 1993).

The HPA axis is a slower response system which has the function of ensuring vital organ functioning in response to ongoing stress for energy expenditure. The HPA axis starts with release of corticotrophin-releasing hormone (CRH) from the hypothalamus to the pituitary gland. After stimulation by CRH (often acting with vasopressin) the pituitary gland releases adrenocorticotrophic hormone (ACTH), which circulates through the bloodstream to the adrenal cortex. ACTH causes the adrenal cortex to synthesize (among other steroid hormones) corticosteroids. Corticosteroids cause the suspension of the digestive system and the immune system, and promote the liver to release sugar and lipids for rapid energy utilization (Harbuz and Lightman, 1992). The main glucocorticoid hormone in humans is cortisol (Engelmann et al., 2004; Gómez et al., 1998). Cortisol is often deregulated in stress related disorders, e.g. PTSD (Olff et al., 2006). However, cortisol is not necessarily an indicator of distress, but rather associates with metabolic challenges (Harbuz and Lightman, 1992; Miller and O'Callaghan, 2002). For example, there is a circadian variation of cortisol release with a rise in cortisol in the first 30 minutes after awakening that is linked to preparation for metabolic demands of the upcoming day after a fastening period of time. Cortisol also rises after other increases of metabolic demand such as sexual intercourse, physical exercise, and food intake (Hoffman-Goetz and Pedersen, 1994). As cortisol is not simply found to be a 'stress' hormone, its relation to psychopathology is not yet clear (Meewisse et al., 2007).

The fast pathways of arousal are also called the 'fight or flight response', as these reactions take place to allow the body to physically deal with the stressors it is confronted with. It was described by Cannon as an evolved response by mammals to threat (Hoffman-Goetz and Pedersen, 1994). However, stress reactions can be maladaptive. For example, today stress responses may arise to psychological problems for which there is no reason for a 'flight' response as was required in the evolutionary past when threats to survival were more prevalent and severe (Christopher, 2004; Peleg and Shalev, 2006; Raison and Miller, 2003). Although Selye's GAS presupposed the same response to a variety of stimuli, later studies showed that specific perceptions of control result in different patterns of neuroendocrine activation (Henry, 1992; Peacock and Wong, 1990). Lazarus and Folkman (1984) proposed a cognitive theory of stress, referring to the meaning of an event to the individual. They developed a model, which focuses on the transaction between people and the external environment (known as the Transaction Model). The stressor may in fact reside in the individual's perception of the event's implications for him, making stress the consequence of appraisal and not the antecedent of stress. According to this theory, the way an individual appraises an event plays a fundamental role in determining the magnitude of the stress response (Lazarus and Folkman, 1984; Olff et al 2005a). This has also physiological implications. For example, exposure to stressors that are viewed as challenging, tend to result in significant elevations of serum catecholamines with no change in serum prolactin or cortisol. It enhances immune function (McEwen, 2000). In contrast, exposure to chronic stressors that are viewed as overwhelming, tend to result in marked elevations of serum prolactin and cortisol with no change in serum catecholamines. Sustained activation of the HPA axis with chronic elevated cortisol levels has been associated with degeneration of the hippocampus, deficits in hippocampus-dependent memory tasks, affective distress, perceived uncontrollability, prolonged major depressive illness and with a range of somatic problems such as

sugar imbalances, decreased bone density, higher blood pressure and lowered immunity (Lupien et al., 1997; Lupien et al., 1998; McEwen 1998, 2006, 2008, Olff, 1999; Olff et all., 2005b). To identify the health consequences of intense, repeated, and sustained activation of bodily systems is currently a major objective for stress research.

One of the psychiatric disorders that can develop after a stressful event is Posttraumatic Stress Disorder (PTSD). The defining characteristic of a traumatic event is "the capacity to provoke fear, helplessness or horror in response to a threat of injury or death" (Yehuda, 2002). A diagnosis of PTSD requires that a person is exposed to an extreme stressor or traumatic event to which he responded with fear, helplessness or horror (Yehuda, 2002). PTSD is not uncommon after many types of traumatic events. Nearly all people experience the acute form of the disorder (Acute Stress Disorder) at some time in their lives (Breslau et al., 1998; de Vries and Olff, 2009). While severe, such reactions usually subside within hours to days, if the stressor does not continue. Interestingly, studies show that the psychological and biological response to a traumatic event is not mainly determined by the characteristics of the event, but by the characteristics of the person involved (Olff et al., 2005b; Yehuda, 2002). The neurophysiologic responses between people are different, as well as their subjective interpretations of the event, which are both influenced by previous experiences (Olff et al., 2005a). This will have an important influence on the stress response. Thus, after a traumatic event, the unique characteristics of the person determine if an individual develops PTSD (Peleg and Shalev, 2006; Yehuda, 2002).

PTSD is diagnosed in adults as well as adolescents and children. Of note, in children responses to stress are age-dependent and strongly influenced by the reactions of adults (mainly their caregivers). One of the coping mechanisms of young children (infants and preschool children) is searching for protection by crying and clinging towards their caregiver. Because they generally lack the developmental skills to effectively cope with stressful situations by themselves, children are particularly dependent on family members for comfort (Lerner and East, 1984; Skinner and Zimmer-Gembeck, 2007). Even for anticipation to danger, the young child will rely on his caregiver by looking at his facial expression, also called 'social referencing' (Boer, 2009). For this reason, on some occasions children in this age-group may be as affected by the reactions of their caregivers as they are by the direct effects of the stressor.

Both the 'fight or flight' reaction in older children and adults, as well as increasing search for comfort by caregivers in younger children in reaction to stress have the function of protection and will increase survival (Boer, 2009; Gunnar and Quevedo, 2007).

2.3. Vulnerability by genetic influences

For years it was believed that statistical associations between environmental risks, such as life events and development of psychopathology, only represented environmentally mediated risks effects. The field of behavioural genetics created a major change in this thinking. A growing body of evidence suggested the importance of G x E interactions and suggested that a considerable degree of the effects of risk environments were actually genetically driven (Plomin, 1994; Plomin and Bergeman, 1991; Rowe, 1994; Rutter and Silberg, 2002; Scarr, 1992).

Results from neurobiological and epidemiological studies have increased our understanding of developmental influences on behaviour, emotion and cognition. Because of the known

high heritability of behavioural traits and intelligence, more genetic studies as well as G x E studies have been undertaken over the past few years. Genome-wide association studies, an approach that relies on the data produced by the International Human HapMap Project (Hardy and Singleton, 2008; Plomin et al., 2009), find associations between genotypic variation and risk factors of psychiatric disorders, which reveal 'candidate genes', associated with particular endophenotypes related to psychiatric disorders. For example, the end phenotype 'high reaction time variability' on continuous performance tests in children with attention deficit hyperactivity disorder (ADHD) is associated with dopamine receptor DRD4 7-repeat allele absence (Kebir et al., 2009). Another example is the association found between the serotonin transporter promotor region (5-HTTLPR) and suicidal behaviour and depression related personality traits (Levinson, 2006).

At a molecular level, DNA interacts with signals from the environment, which together influence the expression of a trait in the context of a particular environment (Bagot and Meaney, 2010). Thus, having a certain genetic variant in itself does not mean that a particular trait will develop. For example, rhesus monkeys with secure early attachment relationships are resilient for the otherwise increased risk for adverse developmental outcome of the 'short allele' of 5-HTT by 'maternal buffering' (Suomi, 2006).

The majority of genes are expressed as the proteins they encode. This process occurs in two steps: first transcription, which creates a complementary RNA copy of a sequence of DNA, followed by translation, the creation of a protein from messenger RNA. DNA methylation involves the addition of a methylgroup to DNA. Methylation is crucial for normal development and has the function of cellular differentiation. DNA methylation alters gene expression by regulation of gene transcription. Methyl groups generate a local chromatin configuration that renders the genes inaccessible, and thus transcriptional inactive (Altwood et al., 2002; Cedar, 1998; Jaenisch and Bird, 2003).

It is suggested that for many disorders, different genetic loci impinge on a common pathway to pathogenesis. Thus, finding risk alleles and protective alleles for a particular disorder will provide clues to other risk loci by which variability at the same pathway can contribute to disease in interaction with the environment.

2.4. Resilience

Individuals differ in their response to stress. Individuals who do not develop disorders and illness despite exposure to risk factors are referred to as resilient. Garmezy, Masten and Tellegen were the first to discuss the concept 'resilience' in 1984 (Garmezy et al., 1984). They described resilience as "manifestations of competence in children despite exposure to stressful events". In 1985 Sir Michael Rutter defined resilience again as "facing stress at a time and in a way that allows self-confidence and social competence to increase through mastery and appropriate responsibility" (Rutter, 1985). In 1994 Masten defined resilience as "successful adaptation despite risk and adversity" and "a pattern over time, characterized by good adaptation despite risk and adversity" (Masten, 1994). Gordon in 1995 defined resilience in the following way: "resilience is the ability to thrive, mature and increase competence in the face of adverse circumstances, including biological, psychological and environmental circumstances" (Gordon, 1995).

However, it is not easy to clearly define the concept 'resilience'. In later studies resilience is often measured as absence of (mental) health problems despite experience of traumatic

events/disasters (Bonanno et al., 2006, 2008). However, most of the times resilience is defined intuitively and not empirically. Resilience differs from both social competence and positive mental health as resilience is defined contextual (Rutter, 2006a). Environmental factors that lead to resilience in one context may not lead to resilience in other circumstances. Hence, 'academic resilience' may be related to other individual characteristics and environmental factors than 'emotional resilience'. Furthermore, because resilience in relation to childhood adversities may be influenced by positive adult experiences, a life span trajectory approach is needed (Rutter, 2006a).

Researchers have documented that protective resources can interact with existing adversities to influence behaviour, emotion and health outcomes (Davey et al, 2003; Fossion and Linkowski, 2007; Heaven and Ciarrochi, 2007; Rew and Horner, 2003). The approach of protecting youth from harm through promotion of protective factors has sparked great interest in resiliency-based research (Rutter, 1993). Resilience is partly influenced by predisposing genetic and biological factors, but is also acquired and developed by for example emotional and secure attachments with parents or significant others, and by social learning (Buckner et al., 2003; Rutter, 2006b). The study of Collishaw et al. (2007) showed that most individuals who reported repeated or severe physical and sexual abuse in childhood develop mental health disorders later in life, however, a small minority of participants did not. This was associated with higher perceived parental care, more adolescent peer relationships, higher quality of adult-love relationships and personality style (Collishaw et al., 2007). Other core psychological factors which have been associated with resilience are Intelligence Quotient (IQ), coping styles (task-oriented instead of emotion-oriented), self esteem, lower anxiety and stress sensibility, personality traits (such as extraversion and conscientiousness) and social support (Buckner et al., 2003; Cameron et al., 2007; Campbell-Sills et al., 2006; Depape et al., 2006; Masten et al., 1999; Rutter, 2006a; Tedeschi and Calhoun, 1996).

2.5. Cultural differences

As causes, expression and prevalence of disorders are different between ethnic groups (Burchard et al., 2003), cultural background cannot be neglected in research. Rather than ignoring cultural differences, scientists should be conscious of the importance of increasing replication studies in other countries. In this thesis one study was performed in a Dutch cohort and 4 studies were performed in a Singaporean cohort.

The Netherlands is a European country with 16.7 million residents. It has a multicultural population, although the majority of the residents is Dutch. The Netherlands is a democratic state, has a capitalist market-based economy and is seen as a liberal country given the drugs legalisation policies and legalisation of euthanasia. The Gross Domestic Product (GDP) in the Netherlands in 2009 was 792.128 billion US dollars (source: World Bank). The Gini coefficient, as a measure of income inequality (value of 0 expressing total equality and a value of 100 maximal inequality) was 30.9 in 2007 (source: CIA World Factbook). The homicide rate in the Netherlands in 2009 was 0.92 per 100.000 inhabitants (source: Dutch homicide statistics). In 2010 the birth rate was 10.3 births per 1000 residents (source: CIA World Factbook). The life expectancy has been quite stable over the last 50 years (slightly increasing) to 80.4 years in 2008 (source: World Bank). Around 3000 psychiatrists are registered, of which quite a large number work part-time. The suicide rate was 0.85 per 100.000 inhabitants in 2009 (source: World Health Organization).

Singapore is a South East Asian island country off the southern tip of Malaysia, with a highly developed market-based economy. It has a multicultural population of 5+ million people, mostly made up by Chinese, followed by Malay and Indians. Singapore is a parliamentary republic and establishes a representative democracy, although one party has dominated the political process since 1959. Singapore laws include judicial corporal punishment in the form of caning, and death penalty for first degree murder and drug-trafficking. Criminality rates are low. In 2009 the homicide rate was 0.38 per 100.000 inhabitants (source: United Nations Office on drugs and crime division for policy analysis and public affairs). The GDP was 182.23 US billion dollars in 2009 (source: World Bank). The Gini coefficient was 48.1 in 2008, representing more income inequality than in the Netherlands (source: CIA World Factbook). The birth rate was 8.65 births per 1000 residents in 2010 (source: CIA World Factbook). The life expectancy has increased over the last 50 years up to 80.7 years in 2008 (source: World Bank). In Singapore 152 psychiatrists are registered, of which most of them work fulltime. The suicide rate was 10.3 per 100.000 inhabitants in 2006 (source: World Health Organization). Of note, in Singapore suicidal attempts and suicide are offenses by law.

There is increasing awareness to incorporate culture and diversity into the study of stress and resilience (Arrington and Wilson, 2000; Ungar, 2006, 2008). Parenting, social learning, coping styles and some other core psychological factors associated with resilience are influenced by culture. Hence, resilience is expected to differ among different cultures.

One important difference between cultures is the way stressors are perceived. For example, although school work and academic performance are major stressors for children worldwide, the level of educational stress varies among different societies (Steinberg et al. 1992). Among them, the highest pressure to succeed and do well academically is found in Asian cultures. For example Steinberg et al. (1992) found that Asian American students spend twice as much time each week on homework than students of other ethnic groups living in the United Sates. The Asian American students also reported that their parents had high expectations and would be disappointed if they came home with any grade lower than A (Steinberg et al., 1992). Also in Korea it was found that children spend twice the time on studies and less time on socializing and leisure activities than their American counterparts (Lee and Larson, 2002). It has been suggested that the focus on studies is related to the belief in Asian countries that graduating from a (high ranking) university is a passport to a good job and a high social status (Chung et al. 1993; Steinberg et al., 1992). As a consequence, Asian families strongly emphasize to their children the need to succeed educationally (Mordkowitz and Ginsburg, 1987). Shame and loss of face are frequently used in Asian cultures as socialization tools to reinforce familial and cultural obligations, societal expectations and proper behaviour (Yeh and Huang, 1996). Not meeting one's own expectations and/or the expectations of significant others is a serious matter that can potentially result in loss of face and loss of confidence as well as support from one's family and even the community (Yeh and Huang, 1996). In Singapore, Isralowitz and Hong found in 220 high school students that 'being pressured to keep up with the schoolwork' was ranked as the top problem or concern of students (Isralowitz and Hong, 1990). In another national youth survey in Singapore, the majority of young people ranked education as the most stressful aspect of their lives (Ho and Yip, 2003).

Two studies showed that Dutch children have a high quality of life and feel less pressured by education in comparison to children in other industrialized countries in Europe.

The most recent publication is from a British study of the University of York, published in 2009, carried out among children in 29 European countries (27 EU countries plus Norway and Iceland). They included 43 life circumstances that influence happiness, such as infant mortality, obesity and poverty. In each category The Netherlands scored best (Child Poverty Group, 2009). A study done of Unicef (Unicef, 2007) examined happiness of children on the basis of 6 criteria: material wealth, health, education, relationships, safety, and children's own feelings of luck. The Dutch children had the best scores in comparison to 17 other European countries plus Canada and the United States. They found that, aside from material quality, Dutch children expressed the greatest amount of 'subjective well being' in response to questions about whether they like school, feel pressured by school and how they rate their own health. Dutch children are generally happy with the compulsory educational system with relatively low pressure and they enjoy the best relationships with their parents (judged by the ease children can talk to their parents about various topics) and friends (Unicef, 2007).
Another important difference between cultures is family bonding. While in most European countries there is a focus on individualisation and self-development, in Asia the focus is on strong family bonding and interdependency. The stigma of mental disorders is high in Asia and many problems are addressed within the family. Although the prevalence of mental disorders in Singapore is roughly the same as found in Europe or Australia (Fones et al., 1998), the outcomes can be different. For example the outcomes for schizophrenic patients are better in Singapore, probably associated with the high family care (Kua et al., 2003). Furthermore, in Singapore 'domestic helpers' are commonly employed by families. This helper typically lives in close proximity with the family providing continuous assistance in household matters, child care or care for elderly. It is important to understand that children raised under these circumstances are likely to have different experiences, with parenting influences not only from their biological parents, but also from living-in grandparents and domestic helpers.

2.6. Limitations of previous developmental studies in gene and environmental influences on childhood outcomes

Individuals, from childhood onwards, respond differently to stress (Broekman et al., 2007; Langeland and Olff, 2008; Olff et al., 2005b). Most developmental studies have focused on children with problem behaviour or psychiatric problems to understand the effect of stress and adversities on socio-emotional and cognitive outcomes. Although it might be more difficult to study the effects of risks and protective studies in a general population, this is of utmost importance, because most children will belong to this group. Hence results of these studies will be more applicable to daily life and can improve the health of a larger group of children. However, these studies are rare.
For example, most PTSD studies have focused on extreme situations such as war and terrorist attacks or natural disasters. However, posttraumatic stress can develop also in more common situations such as traffic accidents or medical surgeries (Kotinimie et al., 1996; Langeland and Olff, 2008; Siegel, 1988). Detection of susceptibility to develop posttraumatic stress symptoms is important for prevention, but is hampered by inherent unpredictability of stressful events. That is why most studies are done in retrospect.

Another example is studies examining the effect of birth weight. To date most of these studies focus on children born with a low birth (i.e. <2500 grams) or very low birth weight (i.e. <1500 grams), or children born premature. Although these studies were important to show the impact of fetal growth on neurodevelopment and socio-emotional outcomes (Bhutta et al., 2002; Black et al., 2008; Victora et al., 2008), they do not apply for most children within the normal birth weight range. Studies conducted in normal birth weight children are rare and often show contradictory results, for example for the influence of birth weight on cognitive function (Christensen et al., 2006; Gale et al., 2004; Lawlor et al., 2006; Matte et al., 2001; Pearce et al., 2005; Shenkin et al., 2004; Tong et al., 2006).
Studies on developmental origins of socio-emotional behaviour reveal pronounced effects of G x E interactions but focused mostly on specific psychiatric problems in adolescents and adults, such as affective disorders (Caspi et al., 2003; Kendler et al., 2005; Risch et al., 2009). However, it is equally important to understand the role of genes in a general population, but G x E studies examining differences in socio-emotional characteristics in 'healthy' children are rare.
Also studies examining resilience are seldom performed in a general population. Most studies focus on high-risk samples of children, such as children who suffer from diseases, children from broken families and children who live under extreme adversities. Although these studies are important, they do not provide a broad perspective of resilience for children in the general population. Moreover, established risk factors have been studied extensively for over many years, but only over the last years studies have started to focus on protective factors as well (Richardson, 2002). Furthermore, it is expected that concepts of resilience are cultural sensitive. So far, there has been no universal resilience scale, which enables a more objective outcome of resilience measured in different cultures. Although population studies on resilience have been done in school aged children living in the United Sates and Europe (Backett-Milburn et al., 2003; Rew et al., 2010; Shapiro and Lebuffe, 2006; Tschann et al., 1996), no resilience studies have been performed to date in Asian school aged children.

3. Outline of the thesis

3.1. Studies in genetic vulnerabilities

The intriguing question is; why do some people develop mental health problems after a stressful or traumatic event, while others do not? **Chapter 2** provides a review of the literature about genes which, in interaction with a traumatic event, may be involved in the development of PTSD. Variation in these genes might explain variation in outcomes across individuals. This article will give a firm overview to understand genetic effects on unique individual outcomes.
In **chapter 4** we examine G x E influences on the outcome of children in socio-emotional development in the general population, measured with the Child Behavior Checklist (CBCL). We study the interaction between birth weight corrected for gestational age (as a reflection of early life influences) and genotypes involved in the serotonergic system. Our hypothesis is that different genotypes will moderate the effect of stress in-utero on the socio-emotional outcome in school aged children.

3.2. Studies in early life stress in-utero

In **chapter 3** a cohort study is described which examines early life adversity in-utero (reflected in birth weight, birth length and head circumference) on IQ in children, measured with the Raven's Progressive Matrices. The hypothesis is that a lower birth weight, a shorter birth length and a smaller head circumference at birth, within the normal birth proportion range, will reflect a less optimal environment in-utero and will predict a lower IQ than children who had a high birth weight.

The study in **chapter 4** describes the influences of the environment in-utero in interaction with genes involved in the serotonergic system on the outcome in socio-emotional development (see studies in genetic vulnerabilities).

3.3. Study in stress during early childhood

Medical procedures, if planned in advance, may provide an opportunity to investigate the stress response in a prospective way because they are stressful in nature, especially for young children. In **chapter 5** we examine the influence of a medical procedure on the outcome of behaviour and emotion (measured with the CBCL) and posttraumatic stress symptoms (measured with the Impact of Event Scale) in Dutch children. Our hypothesis is that stress of a medical procedure will have a negative influence on the behavioural and emotional state of children. We also hypothesize that the neurophysiologic states (measured by Respiratory Sinus Arrhythmia and cortisol) as well as the temperament styles of children (measured with the EAS temperament survey) will predict the outcome of posttraumatic stress, behaviour and emotion of the child after a medical procedure.

3.4. Studies in resilience

In **chapter 6** we describe a cohort study which attempts to approach real life complexity between common risk factors and adversities as well as common assets and protective factors during development on cognition (IQ, measured with Raven's Progressive Matrices) and academic results (report of school) as well as behavioural and emotional outcomes in children (measured with the CBCL) in Singapore. Our hypothesis is, that in comparison to studies done in the USA and Europe, for Singapore different protective factors and risk factors are important in influencing mental wellbeing and academic performance.

In **chapter 7** the development of a resilience scale for a Singaporean population is described.

3.5. General discussion

The main conclusions, implications, limitations and strengths of our studies are described in **chapter 8**, as well as proposals for further research and investigations.

3.6. Summary

An English and Dutch summary of all studies is provided in **chapter 9**.

2

The genetic background to PTSD

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"Abstract" by Linde Leutscher, 1 year old

Abstract

Objective

Although extensive research has already been done on the genetic bases of psychiatric disorders, little is known about polygenetic influences in posttraumatic stress disorder (PTSD).

Methods

This article reviews molecular genetic studies relating to PTSD that were found in a literature search in Medline, Embase and Web of Science.

Results

Association studies have investigated 8 major genotypes in connection with PTSD. They have tested hypotheses involving key candidate genes in the serotonin (5-HTT), dopamine (DRD2, DAT), glucocorticoid (GR), gamma aminobutyric (GABRB), apolipoprotein (APOE2), brain-derived neurotrophic factor (BDNF) and neuropeptide Y (NPY) systems. The studies have produced inconsistent results, many of which may be attributable to methodological shortcomings and insufficient statistical power.

Conclusions

The complex etiology of PTSD, for which experiencing a traumatic event forms a necessary condition, makes it difficult to identify specific genes that substantially contribute to the disorder. Gene-finding strategies are difficult to apply. Interactions between different genes and between them and the environment probably make certain people vulnerable to developing PTSD. Gene-environmental studies are needed that focus more narrowly on specific, distinct endophenotypes and on influences from environmental factors.

1. Introduction

Posttraumatic stress disorder (PTSD) is a condition widely prevalent in people who have had one or more traumatic experiences. It is characterized mainly by symptoms of re-experiencing, avoidance and hyperarousal. PTSD is one of the few mental disorders in DSM-IV for which criteria include an etiological agent, in this case experiencing a traumatic event. Not everyone that has undergone a traumatic event develops PTSD (Costello et al., 2002; Monroe et al., 1991). Although lifetime prevalence of exposure to traumatic events is thought to be between 40% and 90% in the general population, the overall lifetime prevalence of PTSD is estimated at 7–12% (Breslau, 2001; Kessler et al., 1995; Olff and de Vries, 2005). This means that exposure to a traumatic event does not entirely explain the etiology of the disorder. The suspicion is that individuals with an existing genetic vulnerability have a higher risk of developing PTSD once they experience a trauma.

Two sources of evidence for a genetic component have been transgenerational research and epidemiologic studies on twins. Transgenerational studies have reported that PTSD is more likely to occur in certain families (Koenen et al., 2003; Yehuda et al., 1998, 2001, 2002). Twin studies have found that monozygotic twins are more concordant for developing PTSD after trauma exposure than dizygotic twins (Skre et al., 1993; Stein et al., 2002; True et al., 1993; Xian et al., 2000). Other research, including twin studies, has shown that PTSD may also be related to structural brain abnormalities. Smaller hippocampal volume and abnormalities in the septum pellucidum have both been linked to a higher susceptibility to PTSD (Gilbertson et al., 2002; Gross and Hen, 2004; May et al., 2004; Talbot, 2004). Possible confounding factors in twin and adoption studies are unique, non-shared environmental factors, such as personal life events and other psychosocial stress factors. Early prenatal and perinatal environmental factors may also play a role (Figee et al., 2004), as was also suggested in a recent study by Yehuda et al. (2005). The latter authors studied babies of mothers who were pregnant during the 9/11 World Trade Center attack and who later developed PTSD. Like their mothers, the babies were found to have low salivary cortisol. This suggests that stress-induced elevations of glucocorticosteroids during pregnancy may affect foetal brain development and thereby induce permanent changes in the glucocorticoid (GR) programming of the offspring. Hence, although evidence exists for a genetic vulnerability to PTSD, the vulnerability develops in interaction with environmental factors such as stressful life events or a mother's prepartum or even pre-fertilization hormonal status (de Kloet et al., 2005). The fact that monozygotic twins share a more similar intrauterine and postnatal environment than dizygotic twins makes it very difficult to estimate heritability from twin studies. Therefore, the genetic component in twin studies will be overestimated to an unknown degree (Joseph, 2002; Kamin and Goldberger, 2002; Robert, 2000). Although the discordance between monozygotic and dizygotic twin pairs is still seen by many as a 'gold standard', evidence for a genetic contribution is more likely to come from (molecular) association studies such as those summarized in this present review.

1.1. Endophenotypes

The etiology of PTSD is complex and multifactorial (Olff and de Vries, 2005). Like most other mental disorders, the heritable part of PTSD can be viewed as polygenetic. This means that different genes are assumed either to interact or to play an additional role in the disorder's ultimate onset.

The complexity of psychiatric disorders like PTSD makes it difficult to find specific genes that substantially contribute to the disorder. For this reason, genetic research often has its focus on endophenotypes – more elementary underlying traits or facets of clinical phenomena – whereby the number of genes required to produce variations in these traits may be fewer than those involved in producing a psychiatric diagnostic entity. Such basic traits may consist of neurophysiologic, biochemical, endocrinological, neuroanatomical, cognitive and neuropsychological measures (Gottesman and Gould, 2003). Radant et al. (2001) have argued that genes associated with certain endophenotypes may be implicated in PTSD development. These endophenotypes for PTSD include the deregulation of the hypothalamic pituitary adrenal (HPA) axis, the physiology of hyperarousal and the exaggerated acoustic startle response. Other important response measures include autonomic reactivity, such as heart rate variability, and psychological variables like memory problems (Segman and Shalev, 2003).
As PTSD is a complex disorder consisting of different types of symptom clusters, genetic research may be simplified by investigating specific symptoms or clusters. This, too, would improve the chances of identifying distinct contributions by specific genes. Most studies so far have supported the hypothesis that certain facets of PTSD have implications for prognosis and treatment strategy or for comorbidity (Perkonigg et al., 2005). Different symptom clusters have also been associated with different physiological parameters. The specific dimension of emotional numbing, for example, has been linked to low cortisol levels (Asmundson et al., 2004; Hawk et al., 2000; Mason et al., 2001), whereas hyperarousal symptoms show more connections to sympathetic nervous system activation (Olff et al., 2005b; Schell et al., 2004; Woods and Wineman, 2004). Several studies have shown that trauma victims with peritraumatic dissociation, and to a lesser extent with peritraumatic emotional responses, may be at greater risk for developing PTSD (Birmes et al., 2001, 2003; Fullerton et al., 2000, 2001; Koopman et al., 1994; Ozer et al., 2003).
The endophenotypic characteristics or symptom clusters referred to above could contribute to PTSD susceptibility, and each of them separately is likely to better reflect the underlying genetic constitution of a person with PTSD. Hence, genetic research ought to focus primarily on specific, distinct endophenotypes of PTSD (Smoller and Tsuang, 1998).

1.2. Molecular genetic research on PTSD

The major types of molecular genetic research are linkage studies, association studies and microarray analyses (Figee et al., 2004; Vonk et al., 1998; van Waarde et al., 2002). Linkage studies investigate at random the entire genomes of individuals, using DNA markers to locate chromosomal areas that are continuously passed on together with a particular disorder. Natural genetic variants are sought and their prevalence is assessed in subjects with a disorder like PTSD against healthy control groups. No prior hypothesis is required as to which gene could be causing the disorder. Linkage analyses are mainly successful in investigating monogenetic disorders, and they are often carried out on large families. This model is difficult to apply in PTSD research, because the essential condition for PTSD onset – exposure to trauma – is a variable that cannot be influenced. Nonetheless, experimental animal studies in laboratories have already made significant contributions to research on the relative roles that genetic and environmental factors play in stress responses. Most of the hypotheses on candidate genes implicated in PTSD derive from such animal studies (Gass et al., 2001). Candidate genes identified so far are the serotonin transporter gene (5-HTT), the dopamine

receptor gene (DRD2) and the dopamine transporter gene (DAT), the glucocorticoid receptor gene (GR), the GABA (A) receptor gene, the apolipoprotein E gene (APOE), the brainderived neurotrophic factor gene (BDNF) and the neuropeptide Y gene (NPY).

A next step in molecular genetic research is to investigate in association studies whether a connection exists between a genetic variant (polymorphism) and an endophenotype in a disorder. Studies like these can uncover smaller genetic effects. They, therefore, seem the method best suited for molecular genetic studies on complex disorders like PTSD (Sullivan et al., 2001). The presence of genetic polymorphisms in candidate genes in a group of individuals with a particular disorder is compared to a group of healthy individuals. Single nucleotide polymorphisms (SNPs) are small changes in DNA that have no visible effects but which do affect vulnerability. A distinction is made between functional and non-functional SNPs. Functional SNPs have effects on gene expression and/or protein function. No such effects are known from non-functional SNPs, but they can still be of use for association studies. Although they probably do not cause the disorder, they lie on a chromosome near the 'pathogenic' mutation.

Candidate genes investigated in association studies should preferably have already been identified in linkage analyses or should be part of a well-defined prior hypothesis. Because linkage analysis is difficult to perform in PTSD, candidate genes not identified in prior human linkage analyses are often selected for association studies on the basis of hypotheses about their putative functional relationship with PTSD (or after identification in animal studies). Should an association then be found, it merely shows that the polymorphism in question is somehow connected to a particular endophenotype of PTSD, but it does not demonstrate a causal relationship (van Rossum et al., 2005). Other yet undiscovered factors, such as a link between that polymorphism and another polymorphism in the same gene or a nearby gene, could explain the association. The genetic research conducted at present in relation to PTSD involves this type of association studies on candidate genes preselected without prior human linkage analysis (Figee et al., 2004; Vonk et al., 1998; van Waarde et al., 2002).

Another drawback of association studies specific to PTSD is that it remains unclear how many individuals in the 'healthy' group might themselves have a vulnerability to developing PTSD, but have not done so yet because they have never experienced a traumatic event, or for other reasons. This increases the danger of false-negative results in this type of research (Segman and Shalev, 2003). That is why it is important to compare the PTSD group not only with healthy controls but also with trauma-exposed controls without PTSD.

A relatively new method of genetic research is to study expression of genes in specific tissues by using microarray analysis (by using an RNA or cDNA chip). This was recently done for the first time in PTSD. Segman et al. (2005) used oligonucleotide microarrays to measure peripheral blood mononuclear cell (PBMC) gene expression in trauma survivors directly after they presented to a casualty department and four months later. The results showed an overall reduction in expression of transcription activators of PBMC in psychologically distressed victims. This demonstrates the possibility of stress-induced reduction of gene expression.

Despite the tremendous advances in knowledge in the past 5 years in the neurobiology and genetics of other psychiatric disorders like depression, genetic research on PTSD is still rare. Identifying genes that mediate susceptibility to PTSD would greatly improve our un-

derstanding of the disorder and could further uncover the molecular basis of those genes (Segman and Shalev, 2003). This article explores the current molecular genetic findings of association studies on PTSD by reviewing the major candidate genes investigated so far in relation to PTSD, followed briefly by a discussion about the key research results for each of the neurotransmission systems involved and suggests directions for future research.

2. Methods

We carried out searches in Medline, Embase and Web of Science databases (1966–October 2006) using the following medical subject heading terms: "genetics AND PTSD OR serotonin OR dopamine OR glucocorticoid OR GABA OR apolipoprotein OR BDNF OR NPY".
We have chosen those terms because candidate genes from these neurotransmitter systems had already been identified previously in animal research. We also entered the names of the candidate genes themselves as search terms. Additional articles were found by consulting reference lists and publications cited as related articles. We confined ourselves to association studies in humans and to articles published in English. No strict inclusion or exclusion criteria were specified, because our objective was to compile a broad review of publications on the genetics of PTSD.
Placing particular emphasis on literature reviews over the period 1966–2006, we obtained the following results:
-PTSD and genetics: 105 Medline hits 1949–2006, 13 Embase hits 1980–2006 (3 new), 9 Web of Science hits 1988–2006 (3 new).
-PTSD and genetics and review: 27 Medline hits 1949–2006, 7 Embase hits 1980–2006 (1 new), 3 Web of Science hits 1988–2006 (1 new).
-Serotonin and PTSD and genetics (without animal studies): 7 Medline hits 1949–2006 (3 reviews), 0 Embase hits 1980–2006 (0 new), 0 Web of Science hits 1988–2006 (0 new).
-Dopamine and PTSD and genetics (without animal studies): 8 Medline hits 1949–2006 (3 reviews), 0 Embase hits 1980–2006 (0 new), 1 Web of Science hit 1988–2006 (1 new).
-GR and PTSD and genetics (without animal studies): 1 Medline hits 1949–2006 (0 reviews), 3 Embase 1980–2006 (2 new), 1 Web of Science hit 1988–2006 (0 new).
-GABA and PTSD and genetics (without animal studies): 2 Medline hits 1949–2006 (0 reviews), 0 Embase hits 1980–2006 (0 new), 0 Web of Science hits 1988–2006 (0 new).
-APOE and PTSD and genetics (without animal studies): 1 Medline hit 1949–2006 (0 reviews), 1 Embase hit 1980–2006 (0 new), 0 Web of Science hit 1988–2006 (0 new).
-BDNF and PTSD and genetics (without animal studies): 1 Medline hit 1949–2006 (0 reviews), 2 Embase hits 1980–2006 (1 new), 0 Web of Science hits 1988–2006 (0 new).
-NPY and PTSD and genetics: 1 Medline hit 1949–2006 (0 reviews), 0 Embase hits 1980–2006 (0 new), 0 Web of Science hits 1988–2006 (0 new).

3. Results

3.1. Genes involved in the serotonin system

The literature search produced 7 hits in Medline, 3 of them reviews; Embase and Web of Science yielded 0 hits. We thus found a total of 7 articles, including 3 reviews. As two of the articles mainly involved the dopamine receptor (Comings et al., 1996; Lawford et al., 2003), we discuss them in that section below. One study is a receptor binding study instead of an association study (Bonne et al., 2005).

Table 1 summarizes the articles relating to candidate genes for PTSD. Reviews on genes involved in the serotonin system include Gross and Hen (2004); Stahl (2005) (state-of-the-art); Talbot (2004).

Evidence exists that the genes regulating the serotonin system play a role in susceptibility to PTSD or depression in response to various types of stressors over the life course (Stahl, 2005). The ascending serotonin pathway, originating in the dorsal raphe nucleus and innervating the amygdala and frontal cortex, facilitates conditioned fear. The dorsal raphe-nucleus-periventricular pathway inhibits inborn fight-or-flight reactions to impending danger. And finally the pathway connecting the median raphe nucleus to the dorsal hippocampus promotes resistance to chronic unavoidable stress (Graeff et al., 1996). Serotonin may have an inhibitory effect on norepinephrine (NE) neurons at the level of the locus ceruleus. In addition, serotonin terminals from the dorsal raphe and NE terminals from the locus ceruleus converge on the amygdala to mediate fear responses. It is unclear exactly how the serotonin circuits are disrupted in PTSD (Davis et al., 1997). The serotonin transporter gene has been identified in relation to the serotonin system and PTSD. A great deal of research has already been done on genes involved in the serotonin system, and which thereby affect serotonergic transmission. The serotonin transporter, also called 5-HTT (or SERT), is now the best studied biological substrate of depression (Kalia, 2005). Abnormal serotonergic activity may mediate susceptibility to affective disorders, and a relationship has also been found to stress reactions (Caspi et al., 2003; Mendlewicz et al., 2004). Initially, considerable experimental research was done on animals which supported the hypothesis of a 'gene-by-environment' interaction. It showed that variation in the 5HTT transporter gene was linked to altered serotonergic function following stressful early life events (Bennett et al., 2002; Murphy et al., 2001). For example, serotonin played a part in HPA-axis alterations in animals exposed to early life stress (Lauder, 1983).

Although the role of serotonergic transmission in the pathophysiology of PTSD is still unclear, serotonin is thought to be involved in the onset of the PTSD symptoms relating to mood, arousal and sleep. Allelic variation in human 5-HTT expression is caused by functional gene promoter polymorphisms with 2 predominant variant alleles, which are likewise associated with various anxiety responses to stressful events (Glatz et al., 2003). In vitro studies have shown the basal 5-HTT activity in carriers of the 5-HTTPR long (L) allele to be twice as high as in carriers of the short (S) allele, indicating that 5-HTT gene transcription may be modulated by these variants.

An association study in individuals with PTSD versus a healthy control group has found only marginal differences in genotypes between the 2 groups, except that the frequency of the SS genotype was significantly higher in the PTSD group. A limitation was that the controls had never experienced a trauma, so it was unknown whether some might have de-

veloped PTSD on trauma exposure (Lee et al., 2005). The sample was also small and might have been subject to population bias.

A number of studies have found associations between PTSD and depression in the year following trauma (in about 45% of PTSD subjects) (Shalev et al., 1998). Because far more genetic research exists on the impact of a stressful event on the etiology of depression, we will also mention those results here. They are inconsistent. Several studies, including a recent one in a large group of twins, found that individuals with two short alleles (SS or SL) of the 5-HTT polymorphism were more sensitive to depressogenic effects of stressful life events than those with one or two long alleles (LL) (Caspi et al., 2003; Hamet and Tremblay, 2005; Kendler et al., 2005). In contrast to those significant findings, Gillespie et al. (2005) found no associations, nor did a meta-analysis of 14 studies by Lasky-Su et al. (2005) find any clear relationship between the serotonin transporter gene and the onset of affective disorders after stress, except for a weak association between bipolar disorder and the 44-bp polymorphism of the 5-HTT genotype.

A review of neuroimaging studies found that people with SS or SL alleles showed greater amygdala activation in response to fearful stimuli than those with the LL allele (Hariri et al., 2002; Wurtman, 2005). In another study, neurochemical processes were associated with genetic variations in serotonergic neurotransmission relevant to anxiety. Significantly lower hippocampal N-acetylaspartate (NAA) concentrations were found in SL carriers than in individuals with a LL genotype, and NAA concentrations correlated negatively with anxiety traits on the Spielberger State-Trait Anxiety Inventory (STAI; Gallinat et al., 2005). Other studies have looked further into links between the 5-HTT gene and temperament. However, as Schinka et al. (2004) noted in a meta-analysis of 26 studies since March 2003, studies of the association between polymorphisms of the serotonin transporter gene (5-HTT) and trait anxiety have produced inconsistent results, raising questions about the strength of the relationship and the methodological conditions under which the relationship holds. They concluded that no link had been demonstrated between the 5-HTTLPR and trait anxiety.

To conclude, the serotonin transporter gene possibly plays a role in the degree of response to stressful events. Evidence has been found that variation in the 5-HTT gene moderates the sensitivity of individuals to the depressogenic effects of stressful life events. Little research has been done yet on 5-HTT and PTSD, but greater amygdala activation in response to fearful stimuli and lower hippocampal NAA concentrations were more likely in subjects who were homozygote SS or heterozygote SL than in those who were homozygote for LL.

3.2. Genes involved in the dopamine system

The literature search yielded 8 hits in Medline, 3 of which were reviews; Embase yielded 0 hits and Web of Science 1 additional new hit (a state-of-the-art article). The total came to 9 articles, including 3 reviews and 1 state-of-the-art article.

Reviews on genes involved in the dopamine system include Comings and Blum (2000), Gordon and Barnes (2003) (state-of-the-art), Noble (2000), Segman and Shalev (2003). See Table 1.

In animal studies, dopaminergic innervation of the basolateral nucleus of the amygdala, the medial prefrontal cortex and other limbic regions is highly responsive to stress and may be altered by stress (Goldstein et al., 1996; Inglis and Moghaddam, 1999). Also the enhancement of the acoustic startle response, which can be a symptom of PTSD, has been

related to the dopamine D1 receptor agonists in rats (Meloni and Davis, 1999). Genetically determined alterations in dopamine release and dopamine receptor expression in mice have been implicated in behavioural abnormalities induced by chronic stress (Puglisi-Allegra and Cabib, 1997). This finding was interpreted as suggesting that stress-induced alterations of central dopaminergic neurotransmission may be genotype-dependent and expressed in behaviour. Human studies showed that there was a relationship between urinary excretion of dopamine and plasma dopamine and (the severity of) PTSD symptoms (Hamner and Diamond, 1993; Yehuda et al., 1992). Together this has been seen as a relevant role for dopamine in the pathogenesis of PTSD.

There are 2 important PTSD candidate genes that directly affect the dopamine system: the dopamine receptor gene (DRD2) and the dopamine transporter gene (DAT). The D2 dopamine receptor (DRD2) minor (A1) allele (DRD2 A1) has already been linked to ADHD, Tourette's syndrome, conduct disorder and substance abuse (Noble, 2000).

This prompted suppositions that this gene may be involved in stress response in humans. The first study on the role of DRD2 in PTSD was published by Comings et al. (1996). Although this study reported a significant association between the presence of the DRD2 A1 allele and PTSD, this result was not confirmed in a later study (Gelernter et al., 1999). That could have been due to selection bias (from recruitment via a substance abuse treatment centre), comorbidity (with substance abuse), the small size of the earlier study and the difference in control groups. Comings compared the PTSD group with a trauma-exposed group without PTSD, while Gerlenter compared the PTSD group with a healthy control group. Alcohol continued to play a prominent role in the research that followed on DRD2 and PTSD. Although an association was found between the DRD2 A1 allele and PTSD, it was seen only in individuals that drank harmful daily amounts of alcohol (Young et al., 2002). The DRD2 A1 allele has further been linked to improved social functioning in individuals with PTSD being treated with paroxetine. Since only one study was involved, the clinical relevance is yet unclear (Lawford et al., 2003).

There has been only one study on the DAT gene in relation to PTSD. In a large group of twin pairs who had been in the war in Vietnam, it sought to establish a link between PTSD and the DAT SLC6A3 3'-variable number tandem repeat (VNTR), using a trauma-exposed control group without PTSD. Evidence was found that genetically determined changes had occurred in dopaminergic reactivity in the PTSD subjects (Segman et al., 2002). However, another study (not involving PTSD) on the effects of drugs on dopamine found that the VNTR polymorphism was not associated with any increase or decrease in expression of the DAT (Martinez et al., 2001).

To summarize, inconsistent results have been reported on the relationship between the dopamine receptor gene and PTSD. What does become clear from various studies is a connection between the presence of the DRD2 A1 allele and alcohol abuse, which could bias findings on the relationship of the former to PTSD. To gain more clarity about any link between PTSD and the DRD2 gene, it is therefore important to clearly document alcohol consumption in future association studies. Insufficient research has been done on the role of genotypes of the DAT gene in relation to PTSD; the functional role of the DAT SLC6A3 3'-VNTR is still unclear.

3.3. Genes involved in the GR system

The literature search produced 1 hit in Medline, 3 in Embase (2 new) and 1 in Web of Science (0 new), a total of 3 articles, including 2 reviews. Reviews on genes involved in the GR system include Grossman et al. (2002) and Radant et al. (2001). See Table 1.

GRs produced by the stress-responsive HPA axis, are well recognized for their regulatory role in peripheral metabolism and various brain functions. Increased GRs exposure in humans, including exposure to the endogenous GR cortisol, is associated with stress, and decreases memory and learning function. There is evidence that dysregulations in cortisol activity occur in PTSD, but it remains unclear whether these are a cause or a consequence of PTSD (Abelson and Curtis, 1996; Bjorntorp, 2002; Charney, 2004; Holsboer and Barden, 1996; Keck et al., 2004; Olff et al., 2005b; Olff and de Vries, 2005; Schreiber et al., 1996; Zobel et al., 1999). Hippocampal GR receptors play an important role in GR negative feedback. Abnormalities in negative feedback are found in depression and in PTSD and may be involved in the pathophysiology of these disorders (Liberzon et al., 1999). Brain imaging studies have demonstrated a strong relationship between PTSD and a reduction in the volume of the hippocampus. However, the mechanisms that cause such atrophy are not well understood. Recently, Zhang et al. (2006a) proposed the hypothesis that stress-induced changes of mitochondrial membrane potential are regulated by nongenomic and genomic actions of cortisol in hippocampal neurons (Zhang et al., 2006a).

An earlier animal study found that 2 hippocampal GR receptors played a key role in regulating the HPA axis and the cortisol level (Liberzon et al., 1999). These were the mineralocorticoid receptor (MR or type 1) encoded on chromosome 5 and the glucocorticoid receptor (GR or type 2) encoded on chromosome 4 (de Kloet et al., 1998; Veldhuis et al., 1982). Because MRs have high affinity to cortisol (10 times greater than the GRs), it is mainly MRs that are occupied in the absence of stress (basal corticosteroid levels). When stress arrives, cortisol levels increase sharply and the GRs are also occupied. Aldosterone plays a part here; by MRs it can affect the expression of GR mRNA (Chao et al., 1998; de Kloet et al., 1998). The balance between the effects of these 2 corticosteroid receptor types is critical to the stress response and behavioural adaptation thereafter (de Kloet et al., 1998). Both MR- and GR-mediated effects of information processing facilitate behavioural adaptation and thereby stimulate higher brain centers to exert inhibitory control on HPA-axis activity (Gass et al., 2001). Individual differences in the number, the affinity and the efficiency of the signaling cascades activated by these receptors have direct effects on cortisol levels and biological activity (Gass et al., 2001; de Kloet and Derijk, 2004). Corticosteroid receptors function as transcription factors. Most evidence in relevant animal models points towards an involvement of altered GR rather than MR function (Liberzon et al., 1999; Kellner et al., 2002; Yehuda et al., 2004). Much research has already been done on the GR gene and on sensitivity to corticosteroids, and most studies have reported positive associations (Panarelli et al., 1998; Rosmond, 2002). Both the N363S and the BclI polymorphisms of this gene have been linked to GR hypersensitivity (Buemann et al., 1997; Di Blasio et al., 2003; Dobson et al., 2001; Huizenga et al., 1998; van Rossum et al., 2003). Bachmann et al. (2005) went on to test whether variations in the GR gene showed links to PTSD. In a group of PTSD and trauma exposed non-PTSD Vietnam War veterans, they screened for polymorphisms and assessed GR sensitivity using the low-dose dexamethasone suppression test (LD-DST) and the dermal vasoconstrictor assay (DVVA). The researchers concluded that the N363S

and BclI GG genotypes were not more common in PTSD subjects than in the control subjects or in the general population. However, they did find a significant association between the BclI GG genotype and low basal cortisol levels in PTSD. Subjects with PTSD and the GG genotype tended to be more responsive to the DVVA, and their DVVA response correlated with higher scores on the clinician-administered PTSD scale (CAPS). There was insufficient evidence, however, that variation in GR polymorphisms actually increases the susceptibility to PTSD.
In sum, no evidence was found of an association between common GR polymorphisms and PTSD. Only a subgroup of people with PTSD and the BclI GG genotype seemed more responsive to the DVVA and had higher CAPS scores, which in turn showed a significant negative correlation with basal plasma cortisol levels. As the group of individuals with a BclI GG genotype was small, these results will have to be verified in other groups.

3.4. Genes involved in the GABA system

The literature search in Medline delivered 2 hits, none of them reviews, and Embase and Web of Science yielded 0 hits. Of this total of 2 articles, one mainly involved the dopamine receptor (Comings et al., 1996); as it was discussed above, it will not be discussed here. See Table 1.
GABA plays a part in the pathogenesis of anxiety, affective disorders and insomnia; phenomena that also appear in PTSD. Evidence from animal studies suggests that alterations in the benzodiazepine/GABA(A) receptor complex can occur in response to stress and anxiety (Weizman et al., 1990). Clinical evidence in humans also supports a relationship between alterations in benzodiazepine receptor function and anxiety. Studies have shown the efficacy of benzodiazepines in the treatment of a variety of anxiety disorders, such as generalized anxiety disorder and panic disorder, but also in the treatment of symptoms of hyperarousal in PTSD (Braun et al., 1990). Although studies on the connections between GABA type A receptor alpha 6 subunit gene (GABRA6) and cortisol have reported that homozygotes for the T allele had generally higher diurnal cortisol levels (Rosmond et al., 2002a, b), only one study has sought links between GABA and PTSD (Feusner et al., 2001). It focused on the GABA(A) receptor beta 3 subunit gene (GABRB3). Individuals with PTSD who were heterozygote for the G1 polymorphism of the GABRB3 gene were found to have higher total scores than homozygote individuals on the General Health Questionnaire (GHQ), whose 4 subscales pertain to somatic symptoms, anxiety and insomnia, social dysfunction and depression. Comorbidity was not an exclusion criterion, the number of persons studied was small and there was no control group, as a consequence the results have to be interpreted with care. In sum, little is known about the influence of variation of genotypes involved in the GABA system in relation to PTSD.

3.5. Genes involved in the APOE system

The literature search in Medline yielded 1 hit and 0 reviews, and the same hit was found in Embase, and no hit was found in Web of Science, for a total of 1 article. See Table 1.
The APOE mediates the binding of lipoproteins to the low-density lipoprotein (LDL) receptor and plays an important role in the metabolism and redistribution of lipoproteins and cholesterol (Mahley, 1988). In animal studies Raber et al. found higher measures of anxiety following anxiety testing in adult APOE deficient male mice compared to wild-type

controls (Raber et al., 2000, Robertson et al., 2005). In human, APOE4 has been linked to a number of neuropsychiatric disorders, including Alzheimer's disease, stress and depression, as well as to smaller hippocampal volumes and to subjective and objective memory impairment (Cohen et al., 2001; Flory et al., 2000; Gallagher-Thompson et al., 2001; Kuller et al., 1998; Lippa et al., 1997). APOE2 has been linked to lower cortisol levels (Peskind et al., 2001). Since memory impairment also occurs in PTSD and smaller hippocampal volumes are sometimes found too, Freeman et al. (2005) assessed which role APOE genotypes might play in this disorder. They studied 54 male veterans with combat-related PTSD, recruited via a treatment centre. PTSD was assessed with 2 structured interviews, and genotypes were determined through a buccal swab. Memory tests were administered by staff members, who were blind to diagnosis and symptom severity. It was found that the carriership of allele 2, and not of allele 4, was associated with significantly lower scores on the memory test and with more severe re-experiencing symptoms. This study was limited by its small sample and by psychiatric comorbidity and there was no control group. There is, therefore, still insufficient evidence concerning the possible role of APOE genotypes in relation to PTSD.

3.6. Genes involved in the BDNF system

The literature search produced 1 hit in Medline, 2 in Embase (1 new) and 0 in Web of Science, a total of 2 articles, without reviews. See Table 1.

BDNF, a member of the neurotrophin family, promotes neuronal survival and regulates the proliferation and differentiation of nerve cells in the peripheral and central nervous systems. It has important regulatory effects on the serotonergic, glutamatergic and dopaminergic neurotransmitter systems (Zhang et al., 2006b). BDNF is also involved in hippocampal long-term potentiating, which is related to learning and memory (Yamada et al., 2002). There is strong evidence that BDNF may contribute to the pathogenesis of several neuropsychiatric disorders and is also believed to be involved in PTSD. Data of an animal study suggest that psychological, as well as unconditioned physical stress, can decrease hippocampal BDNF mRNA, which could be relevant to the pathogenesis of stress-related disorders, such as depression and PTSD (Rasmusson et al., 2002). Zhang et al. observed the association of gene variants of the BDNF gene and several neuropsychiatric phenotypes. They compared 69 subjects with PTSD with a healthy control group. The SNPs G-712A and C270T and Val-66Met were genotyped. There was only an association between the newly described SNP G-712A and substance dependence, but no association of the SNPs with PTSD. Given the low heterozygosity or the low information content of SNPs C270T and G-712A, these 2 polymorphisms appear to require larger numbers of cases to ensure adequate statistical power. In addition, although the overall study sample was large, the sample for PTSD was much smaller and this limited the power to detect significant associations (Zhang et al., 2006b). In the Korean population Lee et al. (2006) analyzed the genotype and allele frequencies of the BDNF gene Val66Met polymorphism in 106 PTSD patients and 161 unrelated healthy controls using a case-control design. The genotype and allele frequencies for the BDNF gene polymorphism did not differ between the 2 groups (Lee et al., 2006). In summary at this moment there is no evidence concerning the possible role of gene variants involved in the BDNF system in relation to PTSD.

3.7. Genes involved in the NPY system

The literature search in Medline yielded 1 hit and 0 reviews in Medline, and Embase and Web of Science yielded 0 hits. See Table 1.

NPY is a 36-amino acid peptide neurotransmitter. Animal studies have suggested that NPY is involved in the regulation of appetite, reward, anxiety, and energy balance (Lappalainen et al., 2002). NPY is present in extensive neuronal systems of the brain and is present in high concentrations in cell bodies and terminals in the amygdala (Morgan et al., 2000). Morgan et al. found in 2 different studies with soldiers without a control group, that acute stress elicits NPY release and that this release is positively associated with cortisol and NE release. The finding that greater levels of NPY release are associated with less psychological distress suggests that NPY confers anxiolytic activity in humans (Morgan et al, 2000). Individuals with the Pro7/Leu7 genotype have higher maximal increases in the plasma concentration of NPY in response to maximal physiological stress as compared with Leu7/Leu7 individuals (Kallio et al., 2001). Lappalainen et al. tested whether the Leu7Pro allele associated with alcohol dependence in a population study compared to a healthy control group. Population stratification potential and diagnostic specificity was studied by genotyping individuals from additional populations and psychiatric diagnostic classes, such as PTSD. There were 77 PTSD Vietnam combat veterans. Consensus diagnoses were made with the Structural Clinical Interview for DSM-IV diagnosis (SCID). The main outcome measure was the difference in Leu7Pro allele frequencies between alcohol-dependent subjects and controls. There was no association with PTSD (Lappalainen et al., 2002). Until now there is no evidence for the possible role of gene variants involved in the NPY system in relation to PTSD.

4. Discussion

Our review shows that – in contrast to many other psychiatric disorders – no extensive genetic studies have been carried out on PTSD. Association studies have investigated 8 major genotypes in connection with PTSD. They have tested hypotheses involving key candidate genes in the serotonin, dopamine, GR, GABA, APO, BDNF and NPY systems. The results indicate that the serotonin transporter gene possibly plays a role in the degree of response to stressful events, in particular in the sensitivity of individuals to the depressogenic effects of stressful life events. As for the dopamine system, results have been inconsistent and may be dependent on confounding effects of alcohol abuse. GR receptor polymorphisms were not generally found to be more frequent in PTSD. Only in a small subgroup of PTSD patients with the Bcll GG genotype relations were found between PTSD symptoms and basal cortisol levels. For the GABA system and the APO system there is only little evidence associating these systems with PTSD. There was no association found between PTSD and BDNF or the NPY system.

Table 1. PTSD candidate genes

Gene	Function (known or hypothetical)	Chromosomal location	Subjects	Controls	Results	Main author / Year
5-HTT	Serotonergic neurotransmission	17q11.1-q12	100	197	Pos.assoc. betw. SS genotype and PTSD	Lee et al., 2005
DRD2	Dopamine D2 receptor expression	11q22-23	63	None	Pos. assoc. betw. DRD2 A1 allele and response to paroxetine	Lawford et al., 2003
DRD2	Dopamine D2 receptor expression	11q22-23	91	51	Pos. assoc. betw. DRD2 A1 allele and PTSD+alcohol	Young et al., 2002
DRD2	Dopamine D2 receptor expression	11q22-23	52	87	No assoc. betw. DRD2 and PTSD	Gelernter et al., 1999
DRD2	Dopamine D2 receptor expression	11q22-23	37	19	Pos. assoc. betw. DRD2 A1 allele and PTSD	Comings et al., 1996
DAT	Still unknown, possible role in dopaminergic neurotransmission	Unknown	102	104	Pos. assoc. betw. dopaminergic activity and PTSD	Segman et al., 2002
GR	Glucocorticoid receptor expression	5q31-q32	75	33	No assoc. betw. N363S/BclI GR polymorphisms and PTSD	Bachmann et al., 2005
GABR3	GABA(A) receptor beta 3 expression	15q11-13	20 G1G1	39 G1-nonG1 27 NonG1-nonG1	Pos. assoc. betw. heterozygosity of GABA(A) receptor beta 3 subunit gene and high scores for anxiety/insomnia, depression, somatic symptoms+social dysfunctioning	Feusner et al., 2001
APOE	34k Da protein mediating binding of lipoproteins to the LPL receptor	12q13-14	54 APOE2/2 APOE2/3 APOE2/4 APOE3/3 APOE3/4	None	Pos. assoc. betw. APOE2 and poorer memory scores+more severe re-experiencing	Freeman et al., 2005
BDNF	Proliferation and differentiation of nerve cells and regulatory effects on diverse neurotransmitter systems	Unknown	69 G-712A C270T Val66Met	250	No assoc. betw. BNDF G-712A, C270T and Val66Met and PTSD	Zhang et al., 2006a,b
BDNF	Proliferation and differentiation of nerve cells and regulatory effects on diverse neurotransmitter systems	Unknown	106 Val66Met	161	No assoc. betw. BDNF Val66Met and PTSD	Lee et al., 2006
NPY	Vasoconstriction and inhibition of catecholamine release	Unknown	77 Pro7/leu7 Leu7/leu7	267	No assoc. betw. NPY pro7/leu7 and leu7/leu7 and PTSD	Lappalainen et al., 2002

The association studies that have been done have serious limitations. In the first place, studies were based on hypothesis-driven searches for candidate genes rather than on candidate genes already identified in genetic linkage research. That means that any associations found could never provide causal explanations, because they might have been confounded by other factors. Second, some association studies involved candidate genes identified in animal studies. Notwithstanding the important contributions made by experimental studies with animals, a serious shortcoming is that they fail to address a range of other factors that influence the disorder, so that none of the mutations seen in animals can serve as full models for specific psychiatric disorders in humans (Gass et al., 2001; Shekar et al., 2001).

A further difficulty with genetic research on PTSD involves the complexity of this disorder, as seen in the many potential endophenotypes, the precondition of exposure to a traumatic experience, and the high prevalence of comorbidity with other psychiatric disorders. All of these compromise the reliability of the endophenotypes. In doing research on PTSD, it is essential that PTSD groups should be compared not only with a healthy control group, but also with a trauma-exposed group that did not develop PTSD, if the conditional risk of certain genetic markers is to be teased out. Genetic analysis in PTSD is additionally complicated by factors such as incomplete penetrance and pleiotropy (Radant et al., 2001). Incomplete penetrance means that a particular genotype will not always result in a particular endophenotype or phenotype. A gene shows a complete penetrance of 1 only if all individuals of a particular genotype have the same endophenotype. That is not usually the case, and that obviously makes it more difficult to establish a linkage between a particular genotype and an endophenotype. Pleiotropy means that a single gene influences different traits and may thus be linked to different endophenotypes.

Most studies are informed by an assumption of gene-environment interaction deriving largely from animal experiments (Caspi et al., 2003). This could be another reason for inconsistent outcomes. Environmental influences can contribute to the effects a polymorphism has on a particular endophenotype or phenotype (van Rossum et al., 2005). Different types of stressful events, for instance, can have different effects on the expression of genes (de Kloet and Derijk, 2004). Early developmental stress is thought to potentially induce neurobiological changes in humans and thereby to confer a higher risk of psychopathology, in particular anxiety and affective disorders (Heim and Nemeroff, 1999, 2002). Interestingly, though, there are also studies that suggest the possibility of underlying genes, which would actually increase a person's likelihood of being exposed to a trauma in the first place. That would heighten vulnerability to PTSD through a different route (True et al., 1993). An elevated risk of trauma involvement has been linked to factors such as personality traits, substance abuse, patient histories of mood or anxiety disorders, and familial psychopathology (Breslau et al., 1998; Breslau and Davis, 1995; Brewin et al., 2000; Stein et al., 2002). This leads to the conclusion that a genetic predisposition, combined with early stress in critical developmental stages, can result in a phenotype that is neurobiological vulnerable to stress, and which lowers the threshold of an individual for developing a depressive or anxiety disorder once additional stress exposure occurs.

Given the many limitations of the genetic research done on PTSD so far, the current findings provide only very modest explanations for why certain people seem more liable than others to stress-related disorders like PTSD or depression (Glatz et al., 2003). Why one person develops depression and the other PTSD or both is still unexplained, and a good

deal of future research will be necessary to clarify it. Not only is there evidence that both disorders could be linked to reactions to traumatic experiences, but twin research has also produced evidence that both disorders may be part of the same underlying dimension and that the two conditions may be genetically linked. Chantarujikapong et al. (2001) have argued that depression, generalized anxiety disorder and panic disorder could all be part of a 'post-combat response syndrome'. Clear vulnerability has also been found in certain families to the co-occurrence of PTSD with depression or dysthymia (Chantarujikapong et al., 2001; Koenen et al., 2003). The genetic risk factor for trauma exposure also correlates positively with the genetic risk factor for depression (Kendler and Karkowski-Schuman, 1997), which could be evidence for a genetically determined joint confounder (such as personality traits). By conducting further genetic research on both depression and PTSD in response to stressful or traumatic experiences, researchers would be able to study much larger groups than they could up to now, in which they can also seek evidence for a common underlying genetic susceptibility to the 2 disorders.

The distinction we have made here between the genetics of the serotonin, dopamine, GR, GABA, APO, BDNF and NPY systems is an artificial one. In reality, the neurotransmitter systems have considerable influence on one another, and there are presumably many more factors that have not yet been studied. Serotonergic neurotransmission, for example, is involved both in the HPA axis and in reactions to stressful events. In vitro studies suggest that elevated GR concentrations can precipitate depression by inducing an increase in 5-HTT expression, after which increased 5-HT uptake causes a reduction in synaptic 5-HT concentrations. Dexamethasone, a potent glucocorticosteroid hormone, has also been found to induce increased 5-HTT expression in immortalised human B-lymphoblastic cells. This suggests a 5-HTT-genotype-dependent dose response to a glucocorticosteroid hormone antagonist (Glatz et al., 2003). These examples illustrate how many interactions occur between neurotransmitter systems that further increase the complexity of the whole.

In sum, the research to date is still too scarce to warrant any firm conclusions. The relative lack of published studies on the genetics of PTSD could also partly be a consequence of publication bias against negative findings. The positive findings are often difficult to interpret because of the relatively small samples (increasing the chances of false negative outcomes) and the methodological shortcomings. Although some polymorphisms have been described for some genes, little is known about their prevalence or about the relevance of haplotypes (fixed combinations of polymorphisms or mutations) in the development of PTSD.

5. Future research directions

An interaction model appears to exist between genes and other factors, which results in a vulnerability to PTSD, which is in part genetically determined. The genetic contribution lies in 'susceptibility genes' or 'vulnerability genes'. Genetic research on PTSD can, therefore, best be conducted by gene-environmental studies.

Because the clinical picture of PTSD is so complex, further research is needed both on specific endophenotypes and on specific symptoms or subgroups of PTSD. In the field of psychiatric genetics, the concept of endophenotypes is becoming increasingly popular. Neurobiological correlates of the disease which are genetically influenced and stable over

time are considered to be more promising targets of investigation. They are more directly influenced by gene effects and presumably defined by a genetic determination, which is less complex than the phenotype of the disorder. The endophenotype strategy has already been successful regarding other complex disorders, such as alcoholism. For example, one possible endophenotype of alcohol dependence may be related to the P300 waveform of the event-related brain potential (ERP) (Carlson et al., 2004; Hesselbrock et al., 2001). Since endophenotypes are influenced by many different factors, it is more likely that associations can be found between a particular genotype and 'proximal endophenotypes' like gene expression and proteins, which are less subject to influence of environmental factors, than between a genotype and more 'distal endophenotypes' like cortisol levels or DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) diagnoses, which undergo more environmental influences. The success of genetic research will stand or fall with the specificity of diagnoses (to minimize heterogeneity and false-positive results) and the sensitivity of diagnostic criteria (to maximize the statistical power of datasets). The most promising approach may be to focus on functional polymorphisms or genetic variants, which have already been shown to influence neurobiological parameters that play a part in particular endophenotypes or PTSD symptom clusters. Future genetic studies can be facilitated if more specific endophenotypes are first identified. Endophenotypes should be studied that are stable over time, that are more common in individuals with PTSD than without PTSD, and which are genetically associated with PTSD but are not a consequence of it (Radant et al., 2001). Another need is to study large groups of subjects in order to improve statistical power. Comorbid disorders should receive special attention, both as risk factors and as potentially confounding factors.

Gender, age and ethnicity must also be taken into consideration. Animal studies have found that genes, including 5-HTTLPR, modulate the effects of early life stress in female macaques (Barr et al., 2004a, 2004b). Findings like these could help explain why women are more prone to PTSD than men (Olff et al., 2007). Gender should be analyzed in future research where ever possible. Age-related associations with particular polymorphisms have been reported too, and polymorphisms may also have different effects in individuals from different ethnic backgrounds. Polymorphisms may vary in frequency, and different combinations of polymorphisms may result in different endophenotypes or phenotypes (van Rossum et al., 2005). Study samples should, therefore, be comparable in terms of gender, age and ethnicity. If all the above considerations are addressed, association studies can definitely make substantial contributions to the study of genetic variations in relation to PTSD.

The emerging field of epigenetics examens hereditary changes in gene function, which occur without actual changes in DNA structure. Neuroscientists have only recently begun to investigate the possible roles of epigenetic mechanisms in behaviour, physiology and neuropathology. As Levenson and Sweatt explain, "Epigenetics refers to a set of self-perpetuating, post-translational modifications of DNA and nuclear proteins that produce lasting alterations in chromatin structure as a direct consequence, and lasting alterations in patterns of gene expression as an indirect consequence" (Levenson and Sweatt, 2005). Studies in PTSD could clearly benefit from epigenetic research. For example, in animal models, prenatal stress, GR exposure or inhibition/knockout of 11 beta-hydroxysteroid dehydrogenase type 2 (11 beta HSD-2, the fetus-placental barrier to maternal GRs), reduces birth weight and causes permanent hypertension, hyperglycemia, increased HPA axis activity and

behaviour resembling of anxiety. Also in humans, low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming. Besides that, in human pregnancy severe maternal stress affects the offspring HPA axis and associates with neuropsychiatric disorders. PTSD appears to be a variable in the effects. Intriguingly, some of these effects appear to be 'inherited' by a further generation, itself unexposed to exogenous GRs at any point in the lifespan from fertilization, implying epigenetic marks persist into subsequent generations (Seckl and Meaney, 2004, 2006). Longitudinal studies are needed to determine how the association between maternal PTSD symptoms and cortisol levels and infant temperament reflect genetic and/or epigenetic mechanisms of intergenerational transmission (Brand et al., 2006).

Ultimately, genetic studies in PTSD will help to clarify its etiology and to refine the encompassing notion of genetic susceptibility. People who are genetically vulnerable in one of the gene systems implicated in PTSD could have a greater vulnerability to environmental influences and traumatic experiences in particular. More knowledge of genetic and environmental influences and how they contribute to the more 'proximal' or 'distal' endophenotypes, and perhaps to PTSD as a whole, should eventually enable a more precise identification of risk factors. Information on the specific functions of particular genes could improve the treatment of the disorder.

Therapeutic options in the field of genetics are still in their embryonic stages, but they could have implications for both prevention and treatment strategies in the future (Lesch, 1999). If a multiplicity of gene variants could be identified which contribute to chronic or refractory PTSD, this would open unique opportunities for developing new treatment approaches like gene therapies and tailor-made drugs. But that goal is still a distant one. Far more research is needed before we can sufficiently understand the genetic contribution to PTSD.

3

The influence of birth size on intelligence in healthy children

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"Family" by Annabelle Bertina, 5 years old

Abstract

Objective

Birth parameters have been hypothesized to have an influence on IQ. However, studies within the range of normal birth size have been sparse. With this study we examined the associations between birth length, birth weight, head circumference, and gestational age within the normal birth size range in relation to childhood IQ in Asian children.

Methods

A cohort of 1979 of 2913 Asian children aged 7 to 9 years, recruited from 3 schools in Singapore, was followed yearly from 1999 onward. Birth parameters were recorded by health personnel. Childhood IQ was measured with the Raven's Standard Progressive Matrices at ages 8 to 12.

Results

The mean IQ score across the sample (n = 1645) was 114.2. After controlling for multiple confounders for every 1 cm increment in birth length, 1 kg in birth weight, or 1 cm in head circumference, there was a corresponding increase in IQ of 0.49 points (P for trend < .001), 2.19 points (P for trend = .007) and .62 points (P for trend = .003), respectively. These associations persisted even after exclusion of premature children and children with extreme weights and head circumferences.

Conclusions

Longer birth length, higher birth weight, or larger head circumferences within the normal birth size range are associated with higher IQ scores in Asian children. Our results suggest that antenatal factors reflected in altered rates of growth but within the normative range of pregnancy experiences play a role in generating cognitive potential. This has implications for targeting early intervention and preventative programs.

1. Introduction

The impact of early life influences on future neurodevelopmental outcomes has received increasing attention (Larroque et al., 2008). As reported by Victora et al. (2008) and Black et al. (2008), maternal and fetal undernutrition, resulting in smaller born babies, have long-term implications on health and human capital by affecting cognitive development (Black et al., 2008; Victora et al., 2008). This is in concordance with other studies which showed that intelligence quotient (IQ) is consistently correlated with birth weight (Bhutta et al., 2002).
However, there are important gaps in the literature. First, most of these studies have focused on cohorts with low birth weight children rather than across the normal range. In low birth weight children there are multiple pathophysiologies that can arise that do not inform the extent to which the fetal environment within the normative range can affect cognitive potential. In the normal birth size range, the association between birth size and IQ is less conclusive. While some large population-based studies report significant relationships between IQ and birth weight across the normal range (Lawlor et al., 2006; Matte et al., 2001), other studies have not found such relationships (Christensen et al., 2006; Gale et al., 2004; Pearce et al., 2005; Shenkin et al., 2004; Tong et al., 2006). Second, only a few population-based studies have examined birth length (Gale et al., 2004; Shenkin et al., 2004; Tong et al., 2006). Of these, no associations with IQ were found, except in 2 recent studies conducted in men (Bergvall et al., 2006; Eide et al., 2007). Eide et al. (2007) found in a nationwide cohort study of 317.761 men that birth length, birth weight, and gestational age were all associated with intelligence measured with a standardized group intelligence test at age 18 (Eide et al., 2007). Bergvall et al. (2006) also found an association between intellectual performance and birth length for gestational age, but focused more on the very and moderately small sized babies, rather than on the normal range (Bergvall et al., 2006). Third, although combinations of different birth parameters provide a better measure of prenatal growth, only a few studies assessed multiple birth parameters (Gale et al., 2004; Tong et al., 2006; Walker et al., 2007). As a consequence it is not clear which relevant features of fetal growth, as reflected by multiple birth parameters, are most closely associated with neurodevelopmental outcomes. Finally, none of these studies were conducted in Asian populations in which, probably due to ethnic genetic variations, lower birth weights are common and children are often small for their gestational ages (Kierans et al., 2008).
The aim of this study is to examine the association between birth length, birth weight and head circumference adjusted for gestational age at birth, as surrogates of intrauterine fetal growth, to childhood IQ in a large cohort of healthy Singapore children of normal birth size.

2. Methods

2.1. Study design and participants

A cohort of 1979 children out of 2913 Asian children (participation rate: 67.9%), was recruited from 3 'normal' schools in different parts of Singapore between November 1999 and May 2001. Singapore is an urban-city state and the majority of children attend 'normal'

schools; whereas children with mental retardation (IQ < 70) often attend 'special' schools. This cohort was part of the Singapore Cohort Study Of Risk Factors for Myopia (SCORM). The children were in Grades 1 to 3 (7 to 9 years old) at baseline and were followed yearly thereafter. The IQ data from the follow-up in 2002 (children in Grades 2 to 5) were included (Saw et al., 2001; Saw et al., 2004). Children with serious chronic medical conditions (e.g. heart disorders, cancer, chronic eye conditions) were excluded from the study (n = 94). The study was approved by the Singapore Eye Research Institute Ethics Committee and the tenets of the declaration of Helsinki were observed. All parents provided written informed consent and children provided written assent.

2.2. Measures of birth parameters

Birth history data were obtained from documented medical record booklets. In Singapore, the hospital physician or nurse records details of the birth history at or shortly after the time of parturition. Birth parameters include birth length (cm), birth weight (kg), head circumference (cm) and gestational age (weeks). For children without this information from medical record booklets (n = 211), gestational data were obtained from the National Birth and Death Registry. This registry captures information recorded on birth certificates by hospital personnel. A validation study of 911 children with gestational data from both medical record booklets and the National Birth and Death Registry revealed a Spearman correlation coefficient of +0.92.

2.3. Measures of IQ

Children who participated in the SCORM study completed the Raven's Standard Progressive Matrices (RPM), a widely used test of non-verbal reasoning ability, during the follow-up in 2002 (Grades 2 to 6) (Raven et al., 1998). The RPM aims to assess the visual alertness, spatial and abstract pattern-recognition abilities. It was group administered by a team led by a psychologist at the schools' premises during regular school hours; the duration of the assessment was less than 1 hour. Instruction was given in English, which is the language of education in Singapore. The scoring was completed manually by a trained psychologist.

The RPM has shown acceptable test-retest reliability coefficients ranging from 0.76 to 0.91 in various cross-cultural studies of intelligence. While the RPM offers greater brevity, the validity is comparable with conventional tests. Various studies conducted among children and adolescents showed good to excellent correlation, ranging from 0.70 to 0.92, to conventional tests of intelligence such as the Wechsler Intelligence Scale for Children (WISC) (Barrat, 1956).

2.4. Measurement of other variables

Parents completed a baseline questionnaire in English or Chinese. Questions included demographic information, mother's age at birth of the child, family size, birth order, parental smoking status, and indicators of socioeconomic status (parental education level, parental jobs, parental income, housing and school of the child). Ethnicity was assessed by asking the parents to classify his or her ethnicity in the following groups: Chinese, Malay, Indian, Eurasian, or others. Ethnicity of the child was determined by using the father's reported ethnicity, according to the definition adopted by the Singapore Population Census 2000 (www.singstat.gov.sg/statsres/glossary/population.html).

The height of the child was measured against a vertical scale (measuring tape) fixed to the wall. The children were asked to stand without shoes and height was taken with a headpiece held perpendicular to the crown of the head. Body Mass Index (BMI) was calculated as the weight divided by the square of the height (kg/m2).

2.5. Statistics

The association between birth length, birth weight, head circumference, and gestational age and IQ were analyzed as continuous variables in multivariable linear regression models. Adjustments were made for age, gender, ethnicity, gestational age, school, mother's education, mother's age at birth, BMI, parental smoking, family size, and birth order, based on prior knowledge that these variables were potential confounders. Linear trend tests were performed by assigning consecutive integers to each group and regressing the dependent variable on this new score.

All analyses were then repeated among children with 'normal' birth weight (> 2.5 kg and < 4 kg), 'normal' head circumference (> 32 cm and < 36 cm), and 'normal' gestational age (≥ 37 weeks) to verify that the associations were not driven by the lowest or highest birth weights or head circumferences or by prematurity. Besides adjustment for ethnicity, all the analyses were repeated in 1253 Chinese children alone to exclude the influence of ethnicity.

Although this study is not a sibling study, sibship analyses with the paired t-test were performed in all the sibs within this study (n = 125) to exclude the role of residual confounders.

All P values were two-tailed and considered statistically significant when the values were below 0.05. All statistical procedures used SPSS version 16.0 (SPSS Inc, Chicago, USA).

3. Results

Of the 1979 children, 16.8% (n = 334) was lost to follow-up at the 2002 visit for IQ. They were excluded, and thus the total number analyzed was 1645. The children lost to follow-up were not different from the children who remained in the study, except for ethnicity (P = .01), family size and birth order (P < .001) (Table 1). The ethnicity is reflective of the ethnic mix of the Singaporean population. Children without birth weight data had a significantly lower IQ in comparison with children with birth weight data (P = .03). Children with missing birth length data had a significant lower birth weight (P = .02) and gestational age (P < .001) compared with children with birth length data.

For the total population the mean age at the time of the administration of the IQ test was 9.8 (SD: 1.2) years, the mean birth length 49.1 (SD: 2.4) cm, the mean birth weight 3.2 (SD: 0.5) kg, the mean head circumference at birth 33.6 (SD: 1.7) cm, the mean gestational age 38.5 (SD: 1.7) weeks and the mean child BMI 18.0 (SD: 3.6). The mean non-adjusted IQ (at the mean age of 9.8 years) was 114.2 (75.0-129.0). There was no difference by gender between mean IQ scores (P = .68) and gestational ages (P = .87), but boys had significantly higher birth weights (P = .01), birth lengths (P < .000), head circumferences (P < .000) and BMI's (P < .000). The proportion of children born with low birth weight (< 2.5 kg) was 6.7% (n = 110) and the proportion of children with a gestational age < 37 weeks was 8.3% (n = 137) of the total population. The Spearman correlation coefficient of birth weight with birth length was +0.70, of birth weight and head circumference +0.59, and birth weight

with gestational age +0.36 (all P < .01).

The relations between birth length, birth weight, head circumference and IQ are depicted in Table 2. The results of the multivariate adjusted model revealed that, for every 1 cm increment in birth length, 1 kg in birth weight, and 1 cm in head circumference, there was a corresponding increase in IQ of 0.49 points, 2.19 points and 0.62 points respectively. After exclusion of children with extreme birth weights (< 2.5 kg or > 4 kg), extreme head circumferences (< 32 cm or > 36 cm) and prematurity (< 37 weeks), these associations became even stronger; 0.50 points, 2.70 points and 0.96 points, respectively (Table 2). Additional analyses were performed to examine the effects of the confounders plus the base descriptive model; it was found that additional adjustment for specific confounders such as maternal smoking, birth order, family size, and BMI showed an increased strength of the relationships between birth parameters and IQ.

Table 1. Comparison of included and excluded participants

	Included (n=1645)	Excluded (n=334)	P
Child's gender:			
Male	828	172	0.70
Female	817	162	
Child's age (years)	9.84 (7-16)	9.92 (8-14)	0.30
Child's birth length (cm)	49.12 (30 - 61)	49.10 (37-55)	0.92
Child's birth weight (kg)	3.17 (0.57 - 5.00)	3.18 (1.78-6.20)	0.55
Child's head circumference (cm)	33.62 (18 - 49)	33.51 (28-37)	0.33
Child's gestational age (weeks)	38.54 (27 - 43)	38.51 (32-43)	0.86
Child's IQ score	114.24 (75 - 129)		
Child's BMI	17.96 (10.88 - 37.33)	17.66 (9.26-29.22)	0.26
Child's ethnic group:			
Chinese	76.2%	68.7%	0.01
Malay	15.1%	23.8%	
Indian	6.8%	4.7%	
Eurasian	0.3%	0%	
Others	1.5%	2.8%	
Mother's education:			
No formal education	4.8%	7.1%	
Elementary school	69.1%	66.1%	0.26
High school	15.6%	14.2%	
University	10.5%	12.6%	
Family size:			
1	13.1%	14.6%	
2	40.5%	44.2%	< 0.001
3	46.3%	30.1%	
4	0%	11.1%	
Birth order of child:			
1	52.9%	62.9%	
2	8.8%	24.9%	< 0.001
3	38.3%	10.3%	
4	0%	1.8%	

BMI: body mass index

Table 2. Relationship of birth length, birth weight, head circumference and gestational age with childhood IQ

	All children		Excluding BW > 4 kg, BW < 2.5 kg, GA < 37 weeks, HC > 36 cm, HC < 32 cm ‡	
	β coefficient (95 % CI) IQ score, Model 1*	β coefficient (95 % CI) IQ score, Model 2†	β coefficient (95 % CI) IQ score, Model 1*	β coefficient (95 % CI) IQ score, Model 2†
Birth length, per 1 cm increase	0.37 (0.14 – 0.61)	0.49 (0.19 – 0.78)	0.44 (0.08 – 0.79)	0.50 (0.12 – 0.87)
P values	0.002	0.001	0.016	0.010
Birth weight, per 1 kg increase	1.37 (0.24 – 2.49)	2.19 (0.60 – 3.77)	2.19 (0.04 – 4.34)	2.70 (0.43 – 4.98)
P values	0.017	0.007	0.046	0.020
Head circumference, per 1 cm increase	0.42 (0.08 – 0.75)	0.62 (0.21 – 1.04)	0.89 (0.23 – 1.55)	0.96 (0.26 – 1.66)
P values	0.015	0.003	0.009	0.008
Gestational age, per week increase‡	0.05 (-0.32, 0.41)	0.04 (-0.34, 0.43)	-0.32 (-0.94, 0.30)	-0.31 (-0.98, 0.35)
P values	0.805	0.821	0.309	0.351

Table 3. Joint effects of birth weight and birth length and head circumference on childhood IQ

	All children		Excluding BW > 4 kg, BW < 2.5 kg, GA < 37 weeks, HC > 36 cm, HC < 32 cm ‡	
	Mean IQ (Std Error), Model 1*	Mean IQ (Std Error), Model 2†	Mean IQ (Std Error) Model 1*	Mean IQ (Std Error) Model 2†
Both BL and BW < median	109.5 (1.3)	107.8 (2.6)	109.7 (1.9)	108.4 (2.9)
Both BL and BW > median	111.8 (1.3)	110.2 (2.6)	112.0 (1.9)	111.0 (2.9)
Difference	2.30 (95% CI 0.96 – 3.64) P = 0.001	2.42 (95% CI 0.78 – 4.06) P = 0.004	2.32 (95% CI 0.62 – 4.01) P = 0.008	2.67 (95% CI 0.82 – 4.52) P = 0.005
Both BW and HC < median	109.1 (1.5)	107.7 (2.6)	109.7 (1.9)	108.1 (2.9)
Both BW and HC > median	111.8 (1.5)	111.0 (2.6)	112.7 (1.9)	111.9 (2.9)
Difference	2.61 (95% CI 1.25 – 3.97) P < 0.001	3.28 (95% CI 1.59 – 4.98) P < 0.001	3.00 (95% CI 1.27 – 4.74) P = 0.001	3.76 (95% CI 1.89 – 5.64) P < 0.001

*Linear regression model of IQ scores, adjusted for age, gender, ethnicity, school and mother's education
† Linear regression model of IQ scores, adjusted for age, gender, ethnicity, school, mother's education, BMI, mother's age at birth, mother's and father's smoking, family size, birth order and gestational age (‡gestational age not adjusted for in these models)
CI: confidence interval; Std Error: standard error; GA: gestational age, less than 37 weeks is defined as prematurity; BW: birth weight in kg. Low birth weight is defined as a birth weight < than 2.5 kg and high birth weight as > 4 kg; BL: birth length in cm; HC: head circumference at birth in cm.

The mean differences in IQ between the smallest and largest babies were all significant in 4 different models, although the mean difference in the multivariate adjusted model was the greatest. After exclusion of children with extreme birth parameters, the mean difference increased to respectively 2.7 points (P = .005) between the highest and lowest category for birth length and birth weight and 3.8 points (P < .001) between the highest and lowest category for birth weight and head circumference (Table 3). No interaction between birth weight and birth length was found on IQ (P = .49).

IQ was not associated with gestational age (estimate: 0.05 [95% confidence interval (CI): -0.32 to 0.41]), even after adjusting for multiple variables in the populations, including and excluding children with extreme birth parameters and prematurity. The mean multivariate adjusted IQ was 108.7; for children born with a gestational age < 37 weeks 107.7 compared to 108.8 for children born with a gestational age of ≥ 37 weeks (P for trend = .34).

Multivariate regression analyses for 1253 Chinese children alone showed that for every unit increment of birth length, birth weight, and head circumference, the IQ increased by 0.36, 1.78, and 0.37 points, respectively. The same trends were present when children with extreme birth parameters and prematurity were excluded.

An additional analysis was conducted of 165 sibling pairs that were part of this cohort. Notwithstanding the small sample size (n =111), the paired t test analyses (95% CI on age) showed a significant relation between birth length and IQ scores (P = .044) with a mean IQ score of 112.57 (SD: 12.04) for the shortest sib at birth and 115.06 (SD: 11.56) for the longest sib at birth. The relationships between birth weight (n = 123) and birth head circumference (n = 104) showed the same trend of a higher IQ for the sibling with a larger birth size, although statistical significance was not reached (P = .29 and P = .46, respectively), probably due to lack of power. There was no difference in IQ between the eldest and youngest sibling (n = 125, P = .92).

4. Discussion

In this population of Asian children, longer birth lengths, increased birth weights or larger head circumferences within the normal range are significantly associated with higher IQ scores, even after adjustment for multiple potential confounders. This was seen in children with 'normal' birth weight (> 2.5 kg and < 4 kg), 'normal' head circumference at birth (> 32 cm and < 36 cm), and 'normal' gestational age (≥ 37 weeks). Our findings support the concept that improved fetal growth predicts increased IQ at school age, across the entire population.

Our study emphasizes the importance not merely of conditions that severely constrain fetal growth but rather the entire range of factors that influence maternal-fetal health. Hence, even small differences in birth length, birth weight, and head size, within the normal birth size range, could be a sign of alterations in the fetal environment affecting its development. Our findings suggest that antenatal factors reflected in altered rates of growth but within the normative range of pregnancy experiences play a role in generating cognitive potential.

The associations found between childhood IQ and birth weight and head circumference are consistent with some previous Western studies, although this is the first study reporting a positive relationship with IQ later in childhood (IQ beyond 4 years), perhaps due to the

larger sample size (Gale et al., 2004; Gale et al., 2006; Matte et al., 2001). However to date, only Eide et al. (2007) reported a positive association between birth length in the normal range and IQ in a large sample of 18 year old males (Eide et al., 2007), which may not be representative for the whole population. Our finding that birth length, more so than other birth parameters, remains significant in sibship analyses suggests that birth length may better reflect the phases of fetal growth related to later cognitive function. Since fetal length increases mainly in the second trimester (Villar and Belizan, 1982), this suggests that influences operative during this period of fetal development have lasting effects on cognitive ability. Such phases, particularly mid-gestation, are critical in humans for the development of cortical layers that inevitably participate in higher cognitive functions (Kostovic and Rakic, 1990).

Life history theory suggests that developing organisms make tradeoffs in growth, development and reproduction to maximize their potential in the environment they will reproduce in. This concept has been extended to consideration of how the human fetus responds to a disturbed environment (Gluckman and Hanson, 2004; Gluckman et al., 2007). It is suggested that the processes of developmental plasticity operate such that the fetus, in an environment in which nutrient delivery is uncertain or where glucocorticoid levels are high (suggesting maternal stress), makes life history adjustments in anticipation of a threatening environment and a shorter life (Bateson et al., 2004). Such offspring will invest less in growth, repair mechanisms and in reserve tissues such as excess neuron and synaptic capacity.

Evidence from a number of clinical and non-clinical samples suggests that the endocrine conditions that constrain fetal growth might directly compromise neural development, resulting in the observed relation between fetal growth and IQ. One possible pathway is through changes in the hypothalamic-pituitary-adrenal axis. Among human newborns, increased umbilical cord levels of glucocorticoids are associated with growth restriction (Goland et al., 1993; Schneider et al., 2002). Also studies with rodent models reveal that increased glucocorticoid exposure during fetal development recapitulates the clinical physiology associated with low birth weight in humans, including cognitive deficits (Meaney et al., 2007). Glucocorticoids antagonize the anabolic effects of growth hormone through disruption in the insulin-like growth factor (IGF-I) pathway (Seckl and Holmes, 2007). IGF-1 expression in fetal tissues play a critical role in growth regulation and organ development like brain development, and can affect neurogenesis, providing a possible link (Meaney et al., 2007). A preliminary magnetic resonance study reported a significant relation between birth weight and hippocampal volume in humans (Buss et al., 2007). Interestingly, this relation was modulated by measures of postnatal parenting consistent with the idea that the consequences of fetal adversity are subject to subsequent modification by factors operating during later stages of development (Gluckman et al., 2007; Meaney et al., 2007).

The major strengths of this study are the use of several birth parameters, as surrogates of fetal growth, the large sample size with high follow-up rate and the use of a well-validated IQ test, which measures non-verbal abilities, minimizing the effects of language and culture. Limitations include possible selection biases due to lost to follow up, missing birth parameter data and demographic differences between included and excluded children. Previous studies showed that environmental factors seem to weaken the association between birth weight and cognitive skills (Gluckman and Hanson, 2006). Our sibship analyses were done to exclude family environmental factors. Finally, although we adjusted for a range of

covariates which were strongly associated with IQ and birth size in our study, residual confounding may exist as some other covariates of interest, such as maternal height, were not measured.

The findings of the current study reveal the effects of fetal growth on intelligence across the entire range of the population and have implications for designing early intervention and preventative programs, which may increase public health, education, and economic benefits. First of all it stresses the need for interventions based on the improvement of obstetric and neonatal care with enhanced prenatal care services as well as psychosocial support and nutritional support for mothers to enhance the probability of optimal fetal development. Such approaches to maternal-fetal health are critical since it is known that even small differences in IQ may have important societal implications like academic success in childhood, employment success, and income in later life; and even associations with public health and mortality have been reported (Batty and Deary, 2004; Victora et al., 2008). Hence, small shifts in the distribution of birth size in normal size babies may have a huge impact at the population level, because of the large proportion of normal births. In contrast, normal size birth children who are born with a suboptimal birth size may have a greater potential to increase their cognitive enhancement with postnatal interventions such as promotion of breastfeeding (Kramer et al., 2008) and supportive strategies to improve community nutrition.

5. Conclusions

Longer birth length, higher birth weight, or larger head circumferences, within the normal birth size range, are associated with higher IQ scores in Asian children. Our results suggest that antenatal factors reflected in altered rates of growth but within the normative range of pregnancy experiences play a role in generating cognitive potential. This has implications for targeting early intervention and preventative programs (Bhutta et al., 2008; Jolly, 2007). Future studies in normal birth sized children are needed to explore the connections between variations in fetal growth, brain development and the potential influence of gene x environmental interactions.

4

Serotonergic function genes modulate the influence of birth weight on internalizing behaviour

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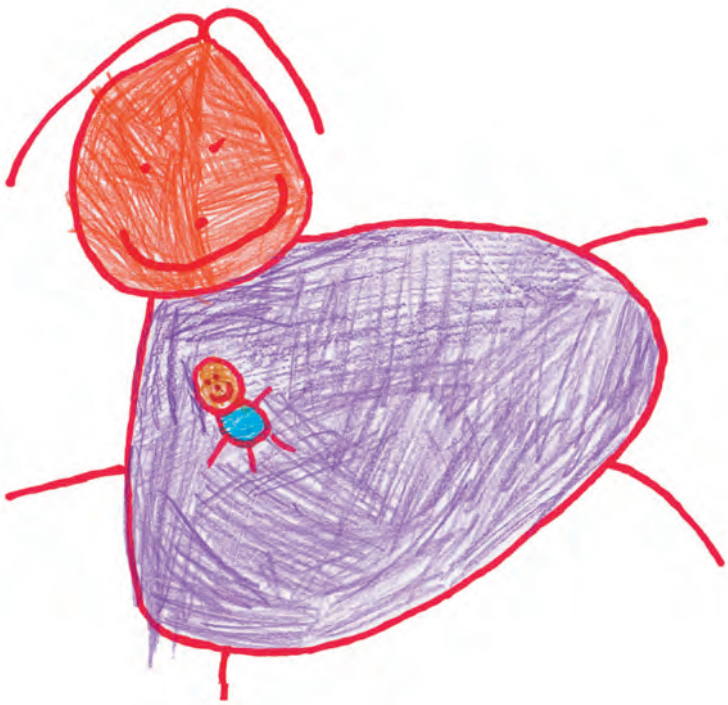

"Pregnant" by Lotte Leutscher, 4 years old

Abstract

Objective

Fetal growth predicts childhood behavioural problems associated with brain serotonergic systems. We hypothesize that allelic variation in genes involved in serotonergic systems will moderate associations between birth weight and internalizing traits in childhood.

Methods

A cohort of 545 healthy Singaporean children was administered the Child Behavior Checklist (CBCL) at 8 to 12 years. The interaction between birth weight, corrected for gestational age, and candidate single nucleotide polymorphisms (SNPs) in the TPH2, HTR2A and SCL6A4 genes on the Internalizing T-scores of the CBCL were investigated.

Results

There was no significant main effect of birth weight on Internalizing T-scores ($F = 1.08$, $P = 0.36$). After multiple corrections, significant main effects were found on Internalizing T-scores for HTR2A rs2296972 ($F = 2.85$, $P = 0.019$ adjusted) and HTR2A rs6313 ($F = 5.91$, $P = 0.0002$ adjusted). However, we found significant interactions between birth weight and SNPs for the TPH2 gene (rs2171363, $P = 0.008$; rs7305115, $P = 0.007$) and the HTR2A gene (rs2770304, $P = 0.001$; rs6313, $P = 0.026$) on Internalizing T-scores. The CC genotype of TPH2 rs2171363, GG genotype of TPH2 rs7305115, CC genotype of HTR2A rs2770304 and CC genotype of HTR2A rs6313 were associated with reduced Internalizing T-scores in children born in the quartile above the mid-point. No significant main effects or interactions were found for SNPs of the SCL6A4.

Conclusions

These findings suggest that sequence variation in genes involved in the serotonergic function modulates the relation between birth weight and development of internalizing traits, and might thus be candidate 'plasticity' mechanisms that determine individual differences to environmental influences over the course of development.

1. Introduction

Quality of fetal growth, reflected by birth weight adjusted for gestational age (BW/GA), predicts the risk for affective disorders (Barker, 1995; Bohnert and Breslau, 2008; Costello et al., 2007; Rice et al., 2007). Such findings suggest that in-utero events affect development of neural systems that regulate emotional and cognitive development. Increased fetal glucocorticoid levels associate with impaired fetal growth, altered neural development, and increased expression of genes associated with behavioural and endocrine stress responses (Challis et al., 2001; Maccari et al., 2003; Meaney et al., 2007; Seckl and Holmes, 2007). This 'fetal programming' hypothesis implies that effects of fetal growth should be apparent in childhood. Indeed, birth weight predicts emotional and cognitive function in childhood, including traits associated with affective illness (Alati et al., 2009; Gale and Martyn, 2004; Lahti et al., 2008; Pesonen et al., 2006; Schlotz and Philips, 2009).

Studies of fetal growth and neurodevelopment focus largely on children born at (very) low birth weight (i.e., <2,500 g). However, epidemiological studies commonly show graded effects of birth weight suggesting an influence across the entire population (Gluckman et al., 2008; Phillips, 1998). Similar effects are apparent in studies of childhood cognitive and emotional function (Broekman et al., 2009; Kelly et al., 2001). More extensive studies of socio-emotional function even suggest a curvilinear relation, with increased anxiety/depressive scores among children born in the lowest and highest quintiles (Alati et al., 2009), comparable to trends in cardiovascular and metabolic health (Huang et al., 2007; Phillips, 1998).

We examine the association between BW/GA and the Internalizing scale of the Child Behaviour Checklist (CBCL) in Singaporean children (Achenbach and Edelbrock, 1991a). The internalizing scores of the CBCL are stable over time and precursors for affective disorders later in life (Caspi et al., 1996; Reinherz et al., 2000). Moreover, internalizing problems are the most common form of mental disorders in children (Kessler et al., 2005) and especially relevant for Singapore where children have higher rates of internalizing than externalizing problems (Woo et al., 2007).

There are pronounced gene x environment interaction (GxE) effects in affective disorders, including genomic variants that influence brain serotonergic function (Caspi et al., 2003; Kendler et al., 2005; Risch et al., 2009; Rutter et al., 2009). Since serotonin is actively involved in the development and function of neural structures that regulate emotional function (Gaspar et al., 2003; Gross and Hen, 2004), it is likely that sequence variants of these genes are important for individual differences in internalizing traits. Considering the association between fetal growth and endophenotypes associated with affective disorders, such effects may associate with birth weight. We focused on candidate genes that code for proteins involved in synthesis (Trytophan Hydroxylase, TPH2), presynaptic reuptake (Solute Carrier family 6 neurotransmitter transporter, serotonin, member 4, SCL6A4) and post-synaptic response of serotonin (5-hydroxytryptamine-2A receptor gene, HTR2A). Sequence variation in these genes is associated with risk for affective disorders (Brezo et al., 2009; Jokela et al., 2007; Lazary et al., 2008; Roche and McKeon, 2009; Strug et al., 2010; Tsai et al., 2009; Unschuld et al., 2007; Wray et al., 2009). Only single nucleotide polymorphisms (SNPs) with published studies of linkage with anxiety, mood disorders or internalizing traits were selected: five TPH2 SNPs: rs2129575, rs2171363, rs7305115, rs1487276 and rs1386483 (Drago et al.,

2009; Lopez et al., 2007; Roche and McKeon, 2009; Tsai et al., 2009; Zhang et al., 2010), three HTR2A SNPs: rs2296972, rs2770304 and rs6313 (Arias et al., 2001; Du et al., 2000; Jokela et al., 2007; McMahon et al., 2006; Unschuld et al., 2007), and three SCL6A4 SNPs: rs3794808, rs140700 and rs6354 (Brezo et al., 2009; Lazary, 2010; Strug et al., 2010; Su et al., 2009; Wray et al., 2009). We hypothesized that above SNPs might modulate the association between birth weight and internalizing traits.

2. Methods

2.1. Study design and participants

A cohort of 1979 out of 2913 Singaporean children (participation rate 67.9%) was recruited for the Singapore Cohort Study of Risk Factors in Myopia (SCORM) between 1999 and 2001 and followed annually. Although the cohort was designed to examine myopia, no particular sampling priorities or risks were assessed, except selection of subjects from 3 schools of different locations in Singapore (Saw et al., 2004). Children with chronic medical conditions were excluded (n = 94). We included only subjects of Chinese descent to minimize population stratification effects of ethnicity. SNP array analyses were performed on DNA samples from 1008 subjects. The sample size was further reduced as CBCL data were not available for subjects of 1 of the 3 schools from which subjects were recruited. Thus, only 545 subjects of the 1008 who completed the CBCL were used for analyses. The protocol was approved by the Singapore Eye Research Institute Ethics Committee and the National University of Singapore Institutional Review Board. The tenets of the declaration of Helsinki were observed. All parents provided written informed consent and children provided written assent.

2.2. Measures of birth parameters

Gestational age was calculated from mothers' last menstrual date and confirmed by fetus' crown rump length during the first trimester ultrasonography. Birth weight was obtained from medical records. Missing birth weight was obtained from the National Birth and Death Registry for children (n = 211), which captures information recorded on birth certificates by hospital personnel. Gestational data from the medical record booklet and the National Birth and Death Registry is highly correlated (n = 911; r = +0.92).

2.3. Measures of internalizing traits

The CBCL/4-18 parental report is a self-administered test of emotional and behavioural difficulties for children between age 4 and 18 (Achenbach and Edelbrock, 1991). The questionnaire consists of 118 items with a 3-point Likert scale using 8 subscales, 2 syndrome groups and a total problem score. The Internalizing subscale is a grouping of social withdrawal, somatic complaints, and anxiety/depressed. Raw scores are converted to age-standardized scores (T scores M = 50, SD = 10). T-scores less than 60 are considered normal, 60-63 represents 'at risk' scores, and scores greater than 63 are in the clinical range (Achenbach and Edelbrock, 1991a). The CBCL has good construct validity and acceptable test-retest reliability coefficients among the subscales, with Cronbach's alphas ranging from 0.62 to 0.92 (Achenbach and Edelbrock, 1991a).

2.4. Measurement of demographic and socioeconomic data

Parents completed a questionnaire in English or Chinese for demographic information and socioeconomic indicators. Ethnicity was determined by father's reported ethnicity (Singapore Population Census 2000; www.singstat.gov.sg/statsres/glossary/population.html). Ethnic Chinese subjects were selected only for those whose father and mother both reported Chinese ethnicity. Socioeconomic status was assessed by maternal education, a frequently used index in studies with children.

2.5. Genotyping

A total of 1116 DNA samples were used for genome-wide SNP genotyping with the Illumina BeadStation and Illumina HumanHap 550 or 550 Duo Beadchips® (http://www.illumina.com/). Details of genotyping and the genomic quality protocol have been described (Li et al., 2010). The final SCORM SNP array data comprised of 1008 samples and 483,212 SNPs (Li et al., 2010).

A total of 11 SNPs for TPH2, HTR2A and SCL6A4 were extracted for this study (see introduction). SCL6A4 rs3794808 and rs140700 were subsequently filtered due to deviation from HWE at a 5% significance level. Most of the SNPs have $r^2 < 0.7$ except for rs2171363 and rs7305115 in TPH2 which are in linkage disequilibrium (LD) with r^2 of 0.945 (Table 1).

2.6. Statistical analysis

Birth weight was corrected for gestation age using linear regression with gestational age as independent variable. CBCL T-scores were used for the internalizing subscale (Achenbach and Edelbrock, 1991a). Differences in age, BW/GA and Internalizing T-scores between gender were analyzed using parametric tests if normality and homogeneity assumptions were satisfied, otherwise the non-parametric Mann Whitney U test was used. Categorical data were analyzed using Chi-square tests.

Birth weight was used as a categorical rather than continuous variable (Table 2), based on findings indicating a j-shaped, curvilinear relation between birth weight and childhood behavioural problems (Alati et al., 2009). Genotype differences and interaction effects of SNP x BW/GA quartiles on Internalizing T-scores were evaluated using a general linear model, adjusting for age, gender and maternal education (Kelly et al., 2001). We used Chi-square analysis across BW/GA quartiles and found no significant association between genotype and BW/GA, suggesting that these are independent factors (data not shown). One-tailed analyses ($P < .05$) were used based on our hypothesized direction for effects for both BW/GA and candidate SNPs. All statistical procedures used SPSS version 17.0 (SPSS Inc, Chicago, USA).

Table 1. Allele frequencies, coding information, LD details and localization of selected SNPs

SNP name	Chr	Coordinate	Source	Db SNP	Variant	MAF asian	MAF SCORM	LD	HWE UNAFF (P)	Gene location	Ensembl gene
Rs2129575	12	70626340	dbSNP	129	G/T	0.47	0.4727		0.1188	Intron	TPH2
Rs2171363	12	70646531	dbSNP	129	C/T	0.476	0.4871	0.945	0.0770	Intron	TPH2
Rs7305115	12	70659129	dbSNP	129	A/G	0.482	0.4841	0.945	0.0221	Exon	TPH2
Rs1487276	12	70691326	dbSNP	129	T/C	0.133	0.0769		0.7173	Intron	TPH2
Rs1386483	12	70698761	dbSNP	129	T/C	0.482	0.4926		0.9173	Intron	TPH2
Rs2296972	13	46326472	dbSNP	129	A/G	0.494	0.4761		0.4659	Intron	HTR2A
Rs2770304	13	46353366	dbSNP	129	C/T	0.344	0.2991		0.2057	Intron	HTR2A
Rs6313	13	46367941	dbSNP	129	C/T	0.482	0.3889		0.3284	Extron	HTR2A
Rs3794808	17	25555919	dbSNP	129	C/T	0.155	0.1809		0.4878	Intron	SCL6A4
Rs140700	17	25567515	dbSNP	129	C/T	0.030	0.0472		1.0000	Intron	SCL6A4
Rs6354	17	25574024	dbSNP	129	G/T	0.137	0.1190		0.8018	Exon	SCL6A4

Table 2. Means and standard deviations of BW/GA quartiles

BW/GA quartiles	BW/GA (grams)	Number	Mean (SD) (grams)
1	≤ 2921.90	138	2692.3 (179.1)
2	2921.91 – 3185.07	141	3048.1 (72.4)
3	3185.08 – 3416.12	132	3293.9 (64.5)
4	> 3416.13	134	3654.1 (217.8)

3. Results

3.1. Demographic data

For Internalizing T-scores there were no outliers > 3SD, and 5 outliers > 2SD. Analyses without outliers did not substantially alter the results and therefore all 545 children were included. Subjects (266 boys and 279 girls) ranged from 8 to 12 years of age (mean = 9.8, SD: 1.0). There were slight differences in age and birth weight, but not in BW/GA or gender between children who did or did not complete the CBCL (Table 3). The distribution for maternal education also differed slightly between these groups.

The birth weight data for the sample (Table 3) conforms to the average values for ethnic Chinese Singaporean babies at term (Chen and Ling, 1984). The proportion of children born at very low birth weight (< 1.5 kg) was 0.2% (n = 1), at low birth weight (< 2.5 kg) 5.9% (n = 32), with a high birth weight (> 4.0 kg) 1.7% (n = 9), and with a very high birth weight (> 4.5 kg) 0.2% (n = 2). Girls showed a slightly lower BW/GA (3143.2, SD: 374.5 g) than boys (3191.0, SD: 386.4 g; P = 0.14). The mean Internalizing T-score was 52.0 (range = 32-85; SD: 11.7) with 82.8% scoring at or below the clinical cut-off of 63. There was no effect of gender on Internalizing T-scores (P = 0.08). Subsequent analyses were performed using the whole sample with gender as a covariate.

3.2. Birth weight effects on CBCL

There was no significant main effect of BW/GA on Internalizing T-scores (F = 1.08, P = 0.36). BW/GA was not predictive of scores in the 'clinical' range for the Internalizing T-score. Likewise, there were no differences in BW/GA when the CBCL was divided between ≤ 63 (Mean = 3159.4, SD: 377.5 g) and above 63 (Mean = 3200.5, SD: 398.2 g; t = 0.95, P = 0.34).

3.3. Genotype

We found significant main effects on Internalizing T-scores for HTR2A rs2296972 (F = 2.83, P = 0.019 unadjusted; F = 2.85, P = 0.019 adjusted) with lower CBCL Internalizing T-scores for GG genotype; and for HTR2A rs6313 (F = 5.81, P = 0.001 unadjusted and F = 5.91, P = 0.0002 adjusted) with lower CBCL Internalizing T-scores for CC genotype. (Table 4).

Table 3. Demographic variables

	Included (n=545)	Excluded (n=498)	P
Gender			
Boys	48.8 %	53.8%	
Girls	51.2 %	46.2%	0.130
Age (years)	Mean = 9.76 SD 1.03 Range (8-12)	Mean = 9.94 SD 1.22 Range (8-15)	0.015
Birth weight (grams)	Mean = 3166.92 SD 431.77 Range (569-4655)	Mean = 3225.59 SD 478.59 Range (1717-6200)	0.039
Gestational age (weeks)	Mean = 38.70 SD 1.46 Range (27-42)	Mean = 38.53 SD 1.61 Range (28-44)	0.164
BW/GA (grams)	Mean = 3166.53 SD 381.05 Range (2099.01-4612.57)	Mean = 3158.66 SD 221.42 Range (1701.19-3912.02)	0.164
CBCL Internalizing T-score	Mean = 52.0 SD 11.67 Range (32-85)	Mean = 51.81 SD 11.74 Range (32-97)	0.819
Maternal age at birth	Mean = 30.43 SD 4.42 Range (17.36-42.49)	Mean = 30.27 SD 4.49 Range (17.72-41.28)	0.587
Mother's education			
No formal education	2.2 %	5.0%	
Elementary school	17.4%	26.9%	
High school	49.4%	42.0%	<0.001
Pre-University	19.6%	15.1%	
University	11.4%	11.0%	

Table 4. Genotype and birth weight effects on Internalizing T-scores

Single nucleotide polymorphisms	Unadjusted		Adjusted for age, gender and maternal education	
	SNP	*SNP*BW/GA*	*SNP*	*SNP*BW/GA*
TPH2 rs2129575	F = 0.505 P = 0.340	F = 1.210 P = 0.149	F = 0.484 P = 0.102	F = 1.140 P = 0.169
TPH2 rs2171363	F = 0.675 P = 0.255	F = 2.730 P = 0.007	F = 0.752 P = 0.236	F = 2.672 P = 0.008
TPH2 rs7305115	F = 0.750 P = 0.235	F = 2.690 P = 0.007	F = 0.910 P = 0.202	F = 2.716 P = 0.007
TPH2 rs1487276	F = 0.924 P = 0.215	F = 0.379 P = 0.416	F = 0.826 P = 0.240	F = 0.423 P = 0.390
TPH2 rs1386483	F = 0.021 P = 0.490	F = 1.810 P = 0.048	F = 0.052 P = 0.475	F = 1.682 P = 0.062
HTR2A rs2296972	F = 2.828 P = 0.019	F = 1.055 P = 0.196	F = 2.848 P = 0.019	F = 1.323 P = 0.119
HTR2A rs2770304	F = 0.144 P = 0.467	F = 2.932 P = 0.001	F = 0.166 P = 0.460	F = 2.957 P = 0.001
HTR2A rs6313	F = 5.813 P = 0.001	F = 2.095 P = 0.026	F = 5.906 P < 0.001	F = 2.094 P = 0.026
SCL6A4 rs6354	F = 0.056 P = 0.473	F = 1.186 P = 0.158	F = 0.017 P = 0.492	F = 1.192 P = 0.157

3.4. BW/GA x genotype effects

We found significant interaction effects between BW/GA and both TPH2 rs2171363 (F = 2.69, P = 0.007) and TPH2 rs7305115 (F = 2.73, P = 0.007) on Internalizing T-scores. These effects remained significant (i.e., P < .01) after correction for age, gender and maternal education (Table 4). There was also a strong trend for rs1386483 (P ~.05). Significant interaction effects with BW/GA were found for both HTR2A rs2770304 (F = 2.93, P = 0.001) and HTR2A rs6313 (F = 2.10, P = 0.03); which remained significant following multiple adjustments. The remaining SNP rs2296972, showed a trend for an interaction with BW/GA following adjustments. When corrected for comparisons within each gene the interaction effects remained significant for TPH2 rs2171363, TPH2 rs7305115 and HTR2A rs2770304, and became borderline significant for HTR2A rs6313.

All significant SNP x BW/GA interactions showed a 'V-shaped' function (Figure 1). Post-hoc analyses using multiple adjusted models revealed significant differences for TPH2 rs2171363 (P = 0.009), TPH2 rs1305115 (P = 0.013) and HTR2A rs6313 (P = 0.007). In each case Internalizing T-scores on the CBCL were significantly lower for selective homozygous genotypes in the 3rd quartile for BW/GA.

We analyzed the outcomes of individual CBCL subscales and found the same SNPs x BW/GA interaction on each Internalizing subscale (social withdrawn, somatic, anxiety/depression). Interestingly, each subscale showed the same V-shaped pattern for the affected genotypes. No significant interactions were found for Externalizing T-scores (data not shown).

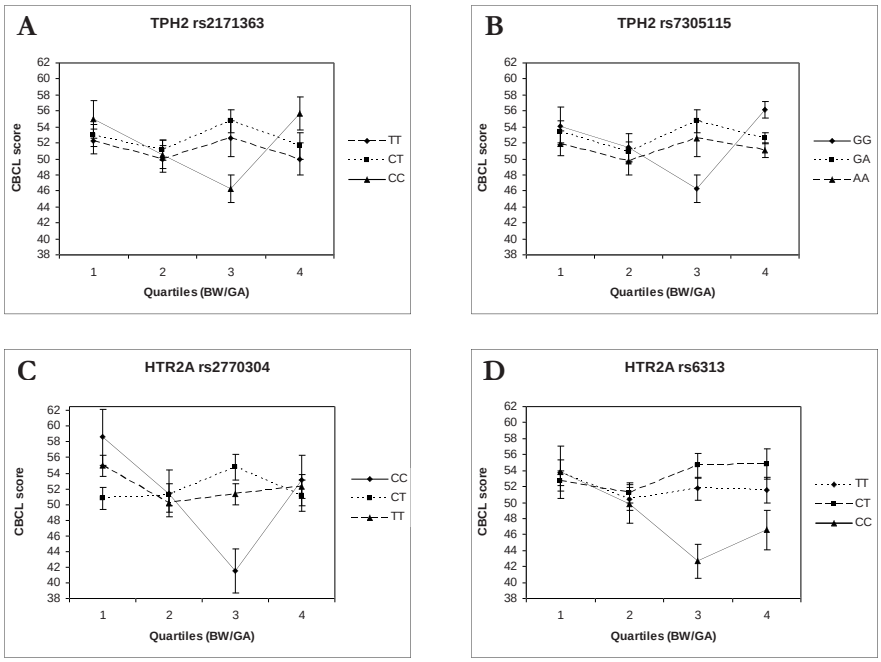

Figure 1. Internalizing T-scores of CBCL 4-18 as a function of BW/GA quartiles and genotypes of TPH2 or HTR2A

a) TPH2 rs2171363 (TT n = 129, CT n = 287, CC n = 129; b) TPH2 rs7305115 (GG n = 130, GA n = 282, AA n = 133) c) HTR2A rs2770304 (CC n = 51, CT n = 223, TT n = 257) d) HTR2A rs6313 (TT n = 205, CT n = 264, CC n = 76)

4. Discussion

Our findings of the effects of BW/GA on Internalizing T-scores are consistent with reports of a curvilinear relation between BW/GA and childhood socio-emotional function (Alati et al., 2009), and suggest modulation by genotype. For 92% of our sample the birth weight fell between 2500 and 4000 grams revealing variation in childhood internalizing traits across the normal range for birth weights as a function of genotype. Among the affected genotypes, there were significantly lower Internalizing T-scores for children in the upper middle range of birth weight.

We found a significant main effect for HTR2A on Internalizing T-scores, with the strongest effect for rs6313 (also known as T102C), which is linked to the risk for affective disorders in adult samples (Arias et al., 2001; Du et al., 2000; Eley et al., 2004; McMahon et al., 2006; Unschuld et al., 2007; Zhang et al., 2008), including ethnic Chinese subjects (Ressler and Mayberg, 2007), although some studies report no association (Bondy et al., 2000; Frisch et al., 1999; Wang et al., 2009). The HTR2A gene encodes the serotonin 2A receptor and is considered a candidate gene for depression and suicidal behaviour (McAuley et al., 2009; Turecki et al., 1999). The serotonin 2A receptor is highly expressed in the prefrontal cortex, a structure closely associated with mood disorders through effects on emotional regulation (Ressler and Mayberg, 2007). We also found a significant interaction between BW/GA and HTR2A rs2770304, for which the TT genotype is linked to anxiety disorders (Unschuld et al., 2007).

We found comparable interactions effects between birth weight and TPH2. TPH2 is a rate-limiting biosynthetic enzyme for 5-HT in brain. TPH2 polymorphisms associate with affective disorders and influence therapeutic responses to SSRIs (Gutknecht et al., 2007; Illi et al., 2009; Lim et al., 2007; Peters et al., 2004; Zhang et al., 2010; Zhou et al., 2005; Zill et al., 2004). Functional TPH2 variants are also linked to personality traits such as emotional instability (Drago et al., 2009; Gutknecht et al., 2007). We found statistically significant interactions between fetal growth and both TPH2 rs2171363 and rs7305115, with a trend (i.e., $.05<p<.10$) for rs1386483. However, TPH2 rs2171363 and TPH2 rs7305115 are in high linkage disequilibrium and thus these findings do not represent independent findings (Table 1). It is interesting to note that the 2 genotypes show a virtually identical interaction effect with BW/GA, thus providing an internal replication of the TPH2 x BW/GA effect on Internalizing T-scores.

These interaction effects suggest that the HTR2A and TPH2 genes modulate effects of fetal growth on internalizing traits in childhood. Interestingly, for both TPH2 and HTR2A, genotypes associated with an increased risk for affective illness (CC for rs2171363, GG for rs7305115, CC for rs6313) showed the greatest sensitivity to fetal growth; individuals born slightly above the mid-point within the normal range (i.e., the 3rd quartile) showed significantly reduced Internalizing T-scores (figure 1). For HTR2A rs2770304 the lowest Internalizing T-scores were associated with the 'low risk' CC genotype. This same pattern was apparent for each of the internalizing subscales of the CBCL (data not shown), indicating broad effects across internalizing traits.

These findings suggest that multiple sequence variants of TPH2 and 5HTR2A genes associated with an increased risk for affective disorders are associated with increased sensitivity to effects of fetal growth. In each instance, the outcome of the interaction with fetal growth

was to decrease the Internalizing T-score on the CBCL among children for whom fetal growth was within the optimal range. Sequence-based variants in genes implicated in 5-HT function associate with individual differences in stress reactivity (Gotlib et al., 2008; Hariri et al., 2005; Peters et al., 2004). Increased stress reactivity associates with context-dependent outcomes, with decreased vulnerability among more reactive children living within enriched contexts (Boyce and Ellis, 2005). Genetic variants that statistically associate with an increased risk for affective illness might enhance sensitivity to environmental context, thus explaining bi-directional outcomes (Belsky and Pluess, 2009; Meaney at al., 2007; Obradovic and Boyce, 2009; Wang et al. 2009). We suggest that the relevant TPH2 and 5HTR2A polymorphisms function to increase the sensitivity of the fetus to the biological signals that influence fetal growth (Boyce and Ellis, 2005). These variants might be candidates for 'plasticity' factors that mediate individual differences in sensitivity to environmental signals.

A strength of this study is the association between BW/GA across a normal range and internalizing traits relevant for affective illness within a normal population. The availability of information on selected polymorphisms with evidence of associations with affective disorders in a reasonably large sample of children is strength. Likewise, the range of scores on the CBCL internalizing scale was substantial enhancing the potential for detecting genotype x birth weight interactions. Finally, this study presents effects of fetal growth uncomplicated by maternal smoking (less than 1% of the mothers were smokers or ex-smokers).

The study has limitations, including reliance on parental report. The conditions that promote fetal growth restraint, such as maternal stress, diet as well as alcohol consumption could reflect maternal adversity. Such adversity might interact with maternal genotype to create emotional states that influence parental assessment of the child's behaviour. It is worth noting that the birth weights for children within this study lay well within the normal range, suggesting that any existing adversity was not extreme. Also, the range of scores and the percentage of children falling within the 'high risk' range are comparable to previous reports using the CBCL. A second limitation is the difference between the included and excluded sample in age, socioeconomic status and birth weight (see Table 3). However, these differences were slight and the variables used for the primary analyzes (BW/GA and CBCL scores) show no significant differences. A third limitation is the use of a candidate polymorphism approach focusing on previously studied SNPs linked to affective illness. Such effects might reflect the importance of undetected variations or gene-gene interactions (Zill et al., 2004). However, the importance of 5-HT systems in perinatal life for the development of cortico-limbic structures that mediate socio-emotional function is well established (Gross and Hen, 2004). A fourth limitation is that the statistical results were not corrected for multiple comparisons. The primary objective of this study was descriptive, and it was not intended, nor should it be interpreted as indicative of novel genotype – phenotype associations. Thus, we selected candidate SNPs in genes involved in serotonergic function associated with affective disorders. Nevertheless, a correction for multiple comparisons within each gene would yield a critical P value of $P = .005$, such that many of the interaction effects and even one main effect (i.e., HTR2A rs6313) would remain significant or near significance. Another limitation, universal among studies of birth weight, is that such measures are proxies for conditions that influence fetal growth and which affect the development of neural systems that regulate socio-emotional function. The response to conditions that produce fetal growth restrictions might actually be dependent upon the genotypes examined in this study.

It is important to note that in our study birth weight was not linked to genotype. However, fetal growth restriction in humans and other mammals is associated with increased exposure to corticosteroids (Chen et al., 2010; Meaney et al., 2007; Seckl and Holmes, 2007).

5. Conclusions

Significant interactions were found between fetal growth and TPH2 and HTR2A genotypes on Internalizing T-scores of the CBCL in Singaporean children. Interestingly, while the effect of fetal growth was clearly dependent upon genotype, the data suggest that the lowest level of Internalizing T-scores occurred among children born within a specific range lying above the mean for birth weight suggesting that, at least with respect to emotional function in childhood, there might be an optimal level of fetal growth. Future studies are needed to replicate these data in other samples, focusing on candidate 'plasticity' genomic variations.

5

The psychological impact of an adenoidectomy and adenotonsillectomy on young children

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"Hospital" by Lucas Berger, 6 years old

Abstract

Objective

Children react differently to surgeries. The purpose of this study is to examine the stress response in young children after an adenoidectomy and adenotonsillectomy, and whether child characteristics of behavioural and neurophysiologic nature can predict this stress response.

Methods

In this prospective cohort study 43 children, aged 2–7 years, scheduled for adenoidectomy or adenoitonsillectomy (response rate 43%) were recruited from the Ear, Nose and Throat Department of the Academic Medical Centre in Amsterdam, the Netherlands. Parents completed questionnaires about temperament 4 weeks before surgery, about behaviour and sleeping problems 4 weeks before and 6 weeks after surgery, and about posttraumatic stress symptoms 6 weeks after surgery. Neurophysiological measurements (cortisol and respiratory sinus arrhythmia) were performed 4 weeks before, directly after and 6 weeks after surgery. Results were compared with a control group of healthy children. The data were analyzed with paired t-tests and one-way repeated ANOVA.

Results

Most children with an indication for an adenoidectomy and adenotonsillectomy had more behavioural and emotional problems before surgery then the control group. After surgery there was an improvement in behaviour and sleep, in respectively 75% and 68% of the children, especially in boys. Posttraumatic stress symptoms were rare. Emotional temperament was associated with more behavioural problems before surgery ($r = 0.53$, $P = 0.02$), after surgery ($r = 0.38$, $P < 0.000$), lower cortisol directly after surgery ($r = -0.49$, $P = 0.05$) and lower respiratory sinus arrhythmia at follow-up ($r = -0.33$, $P = 0.06$). Other temperament styles and pre-surgery levels of respiratory sinus arrhytmia and cortisol did not show associations with any behavioural or neurophysiologic measures.

Conclusions

Adenoidectomy and adenotonsillectomy appear not to be stressful, but rather seem helpful for reducing pre-existing behavioural and emotional problems, possibly associated with the indication for surgery. For those children with an increase of behavioural and sleeping problems after surgery, this can only be partly explained by emotional temperament. There are indications that boys and girls react differently; boys tend to show a better behavioural and emotional improvement after an adenoidectomy and adenotonsillectomy. Other behavioural or neurophysiological child characteristics do not have a predictive value on the outcome.

1. Introduction

Surgeries are stressful in nature, especially for young children. Individuals, from childhood onwards, respond differently to stress (Broekman et al., 2007; Fukuchi et al., 2005; Langeland and Olff, 2008; Olff et al., 2005b). Detection of susceptibility is important for prevention of psychological problems afterwards. Adenoidectomy and adenotonsillectomy (A&ATE) are the most common surgeries in children. Although A&ATE normally reduce the sleeping and behavioural problems that coincide with pre-existent upper airway obstruction, sometimes children exhibit new-onset sleep-related or maladaptive behavioural problems, which may be perceived as signs of posttraumatic stress (Goldstein et al., 2002; Kotinimie et al., 1996; Stradling et al., 1990). Depressive symptoms are also described (Papakostas et al., 2003). Furthermore, parents are often concerned about the possible harmful impact on mental health of their children (Kim et al., 2008).

However, in most of the previous studies responses of children to A&ATE were examined postoperatively (Papakostas et al., 2003). Because A&ATE are often planned in advance, these surgeries provide an opportunity to investigate this stress response in a prospective way. Individual differences in responses to stressful experiences can be studied at behavioural and neurophysiological levels. Two temperamental traits have been found to contribute to anxiety, and may therefore be related with a higher stress response: high emotionality (negative affect) and shyness (behavioural inhibition). High emotionality is generally defined as sensitivity to negative stimuli, causing a broad range of negative moods, including fear, anxiety, sadness and depression (Clark et al., 1994). Shyness is the consistent tendency to display fear and withdrawal in unfamiliar situations (Kagan et al., 1988).

The neurophysiologic part of the stress response involves at least 2 systems; the Autonomic Nervous System (ANS) and the Hypothalamic–Pituitary–Adrenal (HPA) system. The ANS is a quick response system, which prepares the organism to cope with stress. It consists of sympathetic (SNS) and parasympathetic (PNS) branches, with antagonistic effects on the heart, causing variability in heart rate (Porges, 1991). Heart rate variability is typically estimated from respiratory sinus arrhythmia (RSA), respiratory locked oscillations in heart rate, which provides a non-invasive index of parasympathetic function (Beauchaine, 2001; Porges 1991; Porges, 1992). Diminished variability of heart rate is associated with posttraumatic stress (Beauchaine, 2001; Blair et al., 2004; Porges, 1991; Porges, 1992). The HPA system is a slower response system, producing glucocorticoids to maintain energy, mostly assessed by cortisol levels (Young et al., 1998). Excessive activity of the ANS and HPA systems may lead to anxiety or affective disorders (Olff et al., 2006). There are connections between temperament and neurophysiology: children with high emotionality or shyness show stronger neurophysiologic stress responses to fearful situations (Beauchaine, 2001; Tyrka et al., 2006).

The present prospective study was designed to examine whether child characteristics of a behavioural (temperament) and neurophysiologic (RSA, cortisol) nature can predict the stress response. It is hypothesized that children with high emotionality and/or shyness will show a stronger clinical and neurophysiologic stress response to A&ATE. Furthermore, lower levels of heart rate variability and higher levels of cortisol prior to surgery are hypothesized to predict a stronger clinical and neurophysiologic stress response.

2. Methods

2.1. Study design and participants

43 children out of a cohort of 101 children (participation rate 43%), scheduled for A&ATE in day-care at the Ear, Nose Throat clinic of the Academic Medical Centre in Amsterdam (The Netherlands) between April 2005 and December 2005, were recruited by approaching the parents by phone. Subject selection was performed in consideration of the following exclusion criteria: medication (other than paracetamol, saline solution, xylometazoline) (n = 8), psycho-organic disorders (n = 0), mental retardation (n = 2), severe behavioural disorders (n = 1), parents who did not speak Dutch (n = 4). Sixteen families could not be reached before surgery (mainly due to incorrect telephone numbers) and 20 parents refused to participate (20%). Seven families dropped out before the first measurement because of private family issues (n = 1) or time investment (n = 6).

The indications were 'glue ears or sleep disorder' for adenotomy and 'failure to grow or sleep disorder' for adenotonsillectomy. The sluder method was used in all patients to remove the tonsils. The procedures were performed by 3 different registrars under supervision of the same Ear, Nose and Throat surgeon consultant.

Four weeks before A&ATE (preoperative), and 6 weeks after A&ATE (follow-up), parents filled out self-report questionnaires about their children. All children participated in neurophysiologic assessments, which were done 4 weeks before surgery (preoperative), directly after surgery (postoperative; same day for RSA, 2 days after surgery for cortisol to avoid contamination with blood) and 6 weeks after surgery (follow-up). The parents received an information letter, the informed consent paper and the surveys by mail. All parents provided written informed consent. The Institutional Review Board of the Academic Medical Centre, Amsterdam, The Netherlands approved the study. The tenets of the declaration of Helsinki were observed.

2.2. Measures of temperament

Temperament was assessed by parent report with the EAS Temperament Survey for Children, Parental Ratings (Boer and Westenberg, 1994; Buss and Plomin, 1986). Psychometric properties of the EAS have been examined in a sample of parents of Dutch children between 4 and 13 years old and were found satisfactory (Boer and Westenberg, 1994).

2.3. Measures of behavioural problems

The Child Behavior Checklist (CBCL) for ages 1.5–5 years was used to detect behavioural and emotional problems (Achenbach and Rescorla, 2000). Dutch norms are not published yet, but appear to be close to the original American norms (van der Ende, 2009). The CBCL/1.5-5 has demonstrated a very good 8-day test-retest reliability (r = 0.68–0.92, mean r = 0.84), cross-informant agreement (mean mother–father r = 0.61, mean parent-child care r = 0.65) and success in discriminating between referred and non-referred children (Achenbach and Rescorla, 2000). Because this study was designed to show differences in outcome on the same questionnaires within the same child over time, this version was also used for older children in our study.

2.4. Measures of sleeping problems

Sleeping problems were assessed by parent report with the Children's Sleep Habits Questionnaire (CSHQ). The CSHQ screens behaviourally based and medically based sleep problems. The questionnaire is designed for parents of children from preschool to middle childhood, but is also used for children between 2 and 5.5 years of age (Goodlin-Jones et al., 2008). It has shown to have adequate validity and reliability (Owens et al., 2000). A native speaker checked the translation through back translation.

2.5. Measures of posttraumatic stress

To determine the severity of posttraumatic stress, the Children's Impact of Event Scale (CRIES-13) was used (Yule and Williams, 1990). The CRIES-13 is a brief measure designed to screen children at risk for Posttraumatic Stress Disorder. It measures symptoms of intrusive thoughts, avoidance, arousal evoking thoughts, images, and feelings relating to a specific traumatic event, in this case A&ATE, during the past week. A cut-off score of 30 of all the 3 scales and 17 and above on the subscales for Intrusion and Avoidance indicates a likelihood of the presence of Posttraumatic Stress Disorder described by DSM-IV (Perrin et al., 2005). It has shown to have good face and construct validity and stable factor structure, to correlate well with other indices of distress, and has been used to screen very large samples of at-risk children following a wide range of traumatic events (Perrin et al., 2000). The internal consistency of the CRIES-13 lies between 0.60 and 0.90, with a sensitivity of 66% (Ohan et al., 2002; Perrin et al., 2000). A native speaker checked the translation through back translation. In a study of 779 Dutch children the internal consistency of the parent ratings proved to be acceptable to good (intrusion: 0.82; avoidance: 0.79; arousal: 0.73) (Boer et al., 2009).

2.6. Neurophysiologic measurements

2.6.1. RSA

RSA was measured by the Vrije University Ambulatory Monitoring System (Boer et al., 2009; Ohan et al., 2002), developed by the Vrije University of Amsterdam. Testing took place in attendance of one or both parents at the hospital and took 5 minutes. This system monitors heart rate, heart rate variability, respiration rate and RSA with ECG electrodes (Groot et al., 1998a). The RSA was calculated with AMRES software and expressed in milliseconds (Groot et al. 1998b).

2.6.2. Cortisol

Cortisol was measured in saliva, which is a non-invasive procedure. The early morning peak in cortisol is detectable in children from 3 months and above (Groschl et al., 2003). It was measured by parents, who placed a swab with safety thread in the mouth of their children during 5 minutes, 3 times a day; immediately upon awakening, 30 minutes after awakening, and at noon. Parents were instructed not to give any foods or drinks to their children and not to brush their teeth. At the laboratory, saliva samples were centrifuged 3000 U/min for 5 minutes and stored at -20 °C until analysis. The early morning rise (EMR), which is the cortisol response to awakening, was calculated by subtracting the awakening sample from the awakening +30 min sample.

2.7. Statistical analysis

A comparison of preoperative and postoperative scores was performed using the paired t-test. One-way repeated measures ANOVA was used to specify the difference in cortisol and RSA on 3 measurements. Psychological changes related to changes in cortisol and RSA were investigated with Pearson Product–Moment correlation coefficients, controlled for age, by including it as covariate in the model. All P-values were two-tailed and considered statistically significant when the values were below 0.05. All statistical procedures used SPSS (SPSS, USA, version 15.0).

3. Results

43 children participated (37% female, 63% male, mean age 3.7, SD: 1.5) (Table 1). 56% of the mothers and 49% of the fathers were of Dutch origin. 65% of the children underwent an adenoidectomy, 35% underwent an adenotonsillectomy. There was no difference in outcome based upon the individual surgeons. The Q–Q plots showed a reasonable distribution of children according to age in the adenotomy and the adenotonsillectomy group. Although more children were included in the adenotomy group (n = 28) in comparison to the adenotonsillectomy group (n = 15), there was no significant difference in the proportion of boys and girls (P = 0.957). To evaluate the impact of the intervention (adenoidectomy versus adenotonsillectomy) on the behavioural and neurophysiologic stress response, we investigated differences between the 2 intervention groups on all outcome measures with the independent-samples t-test. No significant differences were found; therefore these 2 groups were combined in all further analyses.

Table 1. Demographic data of participants

	Included participants (n=43)
Age (years)	3.7 (SD 1.5)
Gender:	
Boys	27 (63%)
Girls	16 (37%)
Family setting:	
Two-parent family	32 (74%)
One-parent family	11 (26%)
Mother's education:	
No formal education	3 (7%)
Elementary school	2 (2%)
High school	6 (15%)
Vocational training	11 (27%)
Professional training	11 (27%)
University	6 (15%)
Other	3 (7%)
Father's education:	
No formal education	1 (3%)
Elementary school	1 (3%)
High school	7 (19%)
Vocational training	10 (26%)
Professional training	9 (24%)
University	6 (16%)
Other	4 (11%)

95% of the parents completed the EAS. The means and standard deviations on shyness (2.3, SD: 0.70), sociability (3.5, SD: 0.54) and activity (3.6, SD: 0.60) were comparable to Dutch norms (2.4, SD: 0.88; 3.4, SD: 0.74; and 3.5, SD: 0.78 respectively). Only the mean on emotionality (2.6, SD: 0.82) was somewhat lower than the Dutch norms (3.0, SD: 0.82).

The CBCL was completed for 97.7% of the children before surgery, and 93% after surgery. Preliminary analyses were performed to detect violations of assumptions of normality, linearity and homoscedasticity. The outcomes on the CBCL before and after surgery proved to be normally distributed. In general, postoperative scores on CBCL were lower in comparison with preoperative scores (Table 2).

Boys and girls showed higher scores on most of the subscales and on the total score of the CBCL before surgery than the Dutch norm group. Before surgery boys showed significant higher scores on affective problems ($P = 0.007$) and somatic complaints ($P = 0.015$) in comparison to the Dutch norm group (van der Ende, 2009). After surgery for boys all scores on the subscales improved, and some of the scores became even significantly lower than the Dutch norm group; oppositional defiant problems ($P < 0.000$), emotional reactivity ($P = 0.005$), aggressive behaviour ($P < 0.000$), externalizing behaviour ($P = 0.004$) and total problems of the CBCL ($P = 0.050$) (Table 3).

Most of the scores of the CBCL subscales before surgery for girls were also higher than the Dutch norm group, with significant higher scores on affective problems ($P = 0.041$), anxiety problems ($P = 0.006$), pervasive developmental problems ($P = 0.047$), withdrawn behaviour ($P = 0.050$) and sleep problems ($P = 0.010$). In girls the decrease in scores of the subscales after surgery were not that clear in comparison to boys; after surgery the scores on anxiety and withdrawn behaviour were still significantly higher than the Dutch norm group (respectively $P = 0.012$ and $P = 0.016$). The only subscore for girls, which showed a significant lower score than the Dutch norm group after surgery, was oppositional problems ($P = 0.005$) (Table 4). Also after exclusion of the older children (which may have an influence on the mean outcome of the CBCL) the mean CBCL scores of the boys and girls showed the same trend.

To examine the impact of surgery on behaviour of each child, a paired samples t-test was conducted. The mean difference in CBCL total scores was significant after surgery ($P = 0.002$). Most of the children did show a significant improvement in behaviour (75%) according to the CBCL, 10 children (25%) had more behavioural problems on the CBCL after surgery in comparison to before surgery. Overall children had a significant improvement in both internalizing ($P = 0.007$) and externalizing problems ($P = 0.002$), especially for opposite deviant behaviour ($P = 0.001$) and aggressive behaviour ($P < 0.000$). The only subscale, which did not improve after surgery, was anxious/depressive problems ($P = 1.00$) (Table 2).

The CSHQ was completed by 97.7% of the parents before surgery, and by 88% of the parents after surgery. Before surgery 35.7% of the children had a higher total CSHQ score (> 2 SD) in comparison to a Southern New England norm group, after surgery this diminished to 14.3%. A noticeable difference in scores with the norm group occurred with regard to sleep-disordered breathing (100% > 2 SD before surgery, 35.7% > 2 SD after surgery) reflecting that this particular sleeping problem often is one of the reasons to perform A&ATE (van der Ende, 2009).

Table 2. Measurements before surgery, directly after surgery and at follow-up for boys and girls

	4 weeks before surgery		directly after surgery		follow-up, 6 weeks after surgery		
	n	**Mean (SD)**	**n**	**Mean (SD)**	**n**	**Mean (SD)**	**P-values**
Early morning rise cortisol saliva (nmol/l)	26	0.74 (4.45)	22	-1.18 (1.62)	22	1.17 (0.82)	P=0.440
Respiratory sinus arrythmia (msec)	40	107.8 (13.8)	34	109.1 (17.5)	40	106.8 (12.5)	P=0.300
CBCL:							
Affective problems	42	3.26 (2.40)			40	2.38 (2.33)	P=0.002
Anxiety problems	42	3.79 (2.51)			40	3.10 (2.58)	P=0.090
Pervasive developmental problems	42	3.67 (2.86)			40	2.95 (2.78)	P=0.200
Attention deficit hyperactivity	42	4.71 (3.20)			40	4.48 (2.87)	P=0.200
Oppositional deviant problems	42	3.81 (2.46)			40	2.40 (1.95)	P=0.400
Emotionally reactive	42	2.81 (2.37)			40	1.90 (1.85)	P=0.001
Anxious/Depressive	42	2.29 (1.94)			40	2.00 (2.33)	P=0.070
Somatic complaints	42	3.26 (3.06)			40	2.20 (2.36)	P=1.000
Withdrawn behaviour	42	1.95 (1.77)			40	1.62 (2.12)	P=0.050
Sleep problems	42	3.55 (3.03)			40	2.60 (2.65)	P=0.240
Attention problems	42	2.76 (2.24)			40	2.58 (2.29)	P=0.260
Aggressive behaviour	42	10.48 (6.37)			40	7.50 (5.09)	P=0.190
Internalizing problems	42	10.29 (7.40)			40	7.72 (7.16)	P<0.000
Externalizing problems	42	13,24 (8.12)			40	10.08 (6.93)	P=0.007
Total problems	42	37.71 (21.07)			40	28.67 (19.55)	P=0.002
CSHQ (total)	42	47.35 (6.01)			38	44.13 (6.95)	P<0.000
Child impact of event scale (total)					40	28 (8)	
EAS temperament scale (divided to temperament styles)	41	Shyness 2.3 (0.70) Activity 3.6 (0.60) Emotionality 2.6 (0.82) Sociability 3.5 (0.54)					

Table 3. Outcome on CBCL for boys after surgery in comparison to the Dutch control group

CBCL (T-scores)	Boys before surgery (n=16)	Boys after surgery (n=14)	Dutch norm group boys (n=352)
	Mean (SD) P-value difference with norm group	Mean (SD) P-value difference with norm group	Mean (SD)
Affective problems	3.31 (2.33), P = 0.007	1.96 (1.89), P = 0.982	1.97 (1.75)
Anxiety problems	3.31 (2.46), P = 0.134	2.38 (2.42), P = 0.715	2.56 (2.35)
Pervasive developmental problems	3.50 (3.04), P = 0.519	2.31 (2.72), P = 0.146	3.11 (2.86)
Attention deficit hyperactivity	4.88 (3.12), P = 0.815	4.12 (2.82), P = 0.269	4.74 (2.75)
Oppositional deviant problems	3.88 (2.49), P = 0.339	2.38 (2.12), P = 0.000	4.36 (2.74)
Emotionally reactive	2.73 (2.16), P = 0.853	1.73 (1.78), P = 0.005	2.81 (2.68)
Anxious/Depressed	2.04 (1.80), P = 0.488	1.46 (2.10), P = 0.433	1.79 (1.82)
Somatic complaints	3.15 (2.57), P = 0.015	1.69 (1.81), P = 0.680	1.84 (1.99)
Withdrawn behaviour	1.73 (1.51), P = 0.320	1.08 (2.02), P = 0.381	1.43 (1.56)
Sleep problems	2.92 (2.73), P = 0.183	2.19 (2.71), P = 0.997	2.19 (2.18)
Attention problems	2.81 (2.32), P = 0.546	2.38 (2.35), P = 0.775	2.53 (1.94)
Aggressive behaviour	10.27 (6.36), P = 0.569	6.88 (5.12), P = 0.000	10.99 (6.62)
Internalizing problems	9.62 (6.62), P = 0.191	5.96 (6.08), P = 0.122	7.87 (6.16)
Externalizing problems	13.08 (8.03), P = 0.781	9.27 (6.91), P = 0.004	13.52 (7.93)
Total problems	35.85 (18.54), P = 0.301	24.77 (17.93), P = 0.050	32.01 (18.16)

Table 4. Outcome on CBCL for girls after surgery in comparison to the Dutch control group

CBCL (T-scores)	Girls before surgery (n=16)	Girls after surgery (n=14)	Dutch norm group girls (n=317)
	Mean (SD) P-value difference with norm group	Mean (SD) P-value difference with norm group	Mean (SD)
Affective problems	3.19 (2.59), P = 0.041	3.14 (2.91), P = 0.094	1.74 (1.70)
Anxiety problems	4.56 (2.59), P = 0.006	4.43 (2.41), P = 0.012	2.56 (2.25)
Pervasive developmental problems	4.19 (2.59), P = 0.047	4.14 (2.57), P = 0.070	2.79 (2.32)
Attention deficit hyperactivity	4.44 (3.43), P = 0.834	5.14 (2.96), P = 0.520	4.62 (2.55)
Oppositional deviant problems	3.69 (2.50), P = 0.691	2.43 (1.65), P = 0.005	3.94 (2.44)
Emotionally reactive	2.94 (2.74), P = 0.734	2.21 (2.01), P = 0.382	2.70 (2.46)
Anxious/Depressed	2.69 (2.15), P = 0.140	3.00 (2.48), P = 0.106	1.85 (1.80)
Somatic complaints	3.44 (3.83), P = 0.146	3.14(2.98), P = 0.165	1.97 (1.97)
Withdrawn behaviour	2.31 (2.12), P = 0.050	2.64 (1.99), P = 0.016	1.18 (1.37)
Sleep problems	4.56 (3.31), P = 0.010	3.36 (2.44), P = 0.082	2.13 (2.24)
Attention problems	2.69 (2.18), P = 0.405	2.93 (2.20), P = 0.250	2.22 (1.83)
Aggressive behaviour	10.81 (6.58), P = 0.469	8.64 (5.03), P = 0.494	9.59 (5.58)
Internalizing problems	11.38 (8.64), P = 0.110	11.00 (8.06), P = 0.149	7.70 (5.82
Externalizing problems	13.50 (8.52), P = 0.440	11.57 (6.96), P = 0.900	11.81 (6.65)
Total problems	40.75 (24.98), P = 0.085	35.93 (21.00), P = 0.255	29.25 (16.46)

To examine the impact of the surgery on sleep problems of each individual a paired samples t-test was conducted. Sleep improved in most of the children (68%), 8 children (21%) had a worse outcome in sleep patterns, while 4 children (11%) did not improve in sleep after surgery (Table 2).

CRIES was completed by 93%. Scores on this questionnaire were positively skewed, with most parents recording relatively few symptoms of posttraumatic stress in their children. None of the children scored above the cut-off of 30 on all 3 scales, but 9.5% of the children scored above 17 on the 2 subscales Intrusion and Avoidance. This score indicates a likelihood of the presence of posttraumatic stress disorder as described in DSM-IV.

One-way between-group of variances showed no differences between children who had more behavioural and emotional problems after surgery on the CBCL, CSHQ and CRIES on any significant similarities such as age ($P = 0.465$), gender ($P = 0.252$) or type of surgery ($P = 0.439$).

Regarding RSA 93% of the preoperative, 79% of the post-operative, and 93% of the follow-up measurements were completed successfully (Table 2). Most of the participants dropped out for technical reasons ($n = 10$). The scores of RSA were normally distributed. A one-way repeated measures ANOVA was conducted to compare scores on RSA at 3 different measurements. There was no significant effect for time for RSA (Wilks' Lambda = 0.92, $F(2,29) = 1.25$, $P = 0.30$, multivariate partial eta squared = 0.08).

Regarding cortisol, 61% of the preoperative, 49% of the postoperative samples, and 49% of the samples at follow-up was measured successfully (Table 2). Most measurements failed because of small samples of saliva, due to refusal by children to use the cotton swab for at least 5 minutes. The scores of EMR were normally distributed and the average waking values were in accordance with the literature (Silva et al., 2007; Tornhage, 2002). Cortisol measurements were corrected for outliers ($n = 2$), after which one-way repeated measures ANOVA were conducted to compare the levels of cortisol at different measurements. There was no significant effect for time for EMR (Wilk's Lambda = 0.88, $F(2, 13) = 0.879$, $P = 0.44$, multivariate partial eta squared = 0.12).

A high score on temperament shyness, sociability or activity did not correlate with significant higher CBCL scores, CSHQ scores or CRIES scores after surgery. However, a higher score on emotionality was associated with the total score of the CBCL after surgery ($r = 0.38$, $n = 39$, $P = 0.018$), but also before surgery ($r = 0.53$, $n = 41$, $P < 0.000$), which association was even stronger.

A high score on temperament shyness, sociability or activity did not correlate with a significant stronger neurophysiologic stress response after A&ATE. Only children with high scores on emotionality showed a decrease in cortisol levels directly after surgery in comparison with baseline ($r = -0.49$, $n = 17$, $P = 0.045$) as well as a lower RSA at follow-up in comparison with their baseline RSA ($r = -0.33$, $n = 36$, $P = 0.056$), although the last correlation did not reach significance. No significant correlations were found between pre-stress parameters (RSA, cortisol) and neurophysiologic stress responses, behaviour, or posttraumatic stress symptoms.

4. Discussion

To our knowledge this is the first study to report child behaviour in combination with neurophysiologic characteristics in young children before and after A&ATE, investigating individual differences in stress responses to A&ATE measured in a prospective way. Our results show that children with an indication for A&ATE have more behavioural and emotional problems in general in comparison to healthy children of the same age. These findings are in agreement with recent studies, which also found a positive change in behaviour and sleep after A&ATE (Ali et al., 1996; Garetz, 2008; Papkostas et al., 2003; Wei et al., 2007). A small group did experience more behavioural problems (25%), more sleeping problems (21%), and more PTSD symptoms (10%) after surgery. Although this is a minority, it is interesting to understand which factors influence the outcome in this group of children. Our hypotheses that neurophysiologic factors before surgery or temperament are strong predictive factors were not confirmed by our findings. Of note, no relationship was found with shyness, as opposed to previous findings (Doussard-Roosevelt et al., 2003; Kagan et al., 1988). Although we found that emotional children had significantly more behavioural problems after surgery, emotionality was even more strongly related to behavioural problems before surgery. Emotional children showed a decline in cortisol and RSA after surgery, which has been associated with posttraumatic stress symptoms in previous studies (Beauchaine, 2001; Meewisse et al., 2007; Yehuda et al., 2000). However, the CRIES scores were not elevated after surgery. Furthermore the correlation with RSA was weak. More research needs to be done in a larger sample to further examine the role of an emotional temperament in coping with a medical procedure.

Although it was not one of our a priori hypotheses, our findings indicate that gender could be a predictive factor on the outcome. Girls and boys appear to show differences in their psychological improvement after A&ATE in comparison to their pre-surgery behaviour and emotional problems. The benefits of A&ATE seem to be larger for boys than for girls. Particularly for boys most behavioural and emotional problems improved after surgery. However, the number of girls was small. More research is needed to explore the role of gender in the stress response of children to surgery.

This study demonstrates limitations that need to be addressed. There were artifacts in neurophysiologic measurements and missing data (especially cortisol, probably related to the young age of the children) (Bartels et al., 2003). Another limitation of this study is absence of data on the caregiving context. Attachment theory implies that the presence of a caregiver helps children to cope more comfortably with novel and frightening experiences, including painful medical procedures (Prins, 1994). Sensitive parenting can even buffer the reactivity of the HPA system to potentially stressful events and can influence the parasympathetic regulatory system of the child (Meewisse et al., 2007).

But this study also has particular strengths. Whereas research on posttraumatic stress is almost by definition retrospective, a planned medical procedure is potentially useful to prospectively study the impact of a frightening event. In our study we included physiological measures, which allow direct measurement of the child's emotional state in an age group where researchers are dependent upon indirect measuring through parent report (Perrin et al., 2000).

Less than a century ago it was possible for a leading psychiatrist to write about a medical procedure with children: "the anxiety stimulated by such horrors is probably never surpassed in the child's subsequent life" (Lipton, 1962). Observations such as these have led to the awareness that an integral part of A&ATE in children is psychological preparation and support (Papakostas et al., 2003). Pediatric A&ATE, when performed lege artis, now includes this and, as our study shows, makes A&ATE a psychologically acceptable procedure for children, while also offering potential psychological benefits.

5. Conclusions

The results of this prospective cohort study suggest that A&ATE can be a helpful procedure to reduce pre-existing behavioural, emotional and/or sleep problems. It is difficult to predict which children respond with an increase in behavioural and emotional problems to this type of surgery. An increase of behavioural and sleeping problems after surgery could only be partly explained by emotional temperament and gender. Our findings did not show a predictive value for neurophysiologic parameters.

6

Risks and protective factors associated with mental wellbeing and academic performance in primary school aged children in Asia

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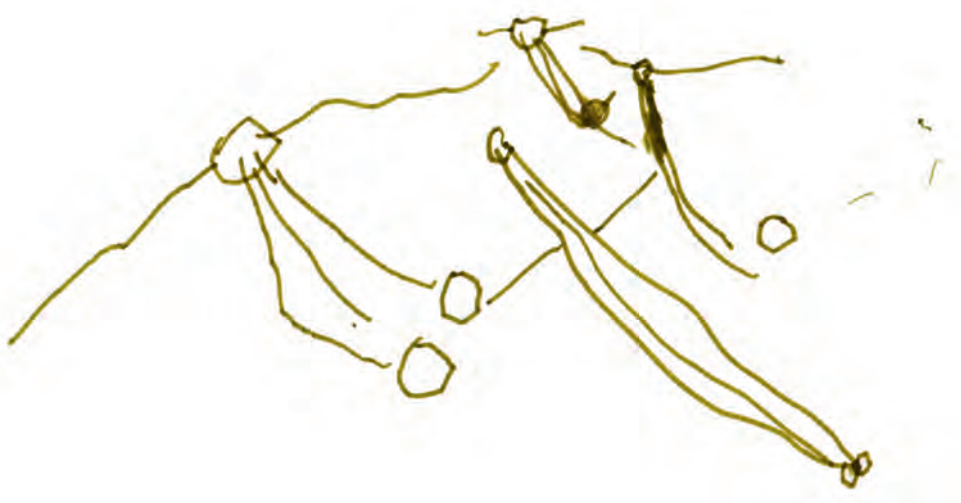
Submitted



"Family" by Charlotte Craig, 3 years old

Abstract

Objective

Studies of risk and resilience in a general population are sparse, especially in Asia. Most previous studies focused on 'high risk' samples and protective factors are often not included.

Methods

This cross-sectional questionnaire study of 1468 Singaporean children aged 7 to 12, with both parent and teacher data, examines the association between risks and protective factors, and mental wellbeing and academic performance in children of the general population.

Results

It was found that protective factors (e.g. intelligence, father's educational and occupation) were strongly associated with fewer emotional and behavioural problems (β = -0.24, T = -2.56) and much less likelihood of poor adaptive functioning and lower academic scores (β = -0.55, T = -7.91), while risks (e.g. negative spousal conflict resolution, negative methods of discipline, chronic health problems, negative life events and developmental delay) showed only a strong positive relationship with emotional and behavioural problems (β = 0.49, T = 8.12).

Conclusions

The empirical understanding of the influence of risks and protective factors in a large normative population is important in improving mental health and psychosocial competence in children.

1. Introduction

Early risk factors and protective factors greatly influence behaviour, emotional wellbeing and psychosocial competence of children. So far, well known risk factors have been studied extensively, such as poverty, poor health, premature birth, divorce, family violence, abuse and single-parent household. Most of these studies showed consistently that these risk factors predict higher rates of negative and undesirable outcomes. Recently McLaughlin et al. (2010) showed that many co-occuring childhood adversities clearly increased the risk of psychiatric disorders later in life (McLaughlin et al., 2010). However, studies in the last decades are more focussing on factors that can be protective and allow adaptation to stressful life experiences, generally termed resilience (Richardson, 2002). Interest in resilience research has been sparked by the potential of protecting youth from harm and improving their mental health and psychosocial competence through a combination of risk reduction and promotion of protective factors (Rutter, 1993; Rutter, 2006a). Although there are conflicting explanatory models about the impact of negative and positive childhood experiences, a body of evidence suggests that the cognitive development of children, as well as their social-emotional development, can be modified to improve wellbeing and competence in the long-term (Hertzman and Wiens, 1996). As emotional wellbeing has been associated to mental health later in life, as well as to socioeconomic outcomes such as academic success and career, this has implications for early intervention and prevention programs (Breslau et al., 2009).

Childhood resilience has been associated with adaptive child attributes, positive family functioning and the availability of external support systems (Garmezy, 1983). Examples of core factors which have been found to be related to mental health, as well as academic outcomes are Intelligence Quotient (IQ), socioeconomic status, elements of parenting (discipline and spousal resolution) and development (health, milestones, separation when young) (Boyle et al., 1987; Janicke et al., 2008; Rydell, 2010; Shaw and Emery, 1987; Tiet et al., 1998).

To study risk and resilience, many studies are focussing attention on high-risk samples of children with traumatic experiences. Although these studies are pivotal in delineating specific processes of stress and adaptation, they do not provide a broad perspective of the influences of negative and positive experiences on wellbeing and competence for children in general. This is better realized in studies which are conducted in normative populations of school children that include a broader range of different kinds and levels of risks and protective factors.

Normative population studies are sparse worldwide. Although the same proportion of primary school aged children with mental problems were found in Singapore (12.5%) as in Western countries (Woo et al., 2007), we assume important differences between cultures. Discipline styles of parents have been described as more controlling and authoritarian in Asia than in Europe and the USA (Lin and Fu, 1990; Steinberg et al., 1992). In Europa and the USA these parenting styles have been found to be predictive of poor school achievements, but Asian youth is performing quite well in school (Chao, 1994). Asian youth is known to face greater socioenvironmental stress in their families due to the high parental expectations on academic achievement (Ho and Yip, 2003; Isralowitz and Hong, 1990; Steinberg et al., 1992). Another difference is that children in Singapore are more often raised by grandparents or the family employs a 'domestic maid'. The domestic maid typically lives

in close proximity with the family providing continuous assistance in household matters and childcare. Despite the knowledge that social support is a protective factor and can benefit mothers and the development of their children, existing evidence for such 'Singapore benefits' is scarce.

Until now only a few studies of risks and resilience have been reported in Asian adolescents (Gau et al., 2005; Leung et al., 2008), but as far as we know hitherto, no studies have been done in Asian school aged children. As prevention is helpful in early age, it is important to explore mental health and academic achievements in primary school aged children in Asia.

The aim of this large population-based study of Singaporean primary school children is to examine risks and protective factors and the relative associations between these factors with mental wellbeing, adaptive functioning and academic outcomes. We hypothesize that in comparison to studies performed in Europe and the USA, in Asia different protective factors and risk factors are important in influencing mental wellbeing and academic performance.

2. Methods

2.1. Participants

Approval to conduct the study was obtained from the Singapore National Healthcare Group Domain Specific Review Board. Singapore is a multi racial country of Chinese, Malay and Indian. Children were assessed in school. A random sample of 18 out of the 178 primary schools in Singapore was obtained using a computerized randomization program. Consent was sought from the principals of the respective schools and all 18 schools agreed to participate. A random sample (proportional to the total number of students in the school) of 3586 children across 18 schools was drawn. Written consent was obtained from the parents of these children; 60% (n = 2139) agreed to participate in the study. In view of the low response rate, data on the demographic profile of the responders (n = 2139) and the non-responders (n = 1447) obtained from the Ministry of Education were analyzed. Compared to non-participants, a significantly higher proportion ($P < 0.05$) of the participants lived in private higher end housing (19% versus 14%) and had mothers with a post-secondary education (32% versus 25%).

Children studying in special education schools for the intellectually disabled, moderately or severely autistic, visually handicapped and hearing-impaired were excluded from the survey.

The study was approved by The Singapore National Healthcare Group Domain Specific Review Board, and the tenets of the declaration of Helsinki were observed. All parents of the included children provided written informed consent.

2.2. Measures

2.2.1. Mental wellbeing

The Child Behavior Checklist (CBCL/4-18) parental report was used to obtain parent-reported information on a broad range of emotional and behavioural difficulties within the last 6 months using 118 items with a 3-point Likert scale (Achenbach and Edelbrock, 1991a). It is self-administered and takes 30 minutes to complete. Summed scores were computed for 8 syndromes (Withdrawn behaviour, Somatic complaints, Anxious/Depressed, Social problems, Thought problems, Attention problems, Delinquent behaviour, Aggressive

behaviour), 2 syndrome groups (Internalizing and Externalizing Problems), and a Total problems score. Internalizing problems include withdrawn behaviour, somatic complaints, anxiety and depression, while externalizing problems include aggressive and delinquent behaviour. Raw scores can be converted to age-standardized scores. Higher scores denote greater severity. T scores less than 60 are considered within the normal range, 60-63 represent borderline scores and scores of 64 and higher are in the clinical range.
The Teacher Rating Form (TRF) (Achenbach and Edelbrock, 1991b), a subsidiary instrument of the CBCL, was also used to obtain teacher-reported information on children's behavioural and emotional problems. The TRF was completed by the teacher who knew the child best. A cut off of 64 was used for both the CBCL and TRF manual. The TRF was administered at school, and the CBCL was mailed to parents. Of 1607 children the CBCL was completed by their parents (response rate 75%) and of 1964 children the TRF was completed by their teachers (response rate 92%). The CBCL has a good construct validity and has shown acceptable test-retest coefficients among the 8 syndromes, with cronbach's alpha's ranging from 0.62 to 0.92 (Achenbach and Edelbrock, 1991a).

2.2.2. Adaptive functioning and academic performance

Adaptive functioning of the child was measured with a survey by teachers in terms of his/her ability to work, to behave, to learn and to be happy in school, compared to other pupils of the same age (much less than others, somewhat less than others, average, slightly more than others, somewhat more than others, much more than others). Poor adaptive functioning was defined as 'much less than others' or 'somewhat less than others' on one or more of these items. Academic performance was assessed using their performance scores on the core academic subjects: English, Mother Tongue, Mathematics and Science, provided by teachers. They were scored as 'above average' for scores at the 76th percentile and above, and 'average' between the 26th and 75th percentile and 'below average' for scores at the 25th percentile and below. Both adaptive functioning (4 items) as well as academic performance (1 item) were also measured with the TRF (below average, average, above average), which was filled out by the teachers. There was a positive correlation between non adaptive behaviour on the survey and a high score (> 64) on the TRF, both filled out by teachers, for all 4 items (not working hard and TRF total: $r = 0.26$, $P = 0.000$; behaviour problems and TRF total: $r = 0.34$, $P = 0.000$; learning problems and TRF total: $r = 0.28$, $P = 0.000$; not happy at school and TRF total: $r = 0.17$, $P = 0.000$).

2.2.3. Demographic data and data about parenting

Variables representing protective and risk factors were created from demographic data provided by mother about the child and family; age, gender, housing (public, private), mother's marital status (married to child's biological father, single, divorced/separated, widowed, deceased), father's educational level (primary or below, secondary/vocational, pre-university/polytechnic, university/postgraduate), father's occupation (managerial/professional, executive/sales, clerical/technical, self-employed, not working, retired) and the primary caregiver (parent, grandparent, sibling, relatives, foster parent, domestic maid, daycare service provider). Other protective factors and risk factors were derived from the questionnaire based on the Family and Household Questionnaire used in the Ontario Child Health Study (Offord et al., 1987), which is a 75-item questionnaire related to family functioning (Copping et al.,

2001). The questionnaire was filled out by mother. Essentially the same questions as in the original Family and Household Questionnaire were asked, only small changes were made to accommodate cultural differences such as language spoken at home. In this questionnaire child characteristics, health characteristics, family issues and life events are explored. The life events were part of the items list used in the Ontario Child Health Study (Boyle et al., 1987). From this questionnaire certain variables were selected as possible protective or risk factors, based on the literature (see appendix I). All these variables were scored dichotomously as a positive or negative screen for a risk factor as follows:

1. Child factors:
   a) lived away from parents for extended period before age 3 (yes, no)
   b) late age walking (yes: > 18 months defined; no: ≤ 18 months)
   c) late age talking (yes: >3 years, no: ≤ 3 years), speech difficulties (yes, no)
2. Medical factors:
   a) health problems (yes, no)
   b) health problems limit participation in school (yes, no)
   c) regularly taking prescribed medication (yes, no)
   d) accidental injury (yes, no)
   e) seen psychiatrist/psychologist/counselor regularly (yes, no).
3. Family factors and life events:
   a) life events (stopped school, lost job, got married, someone moved into home, financial problems, separation, arrival of baby at home, someone moved out of home, serious illness, serious illness of someone dear, left job or retired from fulltime work, started working or changed jobs, death of someone dear) (yes, no)
   b) coping with disagreement with spouse (positive methods of spousal conflict resolution: *discussing calmly and/or, leaving the room to avoid continuing an argument*; negative spousal conflict resolution: *raising one's voice or yelling, refusing to talk, insulting or swearing, crying, smashing objects or throwing things, threatening to hit and/or hitting*)
   c) coping with difficult child behaviour (positive disciplining methods: *reasoning, sending the child to his room and/or removing privileges*; negative disciplining methods: *spanking, caning or hitting with an object, shaking or shoving*).

2.2.4. Intelligence

Children completed the Raven's Standard Progressive Matrices (RPM), a widely used test of non-verbal reasoning ability by assessing the visual alertness and spatial and abstract pattern-recognition abilities by analogy, independent of language and formal schooling (Raven et al., 2000). It was group administered by a research assistant at the schools' premises during regular school hours over less than 1 hour. Instruction for the administration of the RPM was given in English, which is the language of education. The RPM has shown acceptable test-retest reliability coefficients ranging from 0.76 to 0.91 in various cross-cultural studies of intelligence. A cut-off at the 76th percentile was used, with scores of the 76th percentile and above defined as 'above average'. Scores between the 26th and 75th percentile were defined as 'average' and below the 25th percentile as 'below average'. While the RPM offers greater brevity, the validity is comparable with conventional tests such as the Wechsler Intelligence Scale for Children (WISC) (Barratt, 1956).

2.3. Data analyses

Structural equation modelling (SEM), a multivariate regression modelling technique, was performed to construct a heuristic model examining the impact of protective factors and risks on children's mental wellbeing and adaptive functioning and academic performance. Only complete data on all variables were included.

We fit a confirmatory factor model to estimate the amount of variance in CBCL and TRF ratings of internalizing and externalizing problems and the total problems scores (as separate outcome variables), as well as adaptive functioning and academic performance, that were associated with risk factors and protective factors.

Risk factors were defined as negative methods of discipline, negative spousal resolution, chronic health problem(s), developmental delay, child's separation from parents for more than 6 months before 3 years old, negative life events present, mother being non-married (single/divorced, separated, widowed or deceased) and surrogate caregiving (by domestic maids, day-care service providers, foster parents and relatives other than parents or grandparents). The protective factors were defined as intelligence (scoring at or above the 75th percentile on the Raven's Progressive Matrices), positive methods of discipline, positive methods of spousal resolution, father being employed, and father having post secondary/tertiary education. Age and gender were included in the model as neutral demographic factors.

All P values were two-tailed and considered statistically significant when the values were below 0.05. Statistical analysis was performed using SAS System statistical package (Cary, NC: SAS Institute Inc).

3. Results

3.1. Sample characteristics of children

Complete parent and teacher data of 1468 children were available; hence these subjects were included in the analyses. The sociodemographic characteristics of the children in the sample were similar to those shown in the Singapore population census statistics. The majority of the children (76%) were Chinese, and 81% resided in public housing estates. Among their mothers, 8.5% were single, divorced, separated, widowed or deceased (Table 1).

3.2. Heuristic model

The confirmatory factor analysis model fit the data well. See Table 2 (Fit Function = 1.82, Goodness of Fit Index (GFI) = 0.87, Adjusted GFI = 0.84, Chi square < 0.0001, RMSEA Estimate = 0.07), and Table 3 (Fit Function = 1.51, GFI = 0.88, Adjusted GFI = 0.85, Chi square < 0.0001, RMSEA Estimate = 0.07).

Table 1. Characteristics of children in sample

	Included (n=1468)
Child's gender: Male Female	 50.1% 49.9%
Child's age	M 9.4, SD 1.7 (7-12)
Housing type: Public housing Private housing	 80.8% 19.2%
Father's education post-secondary and tertiary	37.5%
Father working	77.8%
Raven's scores ≥ 75th percentile	61.6%
Positive discipline reasoning (sending the child to his room and/or removing privileges)	83.7%
Positive spousal resolution (discussing calmly and/or leaving the room to avoid continuing an argument)	66.7%
Mother is not married (single/divorced/separated/widowed/deceased)	8.5%
Surrogate caregiver is mainly raising child (grandparents/other relatives/ domestic helpers/day-care service providers/foster parents)	14.7%
Negative discipline (spanking and/or caning/hitting with an object and/or shaking/ shoving)	45.9%
Negative spousal resolution (raising one's voice or yelling and/or refusing to talk and/or insulting or swearing and/or crying and/or smashing objects or throwing things and/or threatening to hit, hitting)	48.7%
Negative life events (someone in the household stopped full time school or work, or changed jobs and/or someone in household got married and/or someone moved in or out the home and/or moving homes and/or financial problems and/or separation of parents and/or arrival of baby and/or serious illness or death in family member or close relative)	45.2%
Lived away from parents for more than 6 months before age 3	7.7%
Poor health (health problems and/or regularly taking prescribed medication and/or accidental injuries and/or seen psychiatrist/psychologist/counsellor regularly)	0.5%
Poor development (walking 5 steps > 18 months and/or talking 3 words in a phrase >3 years and/or stuttering or mispronounciations)	31.1%
TRF internalizing scores ≥ 64	4.0%
TRF externalizing scores ≥ 64	3.1%
CBCL internalizing scores ≥ 64	15.1%
CBCL externalizing scores ≥ 64	7.2%
Not working hard in comparison to other pupils according to teacher	11.4%
Not behaving in comparison to other pupils according to teacher	6.4%
Not learning in comparison to other pupils according to teacher	8.4%
Not happy in comparison to other pupils according to teacher	2.0%
Poor english < 25th percentile	17.2%
Poor mother tongue < 25th percentile	12.4%
Poor maths < 25th percentile	20.1%
Poor science < 25th percentile	18.7%

Table 2. Latent factor modeling of protective factors and risks, emotional and behavioural problems, adaptive functioning and academic scores

Variable	Coefficient estimate	Standardized coefficient (β)	T-value
	Protective factors		
Raven's score	0.909	0.545	Infinity
Education father	0.827	0.496	10.597
Occupation father	0.679	0.407	10.174
Positive spousal resolution	0.136	0.082	2.791
Positive discipline	0.098	0.058	2.017
	Risk factors		
Negative spousal resolution	2.097	0.687	30.34
Negative discipline	1.647	0.540	26.65
Poor health	0.944	0.309	18.88
Negative life events	0.832	0.273	16.79
Poor development	0.619	0.203	12.65
Separated when young	0.303	0.099	6.260
Marital status mother	0.281	0.092	5.813
Surrogate caregiver	0.102	0.033	2.129
	Emotional and behavioural problems		
CBCL internalizing	1.632	0.582	21.42
CBCL externalizing	1.588	0.566	21.70
TRF internalizing	0.029	0.010	0.607
TRF externalizing	0.522	0.186	10.49
	Poor adaptive functioning and academic scores		
Not working hard	0.860	0.608	14.99
Not behaving	0.604	0.427	11.60
Not learning	0.924	0.653	15.55
Not happy	0.330	0.233	6.703
Poor English	0.965	0.682	15.83
Poor mother tongue	0.670	0.473	12.62
Poor maths	0.994	0.702	15.98
Poor science	1.084	0.766	Infinity

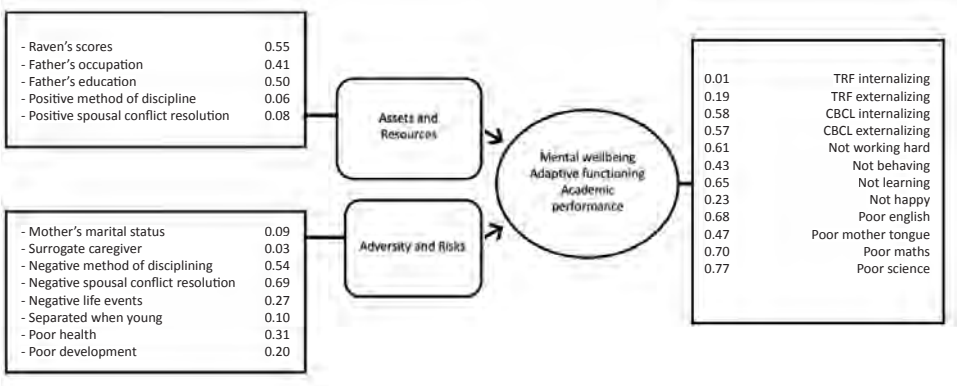


Figure 1. Risks and resources model
The numbers shown are standardized coefficients (β)

As shown in Table 2 and figure 1, protective factors appeared to be determined mainly by intelligence (β = 0.55), father's educational level (β = 0.50) and father's occupation (β = 0.41), and less by positive methods of spousal conflict resolution (β = 0.08) and positive methods of discipline (β = 0.06). Risks appear to be determined mainly by negative methods of spousal conflict resolution (β = 0.69), negative methods of discipline (β = 0.54), chronic health problems (β = 0.31), negative life events (β = 0.27) and developmental delay (β = 0.20), and less by separation from parents for prolonged periods when young (β = 0.10), mother's marital status (β = 0.09) and surrogate care giving (β = 0.03).

As shown in Table 3, older age was associated with fewer emotional and behavioural problems (β = -0.07, T = -3.72) but poorer adaptive functioning and academic scores (β = 0.09, T = 2.60), while male gender was associated with greater emotional and behavioural problem scores (β = 0.15, T = 8.56), without an association with poor adaptive functioning and academic scores.

Protective factors were strongly associated with fewer emotional and behavioural problems (β = -0.24, T = -2.56) and much lesser likelihood of poor adaptive functioning and academic scores (β = -0.55, T = -7.91), while risks showed a strong positive relationship with emotional and behavioural problems (β = 0.49, T = 8.12) but no significant relationship with academic scores (β = 0.004, T = 0.17).

Table 3. Associations of protective factors and risk factors with behavioural and emotional problems and academic scores

	Emotional and behavioural score				Poor adaptive functioning and academic score			
	Coefficient estimate	**Standardized coefficient (β)**	**T-value**	**P**	**Coefficient estimate**	**Standardized coefficient (β)**	**T-value**	**P**
Older Age	-0.187	-0.066	-3.718	0.0002	0.129	0.091	2.595	0.009
Male gender	0.432	0.154	8.559	<0.001	0.041	0.029	0.830	0.407
Protective factors	-0.144	-0.242	-2.559	<0.010	-0.652	-0.553	-7.911	<0.001
Risk factors	0.529	0.487	8.123	<0.001	0.010	0.004	0.172	0.863

4. Discussion

In this study unique and relative contributions of salient risks and protective factors on mental wellbeing and psychosocial competence of Singaporean children are discussed. We attempted to accommodate the complexities and the real-life patterns of these different negative and positive influences (Burack et al., 2007). Uniquely in this research, we ascertained the contributions of each of the salient influences in representing risks or protective factors as well as their relative contribution to mental wellbeing and psychosocial competence.

We found that negative spousal resolution style, negative discipline style, negative life events, poor health and poor development shared heavier representations of risks, whereas separation for prolonged periods when young, mother's marital status and surrogate caregiving showed lesser representations of risks. Previous studies have shown the strong causal significance of each of these individual negative childhood experiences, but their individual contributions relative to the totality of risks may not be the same. Parental strict blame, physical harsh discipline or indifference to the child's misbehaviour have been associated with increased rates of mental health problems (Burack et al., 2007; Keiley et al., 2001; Keiley et al., 2003; Liu et al., 1999) which were in turn perpetuated by parental verbal conflict (Dwyer et al., 2003). This was also found in this Singaporean population (Kong et al., 1988). Other studies showed that poor general health and negative life events were related to mental health problems (Deater-Deckard et al., 1998; D'Imperio et al., 2000; Ford et al., 2004; Liu et al., 1999). These factors were found to be the most important contributors of risks in our study as well.

On the other hand, in contradiction to previous studies which showed that children of single, divorced, separated, widowed or deceased parents and children in foster homes have higher rates of childhood mental health problems (Anglin et al., 2008; Canino et al., 2004; Harden, 2004; Lipman et al., 2002; Munroe-Blum et al., 1988; Thompson et al., 2008), we found that family structure, characterized by mother's marital status and surrogate caregiving, were relatively less important risk factors. This could be related to the fact that the Asian culture, unlike Western cultures, prioritizes family values and extended family systems, such as children being raised by grandparents or live-in maids (Sonuga-Barke and Mistry, 2000). It is known that the risk for mental problems diminishes when other family members take the responsibilities for care of the children after the death of a parent, divorce or other negative events (Masten et al., 1990; Sonuga-Barke and Mistry, 2000).

Conversely, we found that protective factors were determined mainly by intelligence, father's educational level and occupation, and less by positive methods of spousal conflict resolution and positive methods of discipline. High intellectual function has been found to increase coping capacity and stress resistance resulting in fewer psychiatric diagnoses and increase in positive mental health (Campbell-Sills et al., 2006; Cederblad et al., 1995). In Singapore, the fathers' educational level and occupation are correlated with socioeconomic status, a risk determinant for mental health problems (Chia et al., 2004). Although research has shown that emotionally responsive, competent parenting predicts resilience (Wyman et al., 1999), and a combination of rewarding and non-punitive parenting strategies was negatively associated with childhood psychopathology (Vostanis et al., 2006; Wyman et al., 1999), our findings show that positive parenting and positive spousal relationships were relatively less important than intelligence or socioeconomic status in determining protective factors. In

conclusion, we found that the method of discipline and spousal conflict resolution appears to be strongly influential when these methods are negative rather than positive. This is in concordance with earlier studies (Dwyer et al., 2003).

Our findings uniquely show that while risks are only strongly associated with emotional and behavioural health, protective factors are strongly associated with both emotional and behavioural health as well as academic outcomes. It is interesting that, in a country with educational pressure, negative discipline does not have a major influence on academic results. However, these findings are in contradiction to another Asian study done in a small sample of 267 schoolchildren in Thailand (Samchit and Sriyaporn, 2004). This study showed that both protective factors as well as risks were not related to academic results. The different outcome can be related to differences in sample size and methodology.

In our study we found that older age was related to better emotional and behavioural outcomes, possibly because of improving skills of dealing with risks and adversities by shifting from external supports to more internal resources and strengths. The negative association with academic performance was probably linked not to age itself as much as to higher standards of academic performance assessment.

The association of male gender with more emotional and behavioural problems is similar to findings reported elsewhere (Liu et al., 1999, Prior et al., 2005). The lack of association with academic performance outcomes was at variance with other studies which found that boys also performed more poorly academically than girls (Prior et al., 2005).

The study has some limitations. First, the distinction between some risks and protective factors may be problematic, because they may merely represent the negative or positive end of the same dimension. While categorical variables such as negative life experiences, positive and negative methods of disciplining and spousal conflict resolution could be clearly regarded as positive or negative factors, the classification of variables like intelligence, which is measured on a continuum as a positive exposure is arguable. Furthermore, some of the risks and protective factors will be related. For example a low socioeconomic status is in itself associated with a higher risk for negative parenting.

Secondly, this study examined a relatively small subset of possible factors influencing mental health and academic outcome. Based on the literature, we included numerous specific positive and negative factors but they could not be exhaustive. Although it would be ideal to include other variables such as temperament of the child and attachment styles, this was practically not feasible. However, we were able to still measure core risks and protective variables. Although we feel that using a standard Bonferroni correction would be overly conservative, considering the large number of variables that were assessed, a stricter interpretation of the P-values could be considered.

Third, most of our data were collected by self-rating questionnaires and only by maternal reports. Direct causal relationships are not directly inferable from this cross-sectional study, but are well supported by other studies. Furthermore, part of the results obtained may be due to the lack of cultural sensitivity of the instruments used. Studies showed that in Asia sometimes a different cut-off score is necessary for the CBCL and TRF (Woo et al., 2007). The psychometric properties in Asia still need to be evaluated.

Finally, only 60% of parental consent was obtained. As only 1607 of the target sample provided data, and only for 1468 children both parent and teacher data were available, complete data which could be used for analyses was only 40%. However, this number of children with

complete data was adequate for the statistical analyses, and does not jeopardize the internal validity of the findings. However, there were significant differences in socioeconomic status between respondents and non-respondents, with the latter more likely to have a low socioeconomic status, which is a risk factor for developing psychopathology. This limits the generalizability of our findings.

However, this study has particular strengths. We examined a large sample of school-aged children across the island of Singapore, by using a computerized random sampling strategy. Well known and international accepted measurements were used to examine behaviour and emotional wellbeing as well as IQ, and the academic results were objectively measured by inclusions of the performance scores on the core academic subjects. Only well known protective and risk factors which have been found in previous studies and which are common in the general population were included in our model.

Our model sheds light on the complexity of childhood psychopathology by highlighting the influence of an entire range of risks and protective factors and not merely severe adversities on mental health and competence. This is relevant and appropriate for childhood mental health programs. A meta-analysis of primary prevention programs to prevent behavioural and social problems in children showed that most programs had a dual benefit of reducing problems as well as increasing competencies (Durlak and Wells, 1997). Those interventions are critical because a decrease in risks and stimulation of protective factors may have important societal implications like an increase of mental health and academic success in childhood, and consequently employment success and income in later life. Our findings showed that especially stimulation of protective factors could have a dual benefit on mental health as well as academic outcomes. Although not all protective variables are malleable, reducing negative discipline and negative spousal resolution could be helpful to decrease socio-emotional problems. In previous studies parenting skills training has showed to be an effective intervention for reduction of externalizing as well as internalizing symptoms (Cartwright-Hatton et al., 2005).

5. Conclusions

Various risks and protective factors during childhood make unique and varying contributions on mental health and competence. In Singapore protective factors were strongly associated with fewer emotional and behavioural problems and much lesser likelihood of poor adaptive functioning and lower academic scores, while risks showed only a strong positive association with emotional and behavioural problems. Empirical understanding of these associations in a large normative population of school aged children in Asia is important for improving mental health and psychosocial competence in children.

7

The development and validation of the Singapore Youth Resilience Scale (SYRESS)

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"Children of the world" by Fleur Deiters, 6 years old

Abstract

Objective

Resilience research suggests that multiple conceptualisations of resilience are descriptions of the various aspects of the construct. A resilience scale developed on the basis of a particular conceptualisation therefore may not adequately represent all aspects of resilience. Multicultural resilience research also suggests that cross-cultural use of resilience measures developed in Western countries is theoretically unsound.

Methods

In this paper, we report the development and validation of the Singapore Adolescent Resilience Scale (SYRESS), a 50-items 10-domain hybrid scale which not only encompasses the various aspects of resilience but also incorporates the contextual aspects unique to local youths.

Results

The SYRESS showed good internal consistency (Cronbach's alpha = 0.95, $P < 0.01$), test-retest reliability ($r = 0.82$, $P < 0.01$), and convergent validity with the Connor-Davidson Resilience Scale (CD-RISC) ($r = 0.88$, $P < 0.01$), World Health Organisation Quality of Life (WHOQOL-BREF) ($r = 0.55$, $P < 0.01$), and the General Health Questionnaire (GHQ-28) ($r = -0.33$, $P < 0.01$). Factor analyses revealed a 10-factor structure (total variance 63.4%) for the SYRESS and hierarchical analyses showed that SYRESS significantly contributed additional variance to the prediction of the WHOQOL-BREF and GHQ-28 scores over that contributed by CD-RISC alone, suggesting that as a hybrid scale, SYRESS is a more comprehensive measure.

Conclusions

As a hybrid resilience scale the SYRESS showed to be a comprehensive measure for well-being and mental health in a Singaporean population.

1. Introduction

Resilience has been defined as the process, or the achievement, of positive adaptation despite adversities or challenging life conditions (Davydov et al., 2010; Luthar et al., 2000). It has been conceptualised as a set of personality traits that buffers the negative effects of stress (Ahern et al., 2008), the ability to cope with change or stressors (Wagnild and Young, 1993), or simply as a set of personal resources that can be tapped into, to moderate the effects of stressors when the need arises (Davydov et al., 2010). These various conceptualisations have given rise to substantial variations in its measurement and its underlying mechanisms (Luthar et al., 2000). Among these underlying mechanisms are the availability of positive social support, and the presence of certain internal attributes like perseverance and effective coping skills (Ahern et al., 2008; Collishaw et al., 2007; Luthar, 1991; Luthar and Zigler, 1992; Smokowski et al., 1999). Some have suggested that these various conceptualisations and the respective underlying mechanisms are not conflicting views, but merely descriptions of the many different facets of the same construct (Luthar et al., 2000). Given the above, it may be reasoned that the various instruments developed to measure resilience are also measuring the different aspects of resilience. A review of existing scales reveals indeed that these scales measure a plethora of underlying mechanisms ranging from protective resources of healthy adjustment to personality characteristics that enhance adaptation. However, none of the scales measures all of these underlying mechanisms. For instance, the Resilience Scale (RS) (Wagnild and Young, 1993) does not assess the domains of positive acceptance of change and challenges, and emotional regulation, which are assessed in both the Connor-Davidson Resilience Scale (CD-RISC) (Connor and Davidson, 2003) and the Adolescent Resilience Scale (ARS) (Oshio et al., 2003). On the other hand, the ARS does not assess the idea of self-reliance and sense of personal competence, which is assessed in the CD-RISC and RS. And spiritual influences on resilience are assessed only in the CD-RISC. A scale developed based on one particular conceptualisation of the resilience construct, therefore, does not seem to capture the entire possible spectrum of underlying mechanisms of resilience. We hypothesize that a scale with a broad conceptualization of the construct of resilience, that includes more domains and underlying mechanisms, may better capture the various aspects of resilience. To date, no study has yet attempted to develop a hybrid scale that more comprehensively measures resilience.

There is also an increasing awareness of the need to incorporate culture and diversity into the study of resilience (Arrington and Wilson, 2000). Because of different geographical, historical and social environments, various cultures can have different understandings of adversity and positive adaptation (Yu and Zhang, 2007). Indeed, research into the cultural and contextual influences on resilience has revealed that there are global as well as culturally and contextually specific aspects to understanding resilience (Ungar, 2006, 2008), and that cultural beliefs play a significant role in determining an individual's approach to adversities in life (Lee et al., 2010). Thus, although the construct of resilience is believed to be universal, there may be additional specific underlying mechanisms of the development of resilience unique to the culture in question (Ungar, 2008). Since patterns of resilience can be context-dependent, direct wholesale cross-cultural applications of standardized measures may not be viable (Ungar, 2008). For instance, although some measures of resilience developed in Western countries have been validated cross culturally (e.g. CD-RISC), the findings indicate

that the understanding of the construct requires some modification according to the culture in which it is measured (Yu and Zhang, 2007). Thus, to better measure resilience in any culture, the unique contextual and cultural aspects must be considered and incorporated into the measurement scale. However, to date, no scale has been developed to measure resilience in Singapore.

A recent review showed that most existing scales are not suitable for use in adolescent populations (Ahern et al., 2006). Efforts aimed at understanding youth resilience are not new (Luthar et al., 2000). Adolescence is often considered to be a period of rapid development and developmental challenges (Engle et al., 1996; Flisher and Gerein, 2008; Friedman, 1989). Many studies have found that, despite exposure to such stresses, some adolescents exhibit resilience and achieve positive development during this period of transition (Lee et al., 2010; Richter, 2006). This suggests that adolescents can be protected from harm through enhancing their resilience. In Singapore, adolescents face a highly stressful academic environment and are exposed to considerable challenging conditions complicated by a multitude of stresses (Ang and Huan, 2006). The mental health problems caused by these stresses have been well-documented (Ang and Huan, 2006). Despite the key role resilience plays in enhancing adolescents' wellbeing, to date, there is no scale developed to measure resilience in the adolescent population in Singapore.

The current study therefore aims to develop and validate a resilience scale, that not only encompasses the various aspects of resilience, but also incorporates the contextual and cultural aspects unique to the adolescent population in Singapore. In the current study, the CD-RISC (Connor and Davidson, 2003) was used as a convergent validity indicator of SYRESS. Measures of quality of life (World Health Organization Quality of Life, WHOQOL–BREF, WHOQOL-Group, 1998) and general health status (General Health Questionnaire and GHQ-28) (Goldberg and Hillier, 1979) were also used as validity indicators of SYRESS as previous studies have shown that resilience is positively related to positive development (Alriksson-Schmidt et al., 2006; Lee et al., 2010) and general health (Haddadi and Besharat, 2010).

2. Method

2.1. Development of the Singapore Youth Resilience Scale (SYRESS)

The content of the scale was drawn from multiple sources. First, an exhaustive review of the literature was performed, including recent published resilience scales (Adolescent Resilience Scale (ARS), Oshio et al., 2003; Connor-Davidson Resilience Scale (CD-RISC), Connor and Davidson, 2003; Resilience Scale (RS), Wagnild and Young, 1993; Resiliency Scales for Children and Adolescents (RSCA), Prince-Embury, 2007). A focus group comprising researchers, child psychologists and pediatric psychiatrists, with pooled local and international content expertise, then reviewed the domains and question items in these established scales. They identified and selected from among them a comprehensive list of question items that reflect the salient features and global underpinnings of resilience. Items that reflected the local culture but which were not included in the list were added. With an exhaustive list of domains and a saturated pool of associated question items thus generated, an external expert panel with similar content expertise was consulted on the comprehensiveness and

cultural relevance of the items in the list. These experts provided additional feedback and inputs. This generated a pool of 166 items, which was subsequently content-analyzed and classified by domains. Items that lacked face validity for the domain and redundant items that were very similar to a more ideally worded item were eliminated. This resulted in the removal of 71 items, and the remaining 95 items were rewritten or modified where necessary. This prototype scale was reviewed by another external panel of 10 psychiatric experts who did not participate in the focus group discussions. They were similarly asked to review the comprehensiveness and cultural relevance of the question items. Their critical feedback led to a further elimination of 11 items and further changes to the wording of the items. The amended prototype scale of 84 items was then pre-tested in a convenience sample of 20 adolescents to check for clarity of instructions and question wordings. The final prototype scale comprised 84 provisional items reflecting 10 domains of resilience. The provisional domains were: emotional regulation (8 items), spirituality (7 items), family and social support (8 items), self-belief - satisfaction and a sense of purpose in life (15 items), self-confidence during stress (5 items), coping with challenges (6 items), flexibility (6 items), optimism (6 items), humour (5 items), and coping style (18 items). The questionnaire was designed as a self-rating scale that requires the respondent to indicate how much he/she agrees with each statement on a Likert scale ranging from 'never' (1) to 'always' (5). The summed scores across all domains and subscale scores for individual domains required the scores for 8 items to be reversed. Higher scores on the SYRESS denote greater resilience.

2.2. Participants

The 84-item prototype version of the scale was empirically tested for its internal reliability and construct validity among pupils in Grades 7 to 9 of a typical public mainstream secondary level school. The analysis in the present study was performed on data from 190 adolescents: 98 males and 92 females aged between 12 to 16 years (mean age: 12.8 years).

2.3. Consent and procedure

The study was approved by the National University of Singapore Institutional Review Board, and permission to conduct the study at the participating school was obtained from the Ministry of Education and the principal of the school. The purpose of the study was explained to the participants and parents through a participant information sheet and parent information sheet respectively. Both participants and parents provided signed informed consent. The questionnaires were administered in English, the first language of education for all students in Singapore. The questionnaires included brief demographic data, the prototype SYRESS, CD-RISC, WHOQOL-BREF and GHQ-28. The test-retest reliability was assessed two weeks later on a subsample of 30 randomly selected participants who completed the SYRESS for the second time.

2.4. Measures

2.4.1. Connor-Davidson Resilience Scale (CD-RISC)

CD-RISC (Connor and Davidson, 2003) is an established resilience scale that comprises 25 items, each rated on a 5-point Likert scale ranging from 'not true at all' (0) to 'true nearly all the time' (4), with higher scores reflecting greater resilience. The scale has been found to be valid and reliable, with a Cronbach's alpha of .89 (Ahern et al., 2006; Connor and Davidson, 2003). Its Cronbach's alpha in the present study was .94.

2.4.2. World Health Organization Quality of Life (WHOQOL–BREF)

WHOQOL-BREF (WHOQOL-Group, 1998) comprises 26 items measuring the individual's perception of quality of life in each of 4 domains: physical health, psychological, social relationships, and environment. Higher scores denote a perceived higher quality of life. It has been shown to adequately assess the domains in a large number of diverse cultures (WHOQOL-Group, 1998). It has been well validated in Asian participants (Saxena et al., 2001) and found to have good validity and reliability in assessing the quality of life in adolescents (Chen et al., 2006). The WHOQOL-BREF demonstrates good internal consistency, with Cronbach's alpha values ranging from .66 to .84 (WHOQOL-Group, 1998). Its Cronbach's alpha in the present study was .93.

2.4.3. General Health Questionnaire (GHQ-28)

GHQ (Goldberg and Hillier, 1979) is used in many studies to detect minor psychiatric disturbances in community or non-psychiatric clinical settings. The 28 items in the questionnaire measure responses to 4 subscales that examine somatic symptoms, anxiety and insomnia, social dysfunction and severe depression. Symptoms are assessed during the past few weeks on a 4-point scale that ranges from a 'better or healthier than normal' (0) through 'worse/more than usual' (4). Higher scores denote greater severity. Good validity of the GHQ-28 has been reported for adult respondents by the authors (Goldberg and Hillier, 1979), and cross-validated in younger people (Banks, 1983), as well as in Singaporeans (Fones et al., 1998). In the present study, its Cronbach's alpha was .94.

2.4.4. Singapore Youth Resilience Scale (SYRESS)

The development of SYRESS, measuring 10 domains of resilience, is described above in more detail.

2.5. Data analyses

To examine the factor structure of the SYRESS, an Exploratory Factor Analysis (EFA) using the principal components analysis method with an oblique rotation was performed on the scores of the 84-item prototype SYRESS. An oblique rotation was chosen because correlation between factors was expected. The number of factors to retain was evaluated based on the (1) eigenvalues (greater than 1.00), (2) scree plot analysis, and (3) interpretability of the resulting structure. Because the purpose of the EFA was to establish meaningful factors underlying the SYRESS, a minimum loading of .30 was used as a selection criterion. To ensure its comprehensiveness, factors that had at least 3 items loaded on it were retained. To assess the internal consistency of the scale, Cronbach's coefficient alphas were computed for the SYRESS and for each of its factors. The convergent validity of the scale was evaluated by correlating the SYRESS with CD-RISC, WHOQOL-BREF and GHQ-28 using Pearson product-moment correlation coefficients. The scores on the SYRESS were expected to be positively correlated with CD-RISC scores and WHOQOL-BREF scores, and negatively correlated with GHQ-28 scores. The correlations between the SYRESS scores and each of the WHOQOL-BREF domains and GHQ-28 subscale scores were also explored. To examine if the hybrid SYRESS was a more comprehensive measure of resilience than existing established scales, hierarchical multiple regression analysis was performed using the SYRESS and CD-RISC scores as predictor variables and the WHOQOL-BREF and GHQ scores as outcome variables respectively. All statistical analyses were carried out using SPSS 17.0 for Windows.

3. Results

3.1. Preliminary analyses

The Kaiser-Meyer-Olkin measure of sampling adequacy index for the sample was .90 and the Bartlett's test of sphericity was highly significant (R2 = 10586.03, df = 3486, P < .0001).

3.2. Factor analyses

Analysis of the data yielded a total of 10 factors with eigenvalues above 1.0 (See Table 1). Of the 84 items, 34 items were dropped from subsequent analyses because they had loadings of < .30. These procedures resulted in a 50-item scale that accounted for 63.4% of the variance in the SYRESS scores (See Table 1).

Table 1. Eigenvalues and total cumulative variance explained

Factor	Eigenvalue	Total cumulative variance explained (%)
1	16.17	32.33
2	2.65	37.62
3	2.31	42.24
4	2.29	46.81
5	1.72	50.24
6	1.54	53.33
7	1.40	56.14
8	1.30	58.73
9	1.23	61.18
10	1.12	63.42

The pattern matrix is presented in Table 2. The first factor consists of 8 items and accounted for 32.3% of the variance. It represents the individual's perseverance and commitment in the pursuit of goals. The second factor comprises 7 items and relates to the individual's positive self-image and optimism. Factor 3 consists of 5 items and relates to the availability of social support and the individual's ability to seek this support when necessary. Three items load onto Factor 4, which reflects the individual's sense of humour and the ability to think positively. Factor 5 comprises 5 items and reflects the individual's ability to regulate emotions. Factor 6 consists of 6 items and relates to the individual's sense of spirituality and faith. Factor 7 consists of 6 items and relates to a sense of personal confidence and responsibility. Factor 8 consists of 4 items, which reflects a sense of personal control. Factor 9 and 10 consist of each 3 items and relate to the individual's flexibility and coping skills when facing adversity. See Table 3.

Table 2. Rotated factor pattern for the Singapore Youth Resilience Scale

Factor	Item	Item description	Factor loading
1 Perseverance/ Commitment			
	80	I believe I can achieve my goals, even if it is difficult.	.591
	81	I believe by trying hard, things can be different.	.585
	78	When I start doing something I try to finish it.	.490
	79	I know that sometimes I have to make myself do things that I do not like.	.426
	48	Difficult times are an opportunity for me to learn and grow as a person.	.411
	83	I am able to make a decision even when I do not have all the facts.	.373
	46	I am not afraid of challenges.	.345
	71	I put in my best, no matter what the outcome will be.	.334
2 Positive self-image/ Optimism			
	33[a]	I dislike myself.	-.768
	25	I accept myself.	.732
	26	I feel free to be myself.	.710
	13	I feel in harmony with myself.	.466
	57	I am optimistic about my future.	.455
	54	I usually recover quickly after ordinary illness or injuries.	.406
	32	I feel proud about things I have accomplished in life.	.373
3 Relationship/ Social support			
	18	I allow others to help me when I need it.	.731
	17	In difficult times I have at least one close person I can turn to for help.	.670
	16	My family understands how I feel.	.545
	22	I think others find me easy to work with.	.438
	23	I have good friends that I can trust.	.435
4 Humour/ Positive thinking			
	63	I can see the funny side of things.	.862
	64	I can find humor in difficult situations.	.824
	65	I can laugh at myself.	.784
5 Emotional regulation			
	1	I am able to handle unpleasant emotions, like sadness, fear and anger.	.794
	2	I stay calm in difficult circumstances.	.758
	7	I can handle my frustration.	.718
	6	I am able to manage my worries.	.625
	5	I am able to recover emotionally from losses and setbacks.	.377
6 Spirituality/ Faith			
	10	My religious or moral beliefs give me strength and courage for my life.	.761
	12	Good or bad, I believe that most things happen for a reason.	.760
	11	I find strength in a higher meaning when I face problems.	.710
	9	My personal belief gets me through hard times.	.677
	15	I believe my life has meaning and purpose.	.592
	14	Learning lessons from life can bring out the best in me.	.561
7 Personal confidence/ Responsibility			
	3	I think about why I get upset.	.659
	31	I am able to rely on myself when there is no help.	.622
	35	I accept responsibility for what I do with my life.	.531
	53	I would change myself if the situation requires it.	.511
	30	I am confident that I can solve problems in life.	.504
	20	I find strength in my relationships.	.383
8 Personal control			
	73[a]	The problems I have are caused by other people.	-.793
	72[a]	In most situations I worry that something bad will happen to me or those I love.	-.501
	40	When I am under stress I remain calm.	.371
	37	Failure does not easily discourage me.	.317
9 Flexibility			
	45	I can accept it when things are unclear and uncertain.	.831
	82	I do not keep thinking about things I cannot change.	.453
	50	I am able to cope well in unfamiliar situations.	.432
10 Positive coping			
	69	I try to understand the situation before I act on it.	.729
	68	I know which situations I can handle and which I cannot.	.693
	70	I prepare myself mentally when I meet challenges.	.521

Note: Only loadings above 0.3 are shown. [a] reverse scoring

Table 3. Cronbach's alpha for individual factors

Factor	Cronbach's alpha
1 Perseverance/ Commitment	.882
2 Positive self-image/ Optimism	.830
3 Relationship/ Social support	.768
4 Humour/ Positive thinking	.813
5 Emotional regulation	.810
6 Spirituality/ Faith	.859
7 Personal confidence/ Responsibility	.808
8 Personal control	.399
9 Flexibility	.607
10 Positive coping	.835

3.3. Reliability and validity

3.3.1. Internal consistency

Cronbach's alpha for the 50-item SYRESS is .95. With the exception of Factors 8 and 9, the Cronbach's alpha values for the factors are all above .70 (see Table 3).

3.3.2. Test-retest reliability

There is a high level of agreement between the mean SYRESS scores at Time 1: 190.55 (SD: 22.58) and Time 2: 194.45 (SD: 20.42), with a test-retest reliability coefficient of .82 ($P < 0.01$).

3.3.3. Convergent validity

The SYRESS scores strongly correlate with the CD-RISC scores ($r = 0.88$, $P < 0.01$). They also moderately correlate with the WHOQOL-BREF scores ($r = 0.57$, $P < 0$ .01), indicating that a higher level of resilience is related to higher levels of wellbeing. Of the 4 domains, the SYRESS scores most strongly correlate with the psychological domain as measured by the WHOQOL-BREF ($r = 0.55$, $P < 0.01$). The correlation coefficients between SYRESS and each of the WHOQOL-BREF domains are shown in Table 4.

SYRESS negatively correlates with the GHQ-28 ($r = -0.33$, $P < 0.01$), indicating that higher levels of resilience are associated with less psychological morbidity. Of the 4 subscales, they most correlate with the 'severe depression' subscale ($r = -0.33$, $P < 0.01$). The correlation coefficients between SYRESS and each GHQ-28 subscale scores are shown in Table 4.

Table 4. Correlations between the Singapore Youth Resilience Scale (SYRESS) and WHOQOL-BREF, and between SYRESS and GHQ-28

	Correlation coefficient	P value
WHOQOL-BREF		
Physical health	.465	< 0.001
Psychological health	.554	< 0.001
Social relationships	.455	< 0.001
Environment	.511	< 0.001
GHQ-28		
Somatic symptoms	-.247	< 0.001
Anxiety and insomnia	-.232	< 0.001
Social dysfunction	-.289	< 0.001
Depression	-.330	< 0.001

3.4. Hierarchical regression analyses

Results of the hierarchical regression analyses are shown in Table 5. The results indicate that SYRESS accounted for 35.4% of the variance in the WHOQOL-BREF scores, significantly more by 1.8% than that predicted by CD-RISC alone (R2 = 0.336). SYRESS predicted 10.8% of the variance in the GHQ-28 scores. This was significantly more by 3.6% than that predicted by CD-RISC alone (R2 = 0.072).

Table 5. Hierarchical multiple regression analyses using CD-RISC and SYRESS to predict WHOQOL-BREF and GHQ-28 Scores

	Primary predictor	β	ϱ	R^2	Secondary predictor	β	ϱ	R^2
WHOQOL-BREF	CD-RISC	.336	.006	.336**	SYRESS	.279	.023	.354*
GHQ-28	CD-RISC	.062	.596	.072	SYRESS	-.194	.007	.108**

*p < .05
**p < 0.01

4. Discussion

In this study, we developed and tested the internal validity of the SYRESS, a 50-item 10-dimensional resilience scale for use with adolescents in Singapore. The SYRESS demonstrates sound psychometric properties, with good internal consistency and test-retest reliability. The SYRESS strongly correlates (r = 0.88) with another measure of resilience (CD-RISC) and it also relates to higher levels of quality of life and wellbeing, particularly psychological wellbeing, as measured by the WHOQOL-BREF and lower psychiatric morbidity as measured by GHQ-28. The strengths of association with quality of life and

psychiatric morbidity are moderate but expected, and are in line with previous findings of associations between resilience and positive development as well as general health (Alriksson-Schmidt et al., 2006; Haddadi and Besharat, 2010; Lee et al., 2010).

Factor analyses revealed a 10-factor structure that explained a total variance of 63.4%. Perhaps not surprisingly, the factors underlying previously published scales from which some of the items have been used in the present study were found to emerge in the analysis. For example, Factor 1 in SYRESS – perseverance and commitment in the pursuit of goals – is also a factor in CD-RISC (Connor and Davidson, 2003). Social support and relatedness (Factor 3) is a factor in both RSCA (Prince-Embury, 2007) and CD-RISC (Connor and Davidson, 2003). Therefore, the present hybrid scale, SYRESS, is found to encompass all the factors represented separately by other existing scales. This suggests that SYRESS is likely to provide a more comprehensive measure of resilience, reflecting a greater multiplicity of underlying mechanisms of resilience. This is supported by the results from the hierarchical regression analyses. These analyses show that SYRESS significantly contributes additional variance to the prediction of the WHOQOL-BREF and GHQ-28 scores over and above that contributed by CD-RISC alone.

The domains represented by the factors of the resilience construct in SYRESS evidently reflect universal mechanisms and determinants of resilience. Qualities such as perseverance and determination have been found to be salient in helping youths overcome adversities (Smokowski et al., 1999). Self-efficacy and effective coping styles have also been consistently linked to resilience (Campbell-Sills et al., 2006; Carson et al., 1992; Rutter, 1990). Variables such as a sense of humour and the ability to think positively have also been positively related to psychological health (Ciarrochi et al., 2007; Martin et al., 2003). Also, the ability to manage one's negative emotional states and having a healthy selfesteem have been associated with less psychological distress (Kassel et al., 2006; Mäkikangas et al., 2004). Similarly, the relationship between higher perceived social support and better mental health is well-established (Hefner and Eisenberg, 2009; Smokowski et al., 1999), and helpseeking behaviour has been an important resilience strategy (Castro et al., 2010). Self-trust and religiosity have also been found to predict psychological health and adjustment (Ball et al., 2003; van Dyke and Elias, 2007; Johnson, 2004). That the construct of resilience includes universal core dimensions can therefore not be over-emphasized. The contribution of cultural-specific items in the scale to the additional variance due to SYRESS should be discussed. Since no 'new' factor has apparently emerged in the factor analysis, it would appear that the 'cultural-specific' items have either been discarded during the data reduction process, or subsumed under existing factors. The latter appear to be most likely. Rather than any unique cultural domain per se, cultural elements were embedded in the items that were linguistically and semantically validated prior to analysis for the factors representing various domains reflecting universal mechanisms of resilience. However, it is possible that there are cultural-relevant items that were not conceived and included. Also, there may well be a lack of cultural influence because adolescents in Singapore are influenced by Western media and ideals, and Singaporean adolescents have come to adopt many Western perspectives and preferred ways of behaving. However, this does not imply that scales established in a Western context may be directly applied to other local cultural contexts. It is interesting to note that spirituality and faith was a factor that emerged in both the SYRESS and the CD-RISC, but not in either the Chinese version of the CD-RISC (Yu and Zhang, 2007) or in the Resilience Scale for Chinese

Adolescents (developed in China, which assesses adolescents coping with adversities) (Hu and Gan, 2008). The emergence of this factor is reflective of the high level of religiosity in both American and Singaporean societies and cultures. Southeast Asia is the home to most major world religions (Evers, 2004) and Singapore, in particular, is a hub for a multitude of religious faiths (Department of Statistics, 2000). It is not surprising, then, to find spirituality and faith an underlying dimension of resilience in the SYRESS, as it is in CD-RISC (Connor and Davidson, 2003). On the other hand, cross-cultural studies have found that Chinese people from China today do not have strong religious traditions and understandably, the spirituality dimension is absent in these scales (Yu and Zhang, 2007). On the whole, this shows that the underlying mechanisms of SYRESS are still a set of patterns and expressions of resilience that are universal but should be suited for the cultural context of a country.

Some limitations in the study should be noted. First, with a relatively small sample which was recruited from one school, the extent of its generalisability as a measure of resilience is unclear. This should be further investigated in future studies. Second, it is possible that we have missed out other culturally-relevant items. Future studies may adopt an emic approach and generate cultural-specific items from the perspective of youth. In addition, the comprehensiveness of the measure was justified by determining if it makes additional contribution in predicting the scores of the GHQ-28 and WHOQOL-BREF, but this could be done additionally for other correlates of resilience. Further longitudinal studies should validate its use in moderating the effects of life adversities and predicting mental health outcomes.

Notwithstanding the need for further research, the findings from the present study indicate that the SYRESS is a validated tool and a more comprehensive measure of resilience in adolescents. Indeed, as a hybrid scale, the SYRESS has the advantage of encompassing all the underlying mechanisms measured separately by other regular scales but remaining a relatively short and easy scale to administer and score. The findings from the present study also suggest that the resilience construct is multi-faceted and the assessment of resilience should reflect this multiplicity.

8

General discussion

"Helicopter view" by Maas Wentink, 5 years old

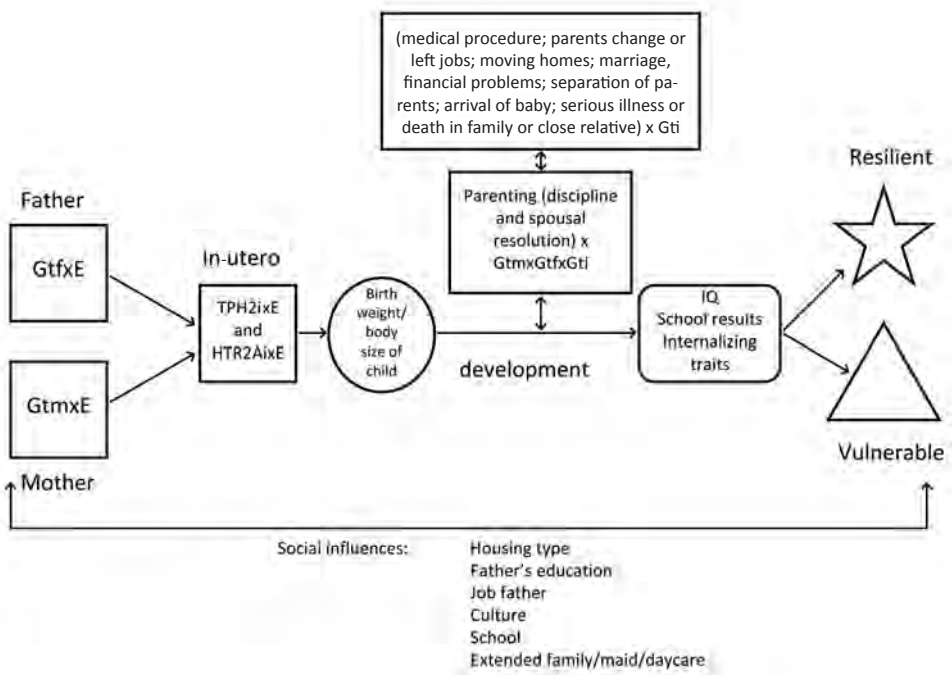
1. General discussion

This thesis consists of studies that attempt to offer a developmental framework to promote greater understanding of common influences and experiences during early life in interaction with genotypes on outcomes later in childhood. In particular how nature (genotypes) and nurture (e.g. environmental risk or protective factors) interact to determine socio-emotional and cognitive outcomes.

The emphasis is on studies done mainly in a general population to examine the impact of (early) life influences in the majority of children instead of children with rare diseases or children living in extreme or aversive situations. We assessed (early) life influences from a neurobiological, social and a psychological perspective using the biopsychosocial model below. Model 2 shows the veriables and results of our studies.

Our studies acknowledge the hypothesis that all experiences during life, including early experiences in-utero, will influence the expression of genes with associated effects on socio-emotional and cognitive outcomes later in life, which determine the vulnerability or resilience of an individual.

In this chapter our findings from clinical, epidemiological and epigenetic studies are discussed, with incorporation of a review of the latest insights gained from relevant research in this field.



Model 2.

Gt = genotype, Gtf = genotype father, Gtm = genotype mother, Gti = genotype infant, E = environment, 'x' = interaction effect, for example: GtxE = interaction between genotype and environment, TPH2i = tryptophan hydroxylase 2 gene of infant, HTR2Ai = serotonin 2A receptor of infant.

1.1. Stress and genetic influences in-utero

Many previous studies have shown that early development in-utero influences later development. For example, in-utero environments, maternal stress and diet, birth weight, and growth by one year of age seem to program offspring growth and metabolic pathways, altering life-long susceptibility to diseases later in life (Bale et al., 2010; Barker 1998, 2007; Fogel, 2003; Gluckman et al., 2005; Gluckman and Hanson, 2005, 2006a, 2006b; Rutter 2006b; Rutter et al., 2006). Although the effect of early in-utero development on physical illnesses was already known, more recent studies relating to psychiatry, psychology and neuroscience have found that early in-utero development also influences the development of vulnerability for mental disorders later in life (Gale and Martyn, 2004; van den Hove et al., 2010; Wiles et al., 2005). For example, a recent study showed an association between smaller gangliothalamic diameters in brains of infants of 6 weeks old and higher internalizing scores on the Child Behavior Checklist (CBCL) at age 18 and 36 months (Herba et al., 2010). Many other studies have described the association between early life stress in-utero and depression later in life (Gale and Martyn, 2004; van den Hove et al., 2006). Hence, an optimal environment in-utero (in interaction with the genotype of the fetus) is important for optimal development, not only physically, but also psychologically (Gluckman et al., 2005).

The stress diathesis model (Francis et al., 1999) proposes that the association between early experiences and health risks later in life, is determined by neurodevelopmental pathways that produce individual differences in neural and endocrine responses to stress (Boer, 2009; Francis et al., 1999; Meaney et al., 2007; Wiles et al., 2005). The hypothesis is that stress in-utero causes excess release of corticotrophin releasing hormone (CRH) as well as cortisol. Increased exposure to these hormones has a negative influence on birth weight and associates with impaired feedback regulation of the HPA axis and serotonin signaling in the key brain areas (Kajantie and Raikkonen, 2010; Weinstock, 2010). This hypothesis has found support in animal studies (Weinstock, 2010). Thus, prenatal stress in rodents results in decreased hippocampal glucocorticoid receptor expression, impaired feedback inhibition and increased HPA responses to acute stress. In line with this, Goland et al. (1993) showed that increased umbilical cord levels of corticosteroids are indeed associated with growth restriction. In conclusion, these findings suggest that unborn babies are capable of making 'life history adjustments' in anticipation to the expected threatening environment by investing less in growth to increase their survival (Bateson et al., 2004; Gluckman et al. 2007; Gluckman and Hanson, 2004).

Our studies in in-utero development showed an effect of fetal growth on Intelligence Quotient (IQ), as well as on socio-emotional traits associated with affective disorders. In chapter 3 we describe our results, which showed a clear linear relation between birth weight, birth length and head circumference and IQ. For children within the normal birth weight range it was found that for every additional kg in birth weight, every additional cm in birth length and every additional cm in head circumference, IQ showed an increase with 0.50 up to 2.70 IQ points in the multiple adjusted model. This suggests that intrauterine growth retardation is associated with a lower IQ. These findings are in accordance with those of other studies in Western countries (Eide et al., 2007; Hollo et al., 2002; Rahu et al., 2010), although our study is the first study to report a positive association with IQ, later in childhood (after 4 years). Interestingly, IQ has been positively related to health later in life (Batty et al., 2007). This suggests that one explanation of the association between fetal growth and health might

be indirectly, through effects on IQ.

Optimal circumstances during early development also seem to be associated with better outcomes in terms of socio-emotional development. Children with a birth weight (corrected for gestational age) just above the mean had lower internalizing scores, modulated by genes involved in the serotonergic system (TPH2, HTR2A). Interestingly, genotyptic variation of TPH2 and HTR2A genes has been associated with an increased risk for affective illness later in life in adults before. Our results suggest that genotypes of TPH2 and HTR2A determine the influence of fetal growth. These findings are consistent with emerging ideas that variation in gene expression might regulate sensitivity to environmental conditions, and that developmental outcomes are context-specific. The hypothesis about change in activity of genes by experiences during life, without alteration of DNA, is seen as 'epigenetic programming' (Meaney and Szyf, 2005).

Another study with robust results showing context-specific effects of genes involved in the serotonergic system is that of Caspi et al. (2003) in New Zealand, who demonstrated in a sample of 847 people that those with a short allele of the serotonin transporter gene were more likely to become depressed when they experienced adverse life events than those with only long alleles. Caspi and his colleagues suggest that the relation between stressful events and depression is modulated by genotypes (long-long, long-short, short-short alleles) of the serotonin transporter gene. A large meta-analysis of 12500 people (including the New Zealand study of Caspi et al.) could not replicate this finding (Risch et al., 2009). However, not all included studies were of good quality. Furthermore, laboratory studies, which were not included in this meta-analysis, have shown that humans with the short allele indeed show greater biological stress responses (Gotlib et al., 2008). In line with this, it has been found that people with the short allele are more vulnerable to disorders such as post-traumatic stress disorder and anxiety (Gotlib et al., 2008).

Epigenetic effects were also demonstrated in an impressive study by McGowan et al. (2009). They examined epigenetic differences in the glucocorticoid receptor promoter in the hippocampus postmortem in suicide victims with a history of childhood abuse in comparison with suicide victims without such a history. The results showed an association between physical and/or sexual abuse and lower expression of the GR gene due to methylation, which inhibits gene expression by blocking transcription factor binding. Decreased function of GR is associated with higher sensitivity for stress and depression (McGowan et al., 2009). This is in line with earlier studies done in rats, which clearly demonstrated the influence of maternal care on hypothalamic pituitary adrenal axis (HPA) function by epigenetic programming of the glucocorticoid receptor expression (Fish et al., 2004). In conclusion, environmental influences lead to alteration in gene expression, either increasing or decreasing the impact of sequence variation.

1.2. Stress during life, resilience and cultural influences

In chapter 5 we describe a study, which examines the influence of a medical procedure on young children. Most children had lesser behavioural and emotional problems after surgery without symptoms of posttraumatic stress, suggesting that these young children are generally quite resilient towards this stressor. There were no significant differences between children with different temperaments, although emotional children did have worse outcomes in general.

Although we initially proposed that children who are highly sensitive to stress do have more behaviour and health problems, Boyce and Ellis (2005) propose that high stress sensitivity is not always negative. They found that children with high stress sensitivity are not only more sensitive to negative experiences, but also to positive experiences. Those children are more likely to develop mental problems when growing up in adversity, while on the contrast, more reactive children were better able to thrive when living in protective circumstances. This suggests that this group of children is more sensitive to the supportive and nurturing qualities of those protective environments (Obradovic et al., 2010). This effect could explain our finding in chapter 5 that high stress reactivity measured by RSA and cortisol was not a predictor in itself, of the response to a surgical procedure. It is likely that other positive influences, such as high quality parental and hospital care, could have had a positive influence on the outcomes. We suggest that more vulnerable children are characterized as those with increased emotional reactivity and environmental adversity, such that emotional reactivity alone is not a sufficient condition for the prediction of vulnerability.

The recent proliferation of research in resilience is not surprising given the well-documented associations between resilience and mental wellbeing. Every individual encounters unavoidable, painful events and hardships in the process of growth and development. The development of an individual is an active process of adaptation within his environment (Nakaya et al., 2006). Resilience is the absence of illness and dysfunction despite exposure to risk. The overarching aim of resilience research has been to increase understanding of underlying mechanisms of resilience to promote resilience-enhancing behaviours, and in turn mental health.

Many studies demonstrate the negative effects of serious life adversity by focusing on low-income children, orphans or abused children. For example studies showed that children living in poverty or children with traumatic experiences were more likely to have problems with academic achievements or social adjustment (Pungello et al., 2010). However, in the study contained in chapter 6 we examined both risk factors as well as protective factors in children from the general population. This study showed that risk and protective factors do have a differing impact on the outcome in IQ and school results along with socio-emotional functioning. Although protective factors (high intelligence, higher education father and occupation father) have an impact on both academic capacities as well as socio-emotional development, risk factors (negative spousal conflict resolution, negative methods of discipline, chronic health problems, negative life events and developmental delay) only influence socio-emotional development in Singaporean children, without having a major effect on academic capacities. These findings parallel those of the effects of poverty in children (Hackman et al., 2010). Thus the quality of the home environment, especially the degree to which it affords cognitive stimulation, predicts academic outcomes. Poverty associates with reduced cognitive stimulation and poorer academic performance. Poverty also associates with marital conflict and parenting problems, such as those described above. Parenting mediates the effects of poverty on socio-emotional, but not academic outcomes (Hackman et al., 2010).

Interestingly, in this study we found some differences in risk factors with earlier findings from the other studies done in European countries. For example, family structure and surrogate caregiving were relatively less important risk factors than in Europe and the USA. This might be related to differences in culture, as Singaporean children are more often raised with surrogate caregiving, by living-in grandparents and/or domestic helpers, while in the USA and Europe parents more often use daycare for children.

Previous studies have found that the social context can influence behaviour directly, as it influences individual beliefs and norms (Pasick et al., 2009). This finding underscores that resilience is a contextual concept and measuring resilience should be culturally sensitive. Although resilience is a worldwide concept and mechanisms and determinants of resilience are universal, local cultural influences cannot be neglected. In chapter 7 we developed a resilience scale (SYRESS) for Singaporean adolescents, in addition to the existing resilience scales developed in the USA and Europe. The fact that patterns and expressions of resilience are context-dependent is discussed. Although the domains of the SYRESS evidently reflect universal mechanisms and determinants of resilience, our findings show that the SYRESS is a more comprehensive resilience scale than existing resilience scales for Singaporean adolescents.

2. Conclusions

In conclusion, research in stress and resilience should always take contextual factors into account. Genetic forces do not operate independently of environmental forces (Meaney, 2010). That means that studies need a dialectical perspective of interconnection between biology and environment, including cultural differences (Sameroff, 2010). This supports a need to understand epigenetic mechanisms as a critical determinant for mental health predisposition. Nature and nurture interact to shape the unique human individuals. A developmental approach is useful to explore those influences.

3. Implications

Understanding the interactions between genotypes and environment during development on cognitive and socio-emotional outcome later in childhood is important for clinical practice as well as public health.

When a child or adolescent is suffering from a mental disorder, parents tend to blame the child: why is our child not listening? Why is he so naughty? Alternatively, many professionals may first react by blaming parents: why is this child not listening, what is wrong with their parenting?

However, it is not an issue of blame, as it is about the interaction between the characteristics of the child as well as the direct environment during their life: "It is both the characteristics of the child and the parents, the neurons and the neighborhoods, the synapses and the schools, the proteins and peers and the genes and government", as Sameroff explains in his article (2010). Adopting a life span perspective will help to emphasize the multiple factors during life which influence the development of the child and help to diagnose problems. The interconnection between genotypes and environment has to be implemented in clinical sciences of psychiatry and psychology. Hence, targets for intervention should focus on child, parents, and the environment.

Experiencing adversity early in life does not always cause developmental problems. The process of interaction between genes and environment is not static. Children have the potential to change and recover. Protective factors are therefore critical to identify and to exploit in the development of new and more effective therapies. Cognitive, temperamental,

health or emotional shortfalls can be compensated for by strengths in other dimensions, and skills associated with positive adaptation can be cultivated and practiced (Richardson, 2002; Richardson and Waite, 2002; Waite and Richardson, 2004). For example, research in neuroscience has shown the enormous plasticity of the prefrontal cortex, which is important for emotion and self-regulation, into the early twenties (Dahl, 2004). Furthermore, although IQ seems to be rather stable from 10 years onwards, there is evidence that interventions during adolescence can affect neurocognitive skills (Cunha et al. 2006). Other studies showed that effects of poverty on emotional and cognitive development can be mediated by parental factors (Conger et al., 1994), and that IQ is subject to the effects of early intervention programs (Ramey et al., 1979).

Although we did not focus on parenting in our studies, it is clear that the role of parenting should not be underestimated. In humans and animal models, both the quality and quantity of early-life maternal care showed to be a predominant signal triggering changes in activity of genes including genotypes involved in the HPA axis, associated with the development of resilient or vulnerable phenotypes. Fish et al. (2004) showed the importance of maternal licking and grooming in mother rats for somatic growth and stress sensitivity in their offspring. In humans, it is known that maternal stress predicts the cortisol levels of the child (Lupien et al., 2000). This finding suggests that parenting skills can influence the activity of genotypes that regulate stress responses and neural development directly, making the child more or less vulnerable to stress. At the same time it is implying that high quality parenting can improve the stress reactions in children by regulating the activity of genes. Indeed, studies have shown that for example hippocampal volume is influenced by parental care, and that hippocampal development remains plastic until late childhood/adolescence, with considerable evidence for reversibility (Belsky and de Haan, 2011; Bredy et al., 2004; Buss et al., 2007).

In conclusion, genotypes influencing neural and endocrine stress responses are 'plastic'. Therefore the impact of prenatal adversity on neural development can be modulated by the quality of postnatal care. This obviously has implications when targeting clinical treatment programs. Parent-child interventions emerge as a target for prevention in high risk populations. Studies have shown that these interventions can be effective (Ramey et al., 1979). Interestingly, these parent-child intervention studies also showed G x E effects. For example Bakermans-Kranenburg et al. (2008) showed that video-feedback interventions, to promote positive parenting and sensitive discipline, were effective in decreasing daily cortisol in children with the dopamine receptor D4 (DRD4) 7-repeat allele, but not in children without DRD4 7-repeat allele (Bakermans-Kranenburg et al., 2008). They also discussed the role of oxytocin and serotonin genes on sensitive parenting and social interactions with offspring (Bakermans-Kranenburg and van Ijzendoorn, 2008). Another study suggests that oxytocin might be even useful in treatment of stress symptoms as it reduces the fear response (decreasing amygdala activation, inhibiting fear response, and enhancing extinction learning) and increases social interaction (activating social reward-related brain regions increasing engagement in the therapeutic alliance) (Olff et al., 2010).

Other helpful clinical interventions can focus on 'building resilience', teaching children stress management skills by adopting problem-focused, cognitive-focused and emotion-focused coping techniques to manage problems.

The idea of a life span approach to psychiatric disorders is also of huge potential importance for public health policy. The concerns of society have always been put in a life-span perspec-

tive. Healthy productive adults are needed to continue a successful culture and economy. Whilst scientists will study cognitive and emotional development for an intellectual interest, governments are interested in the same studies to prevent behavioural problems that are costly for society. Life-time studies are thus often well supported by governments; evidence based interventions are needed to improve life outcomes for human resources. Research in developmental psychology aims to examine origins of individual differences in emotions and behaviour, which is the basis for extensive intervention or prevention programs for mental health problems (Fisher et al., 2000). However, these programs for prevention of mental health problems and resilience building will need to be valued in different cultures and environments. For example, a study showed that behavioural inhibition of toddlers was positively associated with mothers' punishment orientation towards the child and negatively associated with mothers' acceptance and encouragement of achievement towards the child in a Canadian sample, while these directions of associations were opposite in a Chinese sample. Interestingly, in the Chinese sample behavioural inhibition was associated positively with mothers' warm and accepting attitudes towards their children and negatively with rejection and punishment of their children (Chen et al., 1998). Another example of the important influence of the environment on traits is provided by Kerr et al. (1997). They showed that the best trait for survival among males living in high criminal societies was behavioural inhibition, while behavioural inhibition is associated with affective disorders in more safe societies. This finding suggests that temperaments of individuals show different adaptations in different environments and across cultures in particular (Chen et al., 1998). Before developing an intervention program for resilience building (like improving coping skills), it is important to explore the factors associated with resilience in children from different countries and raised under different environments.

One way to prevent psychopathology is by reducing important social risk factors for a given environment, such as poverty and low quality education. Remediation can be offered to disadvantaged children by governmental actions. A study by O'Connor et al. (2000) showed in a study of adopted Romanian infants reared in severely deprived orphanage environments, that the later an orphan was adopted, the lower was his cognitive performance as an adult. Also secondary school classroom remediation programs designed for children with cognitive deficits showed poor outcomes, while empirical literature shows high economic returns for remedial investment in young disadvantaged children (Cunha et al., 2006). This implies that remediation and help for reducing social risk factors should be done as early as possible.

Furthermore socio-emotional skills should be promoted. It is known that socio-emotional skills also foster cognitive skills and promote healthy behaviours. Especially emotionally nurturing environments will produce more capable learners, which is important for human resource (Cunha and Heckman, 2007; Schweinhart et al., 2005). This suggests that family or parenting interventions in disadvantaged families can improve socio-emotional and cognitive function in children.

National interventional programs can be used for resilience building. For example the "Understanding the Adolescent Project" in Hong Kong (Lee et al., 2007; Lee, 2006; Shek et al., 2006; Shek, 2006) seeks to influence problem-solving skills, effective communication with peers and adults, techniques to create win-win situations, and the early identification of the elements of a stressful situation with encouraging results. In the Netherlands several resilience building programs are well accepted. One example is the 'Marietje Kessels Project'

for children of the normal population of 10 to 13 years old, which is given in 12 sessions during school times with the purpose to increase resilience by increasing awareness of abuse and unacceptable behaviour. Research showed that it has a positive effect on resilience building of children (van Helvoort and Clarijs, 2005; van der Vegt et al., 2001).
However, policy should not only focus on improving childhood circumstances, but also on interventions to improve early growth by improving obstetric and neonatal care. This should be available for all mothers, regardless of their socioeconomic status. As mothers of lower socioeconomic status are at risk for less optimal nutrition and lower birth weights (Freisling et al., 2006; Ricciardi and Guastadisegni, 2003; Watson and McDonald, 2010), it is extremely important that this group is included as well. Good infant and maternal health can have a significant positive impact on the future health and wellbeing of offspring. Birth weight of a child is widely accepted as a key indicator of infant health. Birth weight can be affected by a large variety of factors, including age, size, health, stress level and nutritional status of the mother and the use of toxins, which all have a direct influence on the quality of the in-utero environment. Some of these factors can be influenced. Olds et al.(2002) describe perinatal interventions that reduce fetal exposure to alcohol or nicotine with long-term effects on cognition, socio-emotional skills and health in offspring (Olds et al., 2002). Other preventive options to think of are offering nutrient supply, education for mothers-to-be and regular checks during pregnancy on the growth of the baby by scans etc. The evidence for interventions in low-birth-weight children so far suggests that early interventions can be effective (Bonnier, 2008).

4. Strengths

A major strength of the studies in this thesis is that most studies are done in the general population. Previously many studies focused on difficult or disadvantaged circumstances or children with a psychiatric disorder to examine factors relating to genotypes, stress and resilience. It is therefore not easy to generalize these results derived from 'populations at risk'. For example it is clear that low birth weight carries an increased risk for developing mental disorders (Schmidt et al., 2010). However, in this group of children there are multiple pathophysiologies that can arise that do not inform the extent to which the fetal environment within the normal birth weight range can affect children. Our studies in early development show that there is also a clear influence of birth parameters within the normal birth weight range on cognition and, in interaction with genotypes of the serotonin system, on socio-emotional development. We used large sample sizes and well validated instruments and made use of multiple birth parameters.
In our G x E study we used relatively large genetic subgroups, which might have helped us to find independent effects of each genotype. This made it possible to study interaction effects as well, which will be more difficult to demonstrate in smaller subgroups. We selected only polymorphisms with evidence of functionality in previous studies. It is likely that the power was sufficient to detect a large effect size and it is unlikely that the number of significant findings can be explained by statistical chance. On top of that, our results of the involved genotypes which showed a vulnerability to develop internalizing traits, are in accordance with earlier studies which showed associations between those genotypes and af-

fective disorders later in life.

To understand differences in the stress response of children, it is important to be able to study this within the same environmental conditions. For traumatic and stressful experiences, this is obviously a challenge. In our semi-experimental study for children with an indication for an adenotomy or adenotonsillectomy we used a standardized surgery. Subsequently we were able to do pre-assessments of physiological, temperamental and psychological factors as well as post-assessments to study differences in the stress response in a prospective way. We also included physiologic measures, which allow the direct measurement of the emotional state instead of being dependent on indirect measuring through parent report.

While previous studies most of the times focused on older children, adolescents and adults, we examined children of different age groups including pre-school children. The impact of stress will differ according to the developmental stage (Boer et al., 2009). In preschool children more often externalizing symptoms are reported aside from internalizing problems in reaction to a traumatic experience, while in middle childhood and adolescence mainly internalizing problems are reported (Boer et al., 2009). However, studies done in children of pre-school children are rare and rely most of the times on parental report only (Boer et al., 2009). In our ENT study we were able to use more objective measures in addition to the subjective parental report. In this study of young children (M = 3.7 years, SD: 1.5) we found no indications for the development of externalizing or internalizing problems. However, in our resilience study in chapter 6 we examined older children (M = 9.4 years, SD: 1.7) with indeed more children scoring in the clinical range of the internalizing problems (15.1%) than externalizing problems (7.2%).

A strength of our resilience study (chapter 6) is the inclusion of protective factors. Positive factors are often neglected in studies. Combining both risk and protective factors allows improved understanding of 'real life' influences on socio-emotional and cognitive outcomes in children. Another strength of this study is the objective measurement of academic results by performance scores received from the schools.

Finally, an important strength in this thesis is that studies were performed in different cultures and by doing so, we became more aware of the influences of culture on (the measurement of) stress experience and resilience. For example, IQ was measured with the Raven Progressive Matrices, as this is the IQ test which best excludes differences in language and culture. Second, in the epigenetic study, an ethnically homogenous sample was selected. Only Chinese children who had a mother as well as a father from full Chinese heritage were included to exclude any influences of ethnicity on the outcome. Furthermore, in the study of development of a resilience scale, important similarities as well as differences for the concept resilience worldwide were discussed, which has implications for measuring and comparing issues of resilience worldwide.

5. Limitations

In general in all our studies selection biases, loss to follow-up, and demographic differences between included and excluded children could have influenced outcomes. An example of indications for a possible sample bias can be found in the study of early life influences on IQ in chapter 3. The average IQ was relatively high and even the relatively lower birth weight

and shorter birth length siblings had higher IQ's than expected in a general population. An example of differences between included and excluded children was shown in the resilience study in chapter 6, in which the non-responders showed a lower socioeconomic status. This makes us wonder if this sample is representative. Although most studies used a reasonable to large sample, in the ENT study (chapter 5) the number of examined children was limited. Artifacts in neurophysiologic measurements and missing data in this study further reduced the numbers. This could have influenced the power to detect differences between different temperaments and the outcome after surgery.

Studies used self-report questionnaires of behaviour, which render subjective information. For younger children parental self-report was used, in elder children aside from self-report, reports from teachers and school results were included to increase objectivity. In the studies using the CBCL, differences in scores of the CBCL seem not to be that large. However, the CBCL is a subordinate scale, with more differences in scores in the higher range. Our results show an expected percentage in the clinical range, conform previous studies.

Some of the instruments used were not yet validated for the particular cultural population, for example the CBCL norms are validated in the Netherlands, but still need to be evaluated in Singapore. Previous studies done in Asia found different cut-off scores for the CBCL and TRF. Another limitation is that the Raven's Progressive Matrices does not cover such a complete IQ score as can be obtained from other current full batteries, but focuses more on IQ domains of abstract reasoning. On the other hand, the RPM is a well chosen instrument as it is a nonverbal IQ test which minimizes cultural influences, which is needed in a multicultural population such as Singapore. Another limitation is that IQ was measured in early to middle childhood. However, IQ in early to middle childhood is not a strong predictor for later IQ. IQ scores become stable by age 10, suggesting a sensitive period for development of IQ below the age of 10 (Schuerger and Witt, 1989). Previous studies have shown that the association between birth weight and IQ becomes weaker later in childhood, probably due to other factors which play an increasingly important role in the development in cognition of children (Boomsma et al., 2001; Tong et al. 2006). However, we included sibship (the total number of children produced by a pair of parents) analyses to at least exclude the influence of familial environment.

We did not explore differences in gender, with exception of the prospective ENT surgery study. Previous studies, however, have shown that gender is an important factor in stress responses. For example, a recent animal study found that prenatal stress was associated with a clear increase in anxiety-and depression related behaviour in male Sprague-Dawley rats, but not in female rats (van den Hove et al., 2010). In humans it was found that females showed increased depressive symptoms when born at low birth weight, while boys did not (van Lieshout and Boylan, 2010). Also, low birth weight seems to be associated with higher autonomic nervous system response in females, and with higher peripheral vascular resistance and HPA responses in males (Kajantie and Raikkonen, 2010). In our ENT surgery study we found indications for a difference in response between boys and girls. Although the number of girls was small, girls showed more internalizing scores after surgery in comparison to the Dutch norm group, while boys showed more improvement in behavioural and emotional health after surgery. It is well established that gender has an influence on the prevalence of many mental health disorders in adults, with women generally being more prone to internalizing problems such as anxiety and depression and men being more prone

to externalizing problems and substance abuse (Pratchett et al., 2010; Seedat et al., 2009; Smith et al., 2008). Even small gender differences have been found in childhood affective disorders (Franić et al., 2010). Gender specific acute psychobiological reactions to stress and gender differences in perception of control and social support have all been described as important factors in stress reactions (Olff et al., 2007; Zahn-Waxler et al., 2008).

Most of our studies are limited by the chance of residual confounding as it was not feasible to include all environmental factors. Even in the resilience study, in which we included a reasonable number of protective and risk factors. For example, in this study we did not include temperament of the child, although a difficult temperament can have worse outcomes in presence of family conflict, while family conflict has no effect on children with an easy temperament (Rutter, 1999). Another important factor which was missing in our studies was parenting. For example the effect of children's birth weight on the development of psychiatric disorders has shown to be moderated by maternal warmth in low birth weight children (Tully et al., 2004). In line with this, it is known that the association between low birth weight and major depressive disorder is higher in children of depressed parents, which suggests that parental depression may augment the impact of birth weight on offspring (Nomura et al., 2007). Also the duration of breastfeeding has been described as a protective factor against behavioural problems (Golding et al., 1997). A magnetic resonance imaging (MRI) study has shown that parenting modulated the association between birth weight and hippocampal volume in women (Buss et al., 2007).

Although we acknowledge that many more factors are important, the factors are exhaustive and it was not feasible to include them all.

In the resilience study, we assessed risks and protective factors in the whole sample. As a consequence it is unclear if certain children indeed suffered from adversity that carry a markedly increased risk for development of psychopathology, which made it hard to quantify resilience in this study. Besides that, the environmental influences we measured in this study can be part of the same continuum instead of separate entities; in other words the same environmental experience can be a potential protective or a potential risk factor. Another weakness of this study is that we did not correct for multiple measurements.

In chapter 2 a review is presented of candidate genes which might contribute to PTSD. Although important, it only shows candidate genes which have been examined before. Furthermore genotypes can only be evaluated in the context of their environment. The G x E study described in chapter 4 certainly did not cover all genes, which results in a possible genetic bias: some variations remain hidden in the present investigation, such as gene-gene interactions or influences of other genotypes. Secondly, we need to be aware that genetic and environmental risk factors are not independent entities. Bad experiences are not randomly distributed in the population (Rutter, 1999). Individuals shape and select their own experiences determined by the cognitions and behaviour of individuals. For example, individuals who exhibit high levels of internalizing traits may experience relatively more stressful life events, which are partly genetically mediated (Silberg et al., 1999). Also parenting includes both genetic and environmental influences, as parents not only shape their children's upbringing but also pass on their genes (Rutter, 1999). At the same time children will have an influence on their environment (parents) as well (Rutter, 1999). For example the temperament of the child might influence the expression of genes in the mother, and as such influence the behaviour of the mother.

Another important limitation is the fact that a replication study for our findings is lacking. Our genetic findings are only based on one study and were not confirmed in another population. Further research is needed to prove these findings. Finally, the statistical approaches to study G x E interactions are complicated and not yet fully developed. There has been debate about the adequacy of the use of ANOVA models in detecting G x E interactions (Meaney, 2010; Wahlsten, 1990).

In the studies of stress in-utero and stress early in life we did not examine 'timing' of sensitive periods. However, timing seems to be important. For example, there is compelling evidence for critical and sensitive periods in development. This suggests that some traits or skills are more readily acquired at certain stages of childhood than other traits (Knudson et al., 2006). Davis and Sandman (2010) showed that high maternal cortisol in early pregnancy had a negative outcome on mental development at age one, while high cortisol late in gestation was associated with accelerated cognitive development and higher mental development at age one (Davis and Sandman, 2010). This highlights that stress later in pregnancy can even have positive effects. In conclusion, it is not only the exposure to stress in-utero but also the timing of exposure that appears to be relevant. In the studies we used only birth parameters (corrected for gestational age) as a reflection of the environment in-utero. As a consequence, we were not able to postulate further on sensitive periods. Of note, the finding that birth length was correlated with a higher IQ in our study in chapter 3 underscores that timing is indeed important for cognitive development, as birth length mainly increases in the second trimester. This midgestation phase of pregnancy is indeed known for development of cortical layers that participate in higher cognitive functions.

In the studies all ethnicities in Singapore and the Netherlands were included. However, for the genetic study as well as the study of the development of the resilience scale, data were only evaluated among Chinese subjects. It would be interesting to determine whether there are any differences among other ethnic groups in Singapore. Also, because the study was carried out among Chinese adolescents in Singapore, the extent of its generalizability as a measure of resilience in Chinese adolescents in other Asian countries is not known. These should be further investigated in future studies.

6. What's next? Future studies…

We still have to learn if psychopathology can be explained by taking a life span approach, however, recent research shows strong evidence. This research has shown that early life, in-utero and in childhood, is a period of unique sensitivity that, in interaction with genotypes, has long lasting effects throughout life. However, the mechanisms for these effects remain almost as much a mystery today as a century ago. Research in the area has given us a lot of information, but also raised many new questions. This shows us that most of these interactions are still not well understood.

As development is complex and challenging to study, study models should be sophisticated enough to a good understand of what is going on 'in real life'. As Albert Einstein formulated many years ago: "Everything should be as simple as possible, but not simpler"! This is reflected in 'new science', which uses multidirectional models instead of unidirectional ones, with growing emphasis on G x E interactions, epigenome-experience transactions and

brain plasticity. These advances are based on the interconnectedness of genes and environment. Combination of behavioural science and physical science are needed to capture the complete processes underlying developmental change. As a consequence, interdisciplinary collaborations are required for developmental research.

Together with all the previous data in this area, hopefully our previous and planned studies will add to more evidence in the field of developmental psychopathology.

In the Singapore Cohort Study of the Risk factors for Myopia (SCORM) database we are still analyzing data to reveal more G x E interactions. As (early) stress has been associated with changes in the HPA axis, we are currently examining associations between birth weight and socio-emotional development of children in interaction with genotypes of candidate genes involved in the HPA-axis.

Moreover, a follow-up study with the SCORM population has recently started. The children included in this sample are now around 18 years old. In this follow-up study participants are asked to fill out questionnaires, such as the Youth Self Report, the Parental Bonding Instrument, the Parental Authority Questionnaire, the SYRESS resilience scale and the Academic Expectations Stress Inventory (Ang and Huan, 2006). This study will inform us about the socio-emotional development of the children during adolescence and will add important data relating to parenting and academic stress factors, which are currently missing in the data of SCORM. With these data we may be able to assess if parenting styles modify the association between early life experiences and socio-emotional outcomes in children.

At the same time a study is examining the economic outcomes of SCORM. This is part of a larger worldwide project in which the International Healthy Start to Life Project (IHSLP) is included with Prof. Gluckman and his researchers as well as the team of Nobel laureate James Heckman, who developed technology of capability formation. They will examine the cost-effectiveness 'to invest' in early life of children in different developing as well as developed countries over the world. Genetics as well as many biological and social factors will be included in the model. This study, in which the economic costs (in dollars) of early life differences between countries will be made clear, will translate research into practical models for governments. They will also examine when it is more effective to intervene, during early growth versus later in childhood, by defining critical and sensitive periods for investment.

Furthermore, a large translational and clinical research (TCR) flagship program called Developmental Origins: Singapore (DevOS) has been launched to specifically assess the health problems that exist in Singaporeans, especially from pregnancy to infancy as well as adulthood (website: http://www.devos.sg/). The main study under DevOS is Singapore's largest and most comprehensive birth cohort study "Growing Up in Singapore Towards Healthy Outcomes" (GUSTO) (website:http://www.gusto.sg/). GUSTO is a long-term unique cohort study involving Singaporean mothers-to-be aimed at discovering effective prevention and early intervention strategies to reduce the burden of metabolic diseases in Asia and thus providing valuable information about the 'Asian phenotype'. The cohort was completed by September 2010, in over a year, with 1234 eligible mothers who had signed up and 502 babies who have been safely delivered. Many measurements are used, inclusive regular early growth scans in-utero, DNA, MRI studies of newborns, as well as childhood environmental predictors of development and childhood health and growth and behaviour after birth. From this study an enormous amount of data will be provided to continue epigenetic studies examining associations and interactions between early growth,

genotypes and socio-emotional and cognitive outcomes in children. It will help to identify factors that may affect brain development and to create better early intervention programs for children who need them.

Finally, it would be important to collaborate with researchers over the world, to use data of other large birth cohort studies to replicate and compare outcomes, especially when genes are involved.

As described, most of our planned future studies show the integration of behavioural and physical science. Although this is in line with recent developments, for clinicians the importance of the inclusion of 'soft science' need to be underscored: as Greben has stated that "…in times when the pendulum of psychiatric thinking swings more toward the biological and away from the psychological, it is essential to remind ourselves that helping people can never be divorced from listening to and talking with them" (Greben, 1992).

9

Summary

"Print" by Lucas Leutscher, 3 months old

1. Background

This thesis describes a variety of studies using a developmental framework to promote greater understanding of the interaction between genotypes and environmental influences on outcomes later in childhood. We assessed early life influences from a neurobiological, social and a psychological perspective by using a biopsychosocial framework described in chapter 1.
The origins of mental disorders arise often in childhood. Early life is a period of unique sensitivity with long lasting effects on mental health. However, the mechanisms for these effects remain unclear. Biological and environmental factors will naturally be involved. Environmental influences include demanding stressful events from in-utero to adulthood. Individuals are considered resilient when they adapt well, without developing psychopathology or health problems despite experiencing adversity. Although stress reactions can be useful to adapt to situations, prolonged or severe stress reactions can be maladaptive. For example, prolonged stress responses can lead to symptoms of posttraumatic stress and depression, and/or can have an influence on cognitive functioning. During stress, two different physiological stress systems are involved; the sympathetic adrenergic medullary axis (SAM) and the hypothalamic pituitary adrenal axis (HPA).
In this thesis a developmental approach was used to understand how nature (genotypes) and nurture (e.g. environmental risk or protective factors) interact to determine developmental outcomes. The influences of stress in-utero and during early life in interaction with genotypes on socio-emotional and cognitive outcomes are studied in different age groups and of different cultures. Additionally a 'resilience questionnaire' was developed for children in an Asian population.

2. Research questions

With our studies we tried to answer the following questions:

1. What are common candidate genes which, in interaction with environmental influences, have been associated with prolonged stress reactions leading to Posttraumatic Stress Disorder?
2. What is the influence of the interaction between variation in genes involved in the serotonergic system and the environment in-utero (reflected in birth weight corrected for gestational age) on internalizing traits in children (measured with the Child Behavior Checklist, CBCL) at age 8 to 12 years?
3. What is the influence of the early environment in-utero (reflected in weight, length and head circumference of the newborn at birth, corrected for gestational age) on IQ in children (measured with the Raven's Progressive Matrices) at age 8 to 12 years?
4. What is the impact of a standard surgery early in life on the stress response of young children? Are temperamental traits or neurophysiologic characteristics of the child predictors of the stress response?
5. What is the effect of common risks and protective factors during childhood on the outcome of children in terms of socio-emotional development (measured with the CBCL and TRF) and academic outcomes (school results and adaptive functioning) at age 8 to 12 years old?
6. What is the need for developing a comprehensive cultural-sensitive resilience scale and what are the similarities and differences between resilience scales originating in Europe and the USA, and Asia?

3. Summary of the results of the studies

3.1. Studies in genetic vulnerabilities

In chapter 2 a review is given of candidate genes, which in interaction with environmental factors, are associated with Posttraumatic Stress Disorder (PTSD). This study was the first study to give a full overview of candidate genes related to PTSD found with a literature search in Medline, Embase and Web of Science. It shows that – in contrast to many other psychiatric disorders – no extensive genetic studies have been performed on PTSD. Key candidate genes in the serotonin, dopamine, glucocorticoid, GABA, apolipoprotein, brain-derived neurotrophic factor and neuropeptide Y systems are discussed. The results indicate that the serotonin transporter gene possibly plays a role in the degree of response to stressful events, in particular in the sensitivity of individuals to the depressogenic effects of stressful life events. The studies on other candidate genes show inconsistent results, probably due to methodological shortcomings. Gene x environmental (G x E) studies will be needed to fully understand the role of these genes in different environments.

In chapter 4 we describe a G x E study done in a sample of 545 healthy Chinese children from age 8 to 12, recruited from 3 different schools in Singapore. In this study the influence of birth weight corrected for gestational age (as a reflection of the environment in-utero) in interaction with variation of genes involved in the serotonergic system on internalizing traits is examined. Gestational age was calculated from mothers' last menstrual date and confirmed by fetus' crown rump length during the first trimester ultrasonography. Birth weight data were obtained from medical record booklets containing data recorded at parturition. Internalizing traits were measured with the Child Behavior Checklist (CBCL). After correction for multiple confounders, of a total of 9 examined single nucleotide polymorphisms (SNPs), significant interactions are found between birth weight (corrected for gestational age) and 2 SNPs of the *TPH2* gene, which are in high linkage disequilibrium with each other (rs2171363, P = 0.008; rs7305115, P = 0.007), and 2 SNPs of the *HTR2A* gene (rs2770304, P = 0.001; rs6313, P = 0.026). The CC genotype of *TPH2* rs2171363, GG genotype of *TPH2* rs7305115, CC genotype of *HTR2A* rs2770304 and CC genotype of *HTR2A* rs6313 were associated with reduced internalizing scores in children born in the quartile above the mid-point within the normal range for birth weight. In conclusion, this study shows that the effects of fetal growth on socio-emotional traits that associate with affective disorders are modulated by genotypes of the TPH2 and HTR2A genes, suggesting that variation in genes involved in the serotonergic system determine the influence of fetal growth. Second, the results reveal a fetal growth x genotype interaction effect that results in significantly reduced levels of internalizing scores associated with birth weight lying above the average within the normal range. Third, and perhaps most strikingly, 3 of the 4 genotypes that interact with birth weight to reduce internalizing scores are those that in adult populations associate with an increased risk for affective illness. These findings suggest that genetic variants that statistically associate with an increased risk for affective illness might act to enhance sensitivity to environmental context, thus explaining bi-directional outcomes. These findings are consistent with emerging ideas (e.g. Boyce and colleagues) suggesting that effects of such genomic variants may influence environmental sensitivity resulting in context-specific developmental outcomes. Strengths of this study include the

large sample of Chinese children, selected polymorphisms with evidence of associations with affective disorders, and exclusion of important confounders such as maternal smoking. Limitations include missing out other possible restraints of fetal growth because they were not measured (e.g. use of alcohol), the reliance on parental report, and the possibility of undetected variations of gene-gene interactions.

3.2. Studies of in-utero influences

Studies on (very) low birth weight and premature children showed that Intelligence Quotient (IQ) is consistently correlated with birth weight. However, within the normal birth size range, this association has been less conclusive. Population-based studies with large sample sizes are rare, primarily conducted in European-derived populations, and sometimes only done in male participants.

In chapter 3 we describe a cohort study of 1979 Singaporean children, who attended 3 different mainstream schools, which examines the influence of the environment in-utero (reflected by birth weight corrected for gestational age) on IQ at age 8 to 12 years. Birth data were abstracted from children's medical charts. IQ was measured using the Raven's Progressive Matrices. The results show that for every 1 kg increase in body weight; the model predicts a 2.19 ($P = 0.007$) increase in IQ score; for every 1cm increase in length an increase of 0.49 points ($P < 0.001$) in IQ score and for every 1 cm increase in head circumference an increase of 0.62 ($P = 0.003$) in IQ score. These associations persist even after adjustment for multiple confounders, and after exclusion of premature children and children with extreme weights and head circumferences. An analysis of a sub-sample of siblings shows that the taller sibling (at birth) has significantly higher IQs than the shorter sibling as well. In sum, the results show that improved fetal growth predicts increased IQ at school age across the entire population. The implications are important as such findings emphasize the importance of prevention of not merely conditions that severely constrain fetal growth, but rather the entire range of factors that influence maternal-fetal health. The major strengths of this study are the use of multiple birth parameters as surrogates of fetal growth, the large sample size with high follow-up rate, the sibship analyses to exclude important family environmental factors and the use of a well-validated IQ test, minimizing the effects of language and culture. Limitations include possible selection biases due to loss to follow-up, missing birth parameter data, and residual confounding as some other covariates of interest were not measured.

The study in chapter 4 shows the association between the early environment in-utero in interaction with genotypes of the serotonergic system on socio-emotional development. This study is described in more detail under 'studies in genetic influences'. Although the association between birth weight and IQ shows a linear trend, the association between birth weight and internalizing traits shows a non-linear relationship, modulated by genotypes.

3.3. Study in stress during early childhood

Children react differently to a stressor such as a medical procedure. Prospective studies, which examine predictive factors for differences in stress responses to a surgery, are rare. In chapter 5 a prospective cohort study is described in which the influence of a standard medical procedure (adenoidectomy or adenotonsillectomy – A&ATE –) on the stress response is performed in a Dutch sample of 43 children of age 2 to 7 years. It was hypothesized

that temperamental traits and neurophysiologic characteristics of the child have a predictive value on the outcome after A&ATE. This study reports child behaviour and neurophysiologic characteristics in young children before and after surgery, to investigate individual differences in the stress response measured in a prospective way.
Four weeks before surgery parents completed questionnaires on temperamental traits of their child (EAS) and the CBCL (measuring behaviour and emotion). Baseline neurophysiologic measurements, cortisol (measured in saliva) and respiratory sinus arrhythmia (RSA, derived from heart rate variability measured with electrocardiography) were performed 4 weeks before surgery. Directly after surgery and 6 weeks post-surgery cortisol and RSA measurements were repeated to measure the neurophysiologic stress responses. Six weeks post-surgery the child version of the Impact of Event Scale and the Child Sleep Habit Questionnaire were given to parents to measure respectively posttraumatic stress symptoms and sleep problems, and the CBCL was repeated to measure behaviour and emotional changes. The results show that A&ATE is not very stressful for most children. It seems to be a helpful procedure to reduce pre-existing behavioural and emotional and/or sleep problems, in respectively 75% and 68% of the children, especially in boys. Posttraumatic stress symptoms are rare. In contradiction to earlier findings there is no association between shyness and the stress response. Our findings do not show a predictive value for neurophysiologic parameters. However, more research will be needed to examine the role of emotional temperament, which is associated with more behavioural problems before surgery ($r = 0.53$, $P = 0.02$), after surgery ($r = 0.38$, $P < 0.000$), lower cortisol directly after surgery ($r = -0.49$, $P = 0.05$) and lower RSA at follow-up ($r = -0.33$, $P = 0.06$). More research is also needed to explore the role of gender. Girls and boys show different outcomes in this study, with a larger benefit of A&ATE for boys, however the number of girls was small. Strengths of this study include the prospective design and the inclusion of physiologic measures, which allow direct measurements of emotional states of children, aside from self-rating parental psychological questionnaires. Limitations of this study include missing data reducing the sample size, and the possibility of confounders, as we did not include other important covariates such as the caregiving context.

3.4. Studies in resilience

Resilience is often studied in children living under extreme circumstances or having illnesses. However, it is not easy to generalize the findings of children under extreme circumstances to the general population, although all children will face adversities during their development. Furthermore, studies in Asia are rare and many studies do not include protective factors. However, empirical understanding of the influence of risks and protective factors in a large normative population is important in improving mental health and psychosocial competence in children. In chapter 6 we describe a cohort study of 2139 Singaporean children, aged 6 to 12 years, recruited from 18 primary schools. The study was designed to examine relationships between common risks and protective factors in childhood with socio-emotional development and academic performance at age 8 to 12. A variety of questionnaires were used: IQ was measured with the Raven's Progressive Matrices, academic results were based on school results given by the school, and behavioural and emotional problems were measured with the CBCL parental report and the Teacher Rating Form (TRF) to obtain teacher-reported information. The child's adaptive functioning at school was measured with

a survey by teachers in terms of his/her ability to work, to behave, to learn and to be happy in school, all compared to other pupils of the same age. Variables representing protective and risk factors were created from demographic data about the child and family, derived from a questionnaire based on the Family and Household Questionnaire (75-item questionnaire related to family functioning) and derived from a list of life events used in the Ontario Child Health Study, all provided by mother. A multivariate modelling (SEM) was performed to construct a heuristic model examining the impact of protective factors and risks on children's socio-emotional development and adaptive functioning and academic performance.

The results show that some protective factors (intelligence, father's education and occupation) are strongly associated with fewer emotional and behavioural problems in children (β = -0.24, T = -2.56) and a lesser likelihood of poor adaptive functioning and lower academic scores (β = -0.55, T = -7.91). At the same time it shows that some risk factors (negative spousal conflict resolution, negative methods of discipline, chronic health problems, negative life events and developmental delay) are associated with more emotional and behavioural problems (β = 0.49, T = 8.12), without showing an association with academic results. These findings reinforce the importance of both positive resilience building focusing on assets and resources, as well as alleviating risks and adversities. Strengths of this study include the involvement of adversities as well as protective factors, the large sample size and data from different sources (parents, teachers, school reports). Limitations include the fact that only a selection of factors which may influence mental health and academic outcome were measured, the low participation rate, the differences in socioeconomic status between the included and excluded sample, and the lack of known psychometric properties of used instruments in Asia.

Multicultural resilience research has revealed that multiple conceptualisations of resilience are used worldwide, and that patterns of resilience are context-dependent. This suggests that existing resilience scales may not adequately represent all aspects of resilience and that trans-cultural use of standardized resilience measures developed in Europe and the USA, and even for similar ethnic groups in different parts of Asia, is theoretically unsound. Indeed, for those that have been validated cross-culturally, the findings indicate that the understanding of the construct requires some modification according to the culture in which it is measured. To date, no measure of resilience has been developed for use with Chinese adolescents within the cultural context of Singapore. In chapter 7 the development and validation of the Singapore Youth Resilience Scale (SYRESS) is described. The development is based on an exhaustive review of the literature; review of existing domains and items and addition of new domains and items by a focus group of researchers, child psychologists and psychiatrists with local and international content expertise; and additional feedback and contributions obtained from an external expert panel with similar content expertise. It was ensured that the domains and items were comprehensive as well as culturally relevant. The SYRESS included 50 items reflecting 10 domains of resilience; perseverance and commitment, positive self-image and optimism, relationship and social support, humour and positive thinking, emotional regulation, spirituality and faith, personal confidence and responsibility, personal control, flexibility, and positive coping. A test-retest study was done, as well as association studies with other resilience and wellbeing questionnaires. The results show that the SYRESS has sound psychometric properties, with good internal consistency (Cronbach's alpha 0.95) and test-retest reliability (r = 0.82), and convergent validity with

the Connor-Davidson Resilience Scale, WHOQOL-BREF quality of life, and GHQ-28 psychological morbidity. Factor analysis revealed a 10-factor structure for the SYRESS (total variance of 63.4%), and hierarchical analyses showed that the SYRESS significantly contributed additional variance to the prediction of the WHOQOL-BREF and GHQ-28 scores over that contributed by CD-RISC alone, suggesting that the SYRESS is a more comprehensive measure. Limitations include the fact that analyses were restricted to Chinese subjects only, due to the low response rate of Malay and Indian students.

4. Answers to the research questions

1. What are common candidate genes which, in interaction with environmental influences, have been associated with prolonged stress reactions leading to Posttraumatic Stress Disorder?
   Genotypes involved in the serotonin, dopamine, glucocorticoid, GABA, apolipoprotein, brain-derived neurotrophic factor and neuropeptide Y system, in interaction with environmental events, have been associated with Posttraumatic Stress Disorder worldwide. However, results are inconsistent, and G x E studies will be needed to fully understand the role of these genes in different environments.

2. What is the influence of the interaction between variation of genes involved in the serotonergic system and the environment in-utero (reflected in birth weight corrected for gestational age) on internalizing traits in children (measured with the Child Behavior Checklist, CBCL) at age 8 to 12 years?
   The effects of fetal growth on internalizing traits are modulated by genotypes of the TPH2 and HTR2A gene, with a non linear fetal growth x genotype interaction effect that results in significantly reduced levels of internalizing scores for children with a birth weight lying above the average birth weight in Singapore.

3. What is the influence of the early environment in-utero (reflected in weight, length and head circumference of the newborn at birth, corrected for gestational age) on IQ in children (measured with the Raven's Progressive Matrices) at age 8 to 12 years?
   Improved fetal growth predicts increased IQ at school age across the entire population. It shows a linear association between birth weight, birth length and head circumference at birth and IQ scores in children at age 8 to 12 years old on the Raven's Progressive Matrices in Singapore.

4. What is the impact of a standard surgery early in life on the stress response of young children? Are temperamental traits or neurophysiologic characteristics of the child predictors of the stress response?
   A standard Ear Nose and Throat surgery is for most Dutch children not very stressful and can even be helpful to reduce pre-existing behavioural and emotional and/or sleep problems. Temperamental traits and neurophysiologic characteristics are not predictive of the stress response, although more research is needed to examine the role of children with an emotional temperament.

5. What is the effect of common risks and protective factors during childhood on the outcome of children in terms of socio-emotional development (measured with the CBCL and TRF) and academic outcomes (school results and adaptive functioning) at age 8 to 12 years old?
   Some protective factors (intelligence, father's education and occupation) are strongly associated with fewer emotional and behavioural problems in children (β = -0.24, T = -2.56) and a lesser likelihood of poor adaptive functioning and lower academic scores (β = -0.55, T = -7.91), while risk factors (negative spousal conflict resolution, negative methods of discipline, chronic health problems, negative life events and developmental delay) show a strong positive relationship with emotional and behavioural problems (β = 0.49, T = 8.12), but have no influence on academic results in Singapore.

6. What is the need for developing a comprehensive cultural-sensitive resilience scale and what are the similarities and differences between resilience scales originating in Europe and the USA, and Asia?
   Multicultural resilience research has revealed that multiple conceptualisations of resilience has been described worldwide and that patterns of resilience are context-dependent. To date, no measure of resilience has been developed for adolescents within the cultural context of Singapore. In chapter 7, the development of the Singapore Youth Resilience Scale (SYRESS) is discussed. Although cultural items has been added to this scale, the developed SYRESS encompasses a set of underlying variables that appears consistent with universal determinants of resilience, like perseverance and commitment, positive self-image and optimism, relationship and social support, humour and positive thinking, emotional regulation, spirituality and faith, personal confidence and responsibility, personal control, flexibility, and positive coping. The SYRESS showed a better prediction of scores on quality of life and general health questionnaires than an existing resilience scale of the USA. This suggests that the SYRESS may be a more comprehensive measure, that is more useful in a Singaporean population.

5. Conclusions

Our studies support the hypothesis that all experiences during life, including early experiences in-utero, will influence the expression of genes, and in the end the socio-emotional and cognitive development later in life. This model of 'epigenetic programming' suggests the predictive power of the environment in-utero and early childhood on mental health later in life. The stress diathesis model proposes that this association is probably determined by a neurodevelopmental pathway with individual differences in neural and endocrine responses to stress. However, genotypes influencing the neural and endocrine stress responses are 'plastic', which implies that they can be modulated by environmental influences during life.

6. Clinical implications

Our studies underscore the need for a life span strategy. This will help to emphasize multiple factors during life which influence the development of the child and help to diagnose problems. The impact of prenatal adversity on neural development can be modulated by environmental factors later in life. This has implications for targeting clinical treatment pro-

grams, such as parent-child interventions.
Developmental studies are also important for education of the general public and for policy makers. Based on evidence of these studies, programs for prevention can be developed, such as optimalization of fetal and maternal care during pregnancies, remedial teaching, and resilience building. Notably, these clinical as well as prevention programs need to be evaluated in different environments and cultures.

7. Future research

The theory of epigenetic programming with effects on adult (mental) health underscores the need to focus on multidirectional research models in the future, with growing emphasis on G x E interactions and brain plasticity. These advances are based on the interconnectedness of genes and environment. Combination of behavioural science and physical science are needed to capture the complete processes underlying developmental change. Continuing studies in this area are planned for the coming years.

Nederlandse samenvatting

1. Achtergrond

Dit proefschrift beschrijft een aantal studies vanuit een ontwikkelingsperspectief, met als doel de invloed van veel voorkomende omgevingsfactoren en ervaringen op jonge leeftijd op de ontwikkeling van het kind te onderzoeken. Dit is onderzocht vanuit een neurobiologisch, sociaal en psychologisch perspectief met behulp van een biopsychosociaal model, beschreven in hoofdstuk 1.
De oorsprong van psychiatrische stoornissen bevindt zich regelmatig in de kindertijd. De periode in de baarmoeder en in de eerste levensjaren is er een waarin het individu gevoelig is voor invloeden die langdurige effecten kunnen hebben op de (mentale) gezondheid op latere leeftijd. Echter, hoe deze effecten zich manifesteren is nog steeds onduidelijk. In ieder geval zijn biologische invloeden en omgevingsinvloeden beide van belang. Met omgevingsinvloeden bedoelen we met name stressvolle gebeurtenissen vanaf de conceptie tot aan de volwassen leeftijd. Wanneer een individu stress ervaart treden twee verschillende fysiologische systemen in werking, het sympathische adrenerge medullaire systeem (SAM) en het hypothalamus-hypofyse-adrenerge systeem (HPA).
Wanneer iemand in staat is adequaat te reageren op de stressvolle gebeurtenis en, in het geval van een jeugdige, de ontwikkeling normale voortgang vindt, wordt hij als veerkrachtig beschouwd. Maar soms gaat de stressvolle gebeurtenis de verwerkingsmogelijkheden van het individu te boven, met psychologische en lichamelijke problemen tot gevolg. Hierbij moet niet alleen gedacht worden aan symptomen van posttraumatische stress, maar ook aan depressie en/of problemen in het cognitief functioneren.
In dit proefschrift is gebruik gemaakt van een ontwikkelingsperspectief om te begrijpen hoe 'nature' (genotypes) en 'nurture' (omgevingsfactoren zoals stressvolle gebeurtenissen of beschermende factoren) met elkaar interacteren en hoe deze interactie de ontwikkeling beïnvloedt. In de beschreven onderzoeken bestuderen we de invloed van stress gedurende de zwangerschap en in de eerste levensjaren op de sociaal-emotionele en cognitieve ontwikkeling van kinderen van verschillende leeftijden en met verschillende culturele achtergronden. Daarnaast wordt de ontwikkeling van een 'weerbaarheids' vragenlijst voor kinderen van een Aziatische populatie besproken.

2. Onderzoeksvragen

Met onze onderzoeken hebben we geprobeerd de volgende vragen zo goed mogelijk te beantwoorden:

1. Welke kandidaatgenen kunnen, in interactie met omgevingsinvloeden, op grond van onderzoek worden geïdentificeerd in relatie tot ernstige stressgerelateerde problemen, zoals de Posttraumatische Stress Stoornis (PTSS)?
2. Wat is de invloed van de interactie tussen genen die betrokken zijn bij de serotonine huishouding, en de ervaringen van de foetus gedurende de zwangerschap (gemeten met

geboortegewicht gecorrigeerd voor de zwangerschapsduur) op de ontwikkeling van internaliserend probleemgedrag bij kinderen in de leeftijd van 8 tot 12 jaar (gemeten met de Child Behavior Checklist, CBCL)?
3. Wat is de invloed van de ervaringen van de foetus gedurende de zwangerschap (gemeten met gewicht, lengte en hoofdomtrek bij de geboorte, gecorrigeerd voor zwangerschapsduur) op de intelligentie (gemeten met de Raven's Progressive Matrices) van kinderen van 8 tot 12 jaar?
4. Wat is de invloed van een standaard operatieve ingreep (amandelen verwijderen) op jonge leeftijd op de stressreactie? Zijn temperament en neurofyiologische kenmerken voorspellende factoren?
5. Wat is het effect van diverse veel voorkomende risicofactoren en beschermende factoren gedurende de kindertijd op de sociaal-emotionele ontwikkeling (gemeten met de CBCL en de Teacher's Report Form,TRF) en op de schoolprestaties (schoolrapport en aanpassingsvermogen op school) op de leeftijd van 8 tot 12 jaar?
6. Wat is het belang van de ontwikkeling van een cultuur-sensitieve weerbaarheids vragenlijst en wat zijn de overeenkomsten en de verschillen tussen de weerbaarheids vragenlijsten die ontwikkeld zijn in Europa en de VS, respectievelijk Azië?

3. Samenvatting van de resultaten van de onderzoeken

3.1. Onderzoeken naar genetische kwetsbaarheid

In hoofdstuk 2 wordt een review gegeven van onderzochte kandidaatgenen die, in interactie met omgevingsfactoren, geassocieerd zijn met PTSS door middel van een literatuuronderzoek in Medline, Embase en Web of Science. De meest belangrijke kandidaatgenen, coderend voor serotonine, dopamine, glucocorticoid, GABA, apolipoproteine, de brain-neurotrophic factor, respectievelijk het neuropeptide Y systeem worden weergegeven. De besproken onderzoeken laten tegenstrijdige resultaten zien, vermoedelijk op grond van methodologische tekortkomingen en een gebrek aan statistische 'power'. 'Gen-omgevings' onderzoeken zijn nodig om de rol van deze genen onder verschillende omstandigheden te begrijpen.

In hoofdstuk 4 beschrijven we een 'gen-omgevings' onderzoek bij een groep van 545 gezonde Chinese kinderen in de leeftijd van 8 tot 12 jaar, afkomstig van 3 verschillende scholen in Singapore. In deze studie wordt de invloed van geboortegewicht (gecorrigeerd voor de zwangerschapsduur), als een afspiegeling van stress in de baarmoeder, in interactie met serotonerge genen onderzocht op de ontwikkeling van internaliserend probleemgedrag. Zwangerschapsduur werd berekend door middel van de eerste dag van de laatste menstruatie, en nog eens gecontroleerd aan de hand van de foetale kruin-stuit lengte op de met 12-weken gemaakte ECHO. Geboortegegevens werden verkregen uit medische dossiers. Internaliserend probleemgedrag werd gemeten met de CBCL. Na correctie voor verschillende potentiële confounders, worden er van de in totaal 9 onderzochte 'single nucleotide polymorphisms' (SNPs) significante interacties gevonden tussen geboortegewicht (gecorrigeerd voor zwangerschapsduur) en 2 SNPs van het *TPH2* gen, die met elkaar verbonden zijn in 'high disequilibrium' (rs2171363, P = 0.008 en rs7305115, P = 0.007) en 2 SNPs van het *HTR2A* gen (rs2770304, P = 0.001 en rs6313, P = 0.026). Het CC genotype van *TPH2* rs2171363, GG genotype van *TPH2* rs7305115, CC genotype van *HTR2A* rs2770304 en CC genotype

van *HTR2A* rs6313 worden geassocieerd met minder internaliserend probleemgedrag voor kinderen met een geboortegewicht in het 3e kwadrant (net boven het gemiddelde geboortegewicht). Concluderend worden de effecten van foetale groei op de ontwikkeling van sociaal-emotionele karaktertrekken, die geassocieerd zijn met affectieve stoornissen, beïnvloed door genotypes die betrokken zijn in de serotonine huishouding. Dit betekent dat 'serotonerge' genen in staat zijn om de effecten van foetale groei te beïnvloeden. De resultaten laten ook een duidelijke foetale groei x genotype interactie zien, waarbij bepaalde genotypes een lager risico geven op het ontwikkelen van internaliserend probleemgedrag voor kinderen met een geboortegewicht in het 3^{e} kwadrant. Interessant genoeg zijn de genotypes, die geassocieerd zijn met verlaagde scores op internaliserend probleemgedrag, juist geassocieerd met een verhoogd risico op angst en depressie bij volwassenen. Dit betekent dat genetische varianten die statistisch een verhoogd risico voor angst en depressie met zich mee brengen, mogelijk gerelateerd zijn aan een verhoogde gevoeligheid voor omgevingsinvloeden, wat de gedifferentieerde bevindingen (zowel positieve als negatieve gevolgen) verklaart. Deze bevindingen sluiten aan bij recente theorieën (bijvoorbeeld die van Boyce en collega's), die suggereren dat genetische varianten de sensitiviteit voor de omgeving beïnvloeden en resulteren in een context-specifieke ontwikkeling. Sterke punten van dit onderzoek zijn de relatief grote onderzoeksgroep van gezonde Chinese kinderen, de kritisch geselecteerde polymorfismen die al in eerder onderzoek geassocieerd zijn met affectieve stoornissen en het uitsluiten van belangrijke confounders zoals 'roken tijdens de zwangerschap'. Beperkingen zijn dat andere oorzaken van verminderde foetale groei (zoals alcohol, dieet) niet gemeten zijn, dat de meeste data verkegen zijn via de ouders (subjectief) en de mogelijkheid dat andere gen-gen interacties niet aan het licht zijn gekomen.

3.2. Onderzoeken naar de invloed van vroege levenservaringen in-utero

Onderzoeken naar de invloed van een laag geboortegewicht hebben laten zien dat intelligentie gecorreleerd is met geboortegewicht. Deze relatie is minder duidelijk voor kinderen met een normaal geboortegewicht die 'a terme' zijn geboren; onderzoek naar de relatie tussen geboortegewicht en IQ in de normale bevolking is zeldzaam, en is voornamelijk gedaan in westerse landen bij mannelijke deelnemers. In hoofdstuk 3 beschrijven we een cohort onderzoek van 1979 kinderen in Singapore, geworven bij 3 scholen. Dit onderzoek bestudeert de relatie tussen stress in de baarmoeder (gemeten met geboortegewicht gecorrigeerd voor zwangerschapsduur) en IQ op de leeftijd van 8 tot 12 jaar. Geboortegegevens werden verkregen uit de medische dossiers, IQ werd gemeten met de Raven's Progressive Matrices. De resultaten laten zien dat bij Singaporese kinderen voor iedere kg toename in geboortegewicht het IQ toeneemt met 2.19 punten ($P = 0.007$), voor iedere cm toename in geboortelengte het IQ toeneemt met 0.49 punten ($P < 0.001$) en voor iedere cm toename in hoofdomtrek het IQ toeneemt met 0.62 punten ($P = 0.003$), zelfs na correctie voor meerdere potentiële confounders en ook na uitsluiting van premature (< 37 weken) en dysmature kinderen (< 2.5 kg) of macrosome kinderen met een extreme grote lichaamsomvang voor de zwangerschapsduur (> 4 kg). Een analyse van een subgroep van zusjes en broertjes laat dezelfde trend zien, waarin langere zusjes of broertjes een significant hoger IQ hebben dan kortere zusjes of broertjes. Samengevat laten de bevindingen zien dat een betere foetale groei leidt tot een hoger IQ tijdens de schoolleeftijd in de algemene populatie. Dit benadrukt dat we ons niet alleen moeten richten op factoren die de foetale groei op een

extreme wijze remmen (zoals roken, alcohol etc), maar op alle factoren die van invloed kunnen zijn op de zwangerschap. Sterke punten van dit onderzoek zijn het gebruik van meerdere geboortegegevens wat betreft de foetale groei, de grote onderzoeksgroep met een hoog follow-up percentage, de analyse van zusjes en broertjes om omgevingsfactoren binnen het gezin uit te kunnen sluiten en het gebruik van een gevalideerde IQ test die de effecten van taal en cultuur minimaliseert. Beperkingen van het onderzoek zijn de mogelijkheid van selectiebias, omdat bepaalde kinderen niet deel hebben genomen aan de follow-up, incomplete gegevens, en confounding omdat niet alle factoren die van invloed kunnen zijn op de foetus en de ontwikkeling van het kind gemeten zijn.
In hoofdstuk 4 wordt de associatie tussen vroege levenservaringen, in interactie met genen die betrokken zijn bij de serotonine huishouding, met de sociaal-emotionele ontwikkeling beschreven. Dit onderzoek is gedetailleerd beschreven onder 3.1. (onderzoeken naar genetische kwetsbaarheid). Alhoewel het verband tussen geboortegewicht en IQ een lineaire trend laat zien, is het verband tussen geboortegewicht en internaliserend probleemgedrag niet-lineair en wordt dit verband gemoduleerd door genotypes.

3.3. Onderzoeken naar de invloed van stress op jonge leeftijd

Kinderen reageren verschillend op stressoren, zoals bijvoorbeeld medische ingrepen. Prospectieve studies die voorspellende invloeden op de stress reactie bestuderen zijn zeldzaam. In hoofdstuk 5 is een prospectief onderzoek beschreven waarin de invloed van een standaard operatie (een adenoïdectomie – het verwijderen van de neusamandelen – of een adenotonsillectomie – het verwijderen van de neus- en keelamandelen –) op de stressreactie wordt onderzocht bij een groep van 43 kinderen van 2 tot 7 jaar oud in Nederland. De hypothese, dat het temperament van het kind evenals de neurofysiologie van het kind een voorspellende invloed heeft op de psychologische uitkomst na de operatie, werd getoetst. Vier weken voor de operatie werd aan ouders gevraagd om een vragenlijst over het temperament van het kind in te vullen (EAS) evenals de CBCL om het gedrag en de emoties van het kind in kaart te brengen. Tevens werd er een baseline neurofysiologishe meting verricht: cortisol (in speeksel) en respiratory sinus arrythmia (RSA, af te leiden van de hartslag variabiliteit door middel van een electrocardiogram). Direct na de operatie, en nog eens 6 weken na de operatie, werden de cortisol en RSA metingen herhaald om de neurofysiologische stress reactie te kunnen meten. Zes weken na de operatie werd ook de 'Children's Revised Impact of Event Scale', en een slaap vragenlijst afgenomen bij de ouders om respectievelijk posttraumatische stress symptomen en slaapproblemen in kaart te brengen, en eveneens werd de CBCL herhaald om veranderingen in gedrag en emotie vast te kunnen stellen. Voor zover bekend was dit de eerste studie die gedragsmatige en neurofysiologische kenmerken van jonge kinderen zowel voor als na een operatie heeft vastgesteld om de individuele verschillen in de stress response op een prospectieve wijze te kunnen meten. De resultaten laten zien dat een adenoïdectomie of adenotonsillectomie in het algemeen goed verdragen wordt door jonge kinderen en zelfs kan helpen om bestaande sociaal-emotionele problemen (in 75%) evenals slaapproblemen (in 68%) te reduceren, met name bij jongens. Posttraumatische stress symptomen zijn zeldzaam. In tegenstelling tot eerdere bevindingen vinden we geen verband tussen verlegenheid en de mate van de stress reactie. Ook is er geen voorspellende waarde voor de gemeten neurofysiologische parameters aangetoond. Wel lijkt meer onderzoek naar de rol van een emotioneel temperament aangewezen, aangezien een emotioneel temperament

in dit onderzoek geassocieerd is met meer sociaal-emotionele problemen voor de operatie ($r = 0.53$, $P = 0.02$), na de operatie ($r = 0.38$, $P < 0.000$), een lager niveau van cortisol direct na de operatie ($r = -0.49$, $P = 0.05$) en een lagere RSA 6 weken na de operatie ($r = -0.33$, $P = 0.06$). Daarnaast is meer onderzoek nodig om de rol van het geslacht te onderzoeken; meisjes en jongens laten verschillende stress reacties zien, waarbij jongens een betere respons vertonen na de operatie. Hierbij moet wel de kanttekening worden gemaakt dat de groep meisjes erg klein was. Sterke punten van dit onderzoek zijn het prospectieve design en de inclusie van neurofysiologische metingen naast de gebruikelijke rapportages van ouders. Beperkingen van dit onderzoek betreffen de relatief kleine onderzoeksgroep, incomplete gegevens en de mogelijkheid van confounders, omdat variabelen zoals de kwaliteit van het zorgsysteem, niet meegenomen zijn in de metingen.

3.4. Onderzoeken naar weerbaarheid

Weerbaarheid wordt vaak onderzocht in onderzoeksgroepen die leven onder extreme omstandigheden, zoals kinderen in weeshuizen of chronisch zieke kinderen. Echter, alle kinderen zullen in zekere mate moeten leren omgaan met tegenslagen in het leven, en het is niet eenvoudig om de bevindingen van onderzoek onder extreme omstandigheden naar de algemene bevolking te vertalen. Bovendien hebben veel onderzoeken zich alleen gericht op tegenslagen en niet op beschermende factoren. Daarnaast zijn de meeste onderzoeken in Europa en de VS uitgevoerd, en zijn deze onderzoeken in Azië zeldzaam. Empirisch onderzoek naar de invloed van risicofactoren evenals beschermende factoren in een grote algemene populatie is belangrijk om de geestelijke gezondheid en de psychosociale competentie van kinderen te vergroten. In hoofdstuk 6 beschrijven we een cohort onderzoek bij 2139 Singaporese kinderen geworven via 18 basisscholen in Singapore. Het onderzoek had als doel de relatie tussen veel voorkomende risicofactoren en beschermende factoren in de kindertijd en de uitkomst op sociaal-emotioneel en academisch functioneren op 8 tot 12 jarige leeftijd te onderzoeken. Verschillende vragenlijsten werden gebruikt: IQ werd gemeten met de Raven's Progressive Matrices, academische resultaten werden gebaseerd op schoolresultaten die verstrekt werden door de scholen zelf, en sociaal-emotionele problemen werden gemeten met zowel de ouderversie van de CBCL als de leerkrachtversie (TRF). Het aanpassingsvermogen van het kind op school (het vermogen om zich te kunnen concentreren, zich te gedragen, te leren en plezier te hebben op school, in vergelijking met andere leerlingen van dezelfde leeftijd) werd gemeten met een vragenlijst ingevuld door de leerkracht. Bij de moeder werden de risicofactoren en beschermende factoren in kaart gebracht evenals demografische gegevens over het kind en het gezin, met een vragenlijst die gebaseerd is op de 'Family and Household Questionnaire' (vragenlijst over gezinsfunctioneren), terwijl life events werden gemeten met een vragenlijst afkomstig van 'the Ontario Child Health Study'. Een multivariate statistische analyse (SEM) werd verricht om de invloed van risicofactoren danwel beschermende factoren op de sociaal-emotionele ontwikkeling en het aanpassingsvermogen op school en de schoolresultaten te bepalen. De resultaten laten zien dat sommige beschermende factoren (intelligentie, opleidingsniveau vader, beroep vader) in hoge mate geassocieerd zijn met minder sociaal-emotionele problemen ($\beta = -0.24$, $T = -2.56$) en minder kans op problemen in het aanpassingsvermogen of slechte schoolresultaten ($\beta = -0.55$, $T = -7.91$). Tegelijkertijd laten de resultaten zien dat sommige risicofactoren (conflicten tussen ouders, het toepassen van negatieve disciplinaire maatregelen, chronische

gezondheidsproblemen, negatieve life events en vertraging in de ontwikkeling) geassocieerd zijn met meer sociaal-emotionele problemen ($\beta = 0.49$, $T = 8.12$), zonder dat het invloed heeft op het academisch presteren. Deze resultaten benadrukken dat het voor het opbouwen van weerbaarheid van belang is om zowel de beschermende factoren te vergroten, als risicofactoren te vermijden of te verlichten. Sterke punten van dit onderzoek zijn dat zowel beschermende- als risicofactoren zijn meegenomen in de analyses, de grote onderzoeksgroep en het gebruik van gegevens die niet alleen gebaseerd zijn op rapportage van ouders, maar ook op rapportage van leerkrachten en objectieve schoolresultaten. Beperkingen zijn dat het niet mogelijk was om alle mogelijke factoren – die van invloed kunnen zijn op de sociaal-emotionele ontwikkeling en de academische resultaten – onderzocht konden worden, de lage deelname, en het feit dat niet alle gebruikte vragenlijsten gevalideerd zijn voor Aziatische populaties.

Multicultureel onderzoek naar weerbaarheid heeft laten zien dat er wereldwijd multipele conceptualiseringen voor weerbaarheid worden gebruikt, en dat weerbaarheid omgevingsafhankelijk is. Dit betekent dat gestandaardiseerde weerbaarheids vragenlijsten, die ontwikkeld zijn in westerse culturen, niet zonder meer voor gebruik in andere culturen geschikt zijn. Daarnaast is aangetoond dat weerbaarheids vragenlijsten die wel gevalideerd zijn, vaak niet alle aspecten van weerbaarheid dekken, en in andere culturen vaak enige aanpassing behoeven, afhankelijk van de cultuur. Tot op heden is er geen weerbaarheids vragenlijst ontwikkeld voor adolescenten in Singapore. In hoofdstuk 7 wordt de ontwikkeling en validatie van de Singapore Youth Resilience Scale (SYRESS) beschreven. De ontwikkeling is gebaseerd op een uitgebreid literatuuronderzoek; een review van domeinen en items van bestaande weerbaarheids vragenlijsten en toevoeging van nieuwe domeinen en items over weerbaarheid door een focusgroep van onderzoekers, kinderpsychologen en psychiaters met een lokale en internationale expertise. Ook werd feedback gevraagd van een extern expert panel met een gelijke inhoudsexpertise. Er werd gewaarborgd dat domeinen en items veelomvattend en cultureel-relevant waren. De SYRESS bestaat uit 50 items verdeeld over 10 domeinen van weerbaarheid te weten; doorzettingsvermogen en commitment, positief zelfbeeld en optimisme, relaties en sociale steun, humor en positief denken, emotionele regulatie, spiritualiteit en geloof, zelfvertrouwen en verantwoordelijkheid nemen, gevoel van controle, flexibiliteit, en positieve coping.

Er werd een test-hertest onderzoek gedaan en het verband tussen de SYRESS en andere weerbaarheids- en welzijnsvragenlijsten werd onderzocht. De resultaten laten zien dat de SYRESS over goede psychometrische kwaliteiten beschikt, met een goede interne consistentie (Cronbanch's alpha 0.95) en goede test-hertest resultaten ($r = 0.82$). Ook vertoont de SYRESS een overeenkomstige validiteit met weerbaarheids vragenlijsten uit Westerse culturen zoals de Connor-Davidson Resilience Scale (CD-RISC), de WHOQOL-BREF quality of life vragenlijst en de GHQ-28 psychologische problemen vragenlijst. Factoranalyse wees uit dat de SYRESS een 10-factoren structuur bezit (met een totale variantie van 63.4%). Uit hiërarchische analyses blijkt dat the SYRESS weerbaarheids vragenlijst aanzienlijk additioneel bijdraagt aan de voorspelling van scores op de WHOQOL-BREF en GHQ-28 vragenlijst, in vergelijking met de CD-RISC. Dit suggereert dat de SYRESS een meer omvattende weerbaarheids vragenlijst is dan de CD-RISC. Een beperking van het onderzoek is dat de analyses alleen uitgevoerd werden onder Chinese adolescenten, omdat de Maleisische en Indiase studenten ondervertegenwoordigd waren in dit onderzoek.

4. Antwoorden op de onderzoeksvragen

1. Welke kandidaatgenen kunnen in interactie met omgevingsinvloeden, op grond van onderzoek worden geïdentificeerd in relatie tot ernstige stressgerelateerde problemen, zoals PTSS?
   Variaties in genen die coderen voor de serotonine, dopamine, glucocorticoid, GABA, apolipoproteine, brain-derived neurotrophic factor en neuropeptide Y huishouding blijken, in interactie met omgevingsinvloeden, wereldwijd geassocieerd te zijn met PTSS.

2. Wat is de invloed van de interactie tussen genen die betrokken zijn bij de serotonine huishouding, en de ervaringen van de foetus gedurende de zwangerschap (gemeten met geboortegewicht gecorrigeerd voor de zwangerschapsduur) op de ontwikkeling van internaliserend probleemgedrag bij kinderen in de leeftijd van 8 tot 12 jaar (gemeten met de Child Behavior Checklist, CBCL)?
   Het effect van foetale groei op internaliserend probleemgedrag wordt gemoduleerd door genotypes van de TPH2 en HTR2A genen. Het betreft een non-lineaire foetale groei x genotype interactie effect dat resulteert in significant minder internaliserende problemen voor kinderen met een geboortegewicht net boven het gemiddelde geboortegewicht in Singapore.

3. Wat is de invloed van ervaringen van de foetus gedurende de zwangerschap (gemeten met gewicht, lengte en hoofdomtrek bij de geboorte, gecorrigeerd voor zwangerschapsduur) op de intelligentie (gemeten met de Raven's Progressive Matrices) van kinderen van 8 tot 12 jaar?
   Binnen de algemene bevolking is een betere foetale groei geassocieerd met een toename in IQ tijdens de schoolleeftijd. Er is een lineaire associatie tussen geboortegewicht, geboortelengte en hoofdomtrek bij de geboorte met IQ scores van de Raven's Progressive Matrices in Singapore.

4. Wat is de invloed van een standaard operatieve ingreep (amandelen verwijderen) op jonge leeftijd op de stressreactie? Zijn temperament en neurofyiologische kenmerken voorspellende factoren?
   Een standaard KNO operatie in Nederland wordt door de meeste kinderen niet als stressvol ervaren en kan zelfs bestaande sociaal-emotionele problemen en slaapproblemen reduceren. Zowel temperament als neurofysiologische kenmerken zijn geen voorspellende factoren voor de stress reactie, hoewel meer onderzoek moet worden gedaan om de rol van een emotioneel temperament te bepalen.

5. Wat is het effect van diverse veel voorkomende risicofactoren en beschermende factoren gedurende de kindertijd op de sociaal-emotionele ontwikkeling (gemeten met de CBCL en TRF) en op de schoolprestaties (schoolrapport en aanpassingsvermogen op school) op de leeftijd van 8 tot 12 jaar oud?
   Sommige beschermende factoren (intelligentie, educatie van vader, beroep van vader) zijn sterk geassocieerd met minder sociaal-emotionele problemen (β = -0.24, T = -2.56) en minder kans op aanpassingsstoornissen en lagere schoolresultaten (β = -0.55, T = -7.91), terwijl risicofactoren (conflict situaties tussen ouders, het hanteren van negatieve disciplinaire maatregelen, chronische gezondheidsproblemen, negatieve life events en vertraging in de ontwikkeling) geassocieerd zijn met meer sociaal-emotionele problemen (β = 0.49, T =8.12), zonder dat zij invloed hebben op de schoolresultaten.

6. Wat is het belang van de ontwikkeling van een cultuur-sensitieve weerbaarheids vragenlijst en wat zijn de overeenkomsten en de verschillen tussen de weerbaarheids vragenlijsten die ontwikkeld zijn in Europa en de VS, respectievelijk Azië?
   Multicultureel onderzoek naar weerbaarheid heeft bevestigd dat er wereldwijd meerdere conceptualisaties bestaan van weerbaarheid en dat weerbaarheid een omgevings-afhankelijk concept is. Tot op heden was er geen weerbaarheids vragenlijst ontwikkeld voor adolescenten in Singapore. De ontwikkeling van de Singapore Youth Resilience Scale (SYRESS) wordt beschreven in hoofdstuk 7. Alhoewel er cultureel-specifieke items zijn toegevoed aan deze vragenlijst, bestaat de ontwikkelde SYRESS uit onderliggende variabelen die consistent zijn met universele determinanten van weerbaarheid, te weten: doorzettingsvermogen en commitment, positief zelfbeeld en optimisme, relaties en sociale steun, humor en positief denken, emotionele regulatie, spiritualiteit en geloof, zelfvertrouwen en verantwoordelijkheid nemen, gevoel van controle, flexibiliteit, en positieve coping.
   The SYRESS bleek een betere voorspeller te zijn van scores op vragenlijsten over kwaliteit van leven en algemene psychische gezondheid, dan een reeds bestaande weerbaarheids vragenlijsten uit de VS. Dit suggereert dat de SYRESS mogelijk een meer omvattende weerbaarheids vragenlijst is, die beter bruikbaar is in een Singaporese populatie.

5. Conclusies

Onze onderzoeken bevestigen de hypothese dat alle ervaringen gedurende het leven, inclusief de eerste ervaringen in de baarmoeder, invloed hebben op de expressie van genen, en uiteindelijk op de sociaal-emotionele en cognitieve ontwikkeling. Dit komt overeen met de theorie van 'epigenetic programming', die suggereert dat de omgeving in de baarmoeder en vroeg tijdens het leven een duidelijke invloed heeft op de (mentale) gezondheid later in het leven. Het stress-diathese model stelt dat deze associatie waarschijnlijk bepaald wordt door een 'neurodevelopmental pathway', die de verschillen tussen individuen in neurale en endocriene stressreacties vastlegt. Echter, genen die deze neuronale en endocriene stress reacties beïnvloeden zijn 'plastisch', en dit impliceert dat de effecten uiteindelijk gemoduleerd kunnen worden door omgevingsinvloeden gedurende het leven.

6. Klinische implicaties

Dit proefschrift onderstreept het belang van het in acht nemen van de levensloop van het individu. Een levensloop strategie zal helpen om meerdere factoren gedurende het leven, die van invloed zijn op de ontwikkeling van het kind, in kaart te brengen en problemen beter te kunnen diagnostiseren. Aangezien de effecten van prenatale risicofactoren op de neurale ontwikkeling gemoduleerd kunnen worden door omgevingsfactoren later in het leven, is het ontwikkelen van klinische behandelprogramma's, zoals ouder-kind interventies, van belang. Uitkomsten van onderzoeken met een ontwikkelingsperspectief zijn ook van belang voor de voorlichting van de bevolking en voor de beleidsmakers. Gebaseerd op empirische bevindingen kunnen preventieve programma's, ontwikkeld worden; zoals optimalisatie van foetale zorg en zorg voor zwangeren, het bieden van extra begeleiding aan kinderen op school, en programma's gericht op het ontwikkelen van weerstand en veerkracht. Deze klinische en

preventie programma's moeten echter wel geevalueerd worden onder verschillende omstandigheden en culturen.

7. Toekomstig onderzoek

De theorie van 'epigenetic programming' met effecten op de (mentale) gezondheid van volwassenen onderstreept de noodzaak om toekomstig onderzoek te baseren op multidirectionele modellen, met meer nadruk op gen-omgevings interacties en onderzoek naar de plasticiteit van ons brein. Daarbij is het van belang om gedragswetenschappelijk en natuurwetenschappelijk onderzoek te combineren, om aan het hele proces van ontwikkeling recht te kunnen doen. Door middel van vervolgonderzoek, hopen wij hieraan de komende jaren bij te dragen.

References

Abelson JL, Curtis GC. Hypothalamic-pituitary-adrenal axis activity in panic disorder. 24-hour secretion of corticotrophin and cortisol. *Arch Gen Psychiatry.* 1996;53:323-331.

Achenbach TM, Edelbrock C. *The child behavior checklist and revised child behavior profile.* Burlington, VT: University Associates in Psychiatry; 1991a.

Achenbach TM, Edelbrock C. *Manual for the Teacher's Report Form and 1991 profile.* Burlington, VT: University of Vermont; 1991b.

Achenbach TM, Rescorla LA. *Manual for ASEBA preschool forms & profiles.* Burlington, VT: University of Vermont; 2000.

Ahern NR, Ark P, Byers J. Resilience and coping strategies in adolescents. *Paediatr Nurs.* 2008;20:32-36.

Ahern NR, Kiehl EM, Sole ML, Byers J. A review of instruments measuring resilience. *Issues Compr Pediatr Nurs.* 2006;29:103-125.

Alati R, Najman JM, O'Callaghan M, Bor W, Williams GM, Clavarino A. Fetal growth and behaviour problems in early adolescence: findings from the Mater University Study of Pregnancy. *Int J Epidemiol.* 2009;38:1390-1400.

Ali NJ, Pitson D, Stradling JR. Sleep disordered breathing: effects of adenotonsillectomy on behaviour and psychological functioning. *Eur J Pediatr.* 1996;155:56-62.

Altwood JT, Yung RL, Richardson BC. DNA methylation and the regulation of gene transcription. *CMLS.* 2002;59:241-257.

Alriksson-Schmidt AI, Wallander J, Biasini F. Quality of life and resilience in adolescents with a mobility disability. *J Pediatr Psychol.* 2006;32:370-379.

Ang RP, Huan VS. Academic Expectations Stress Inventory: development, factor analysis, reliability, and validity. *Educ Psychol Meas.* 2006;66:522-539.

Anglin DM, Cohen PR, Chen H. Duration of early maternal separation and prediction of schizotypal symptoms from early adolescence to midlife. *Schizophr Res.* 2008;103:143-150.

Arias B, Gasto C, Catalan R, Gutierrez B, Pintor L, Fananas L. The 5-HT(2A) receptor gene 102T/C polymorphism is associated with suicidal behavior in depressed patients. *Am J Med Genet.* 2001;105:801-804.

Arrington EG, Wilson MN. A re-examination of risk and resilience during adolescence: incorporating culture and diversity. *J Child Fam Stud.* 2000;9:221-230.

Asmundson GJ, Stapleton JA, Taylor S. Are avoidance and numbing distinct PTSD symptom clusters? *J Trauma Stress.* 2004;17:467-475.

Bachmann AW, Sedgley TL, Jackson RV, Gibson JN, Young RM, Torpy DJ. Glucocorticoid receptor polymorphisms and post-traumatic stress disorder. *Psychoneuroendocrinology.* 2005;30:297-306.

Backett-Milburn K, Cunningham-Burley S, Davis J. Contrasting lives, contrasting views? Understandings of health inequalities from children in differing social circumstances. *Soc Sci Med.* 2003;57:613-623.

Bagot RC, Meaney MJ. Epigenetics and the biological basis of gene x environment interactions. *J Am Acad Child Adolesc Psychiatry.* 2010;49:752-771.

Bakermans-Kranenburg MJ, van Ijzendoorn MH. Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. *Soc Cogn Affect Neurosci.* 2008;3:128-134.

Bakermans-Kranenburg MJ, van Ijzendoorn MH, Mesman J, Alink LR, Juffer F. Effects of an attachment-based intervention on daily cortisol moderated by dopamine receptor D4: a randomized control trial on 1- to 3-year-olds screened for externalizing behavior. *Dev Psychopathol.* 2008;20:805-820.

Bale TL, Baram TZ, Brown AS, et al. Early life programming and neurodevelopmental disorders. *Biol Psychiatry.* 2010;68:314-319.

Ball J, Armistead L, Austin B. The relationship between religiosity and adjustment among African-American, female, urban adolescents. *J Adolesc.* 2003;26:431-446.

Banks MH. Validation of the General Health Questionnaire in a young community sample. *Psychol Med.* 1983;13:349-353.

Barker DJ. Intrauterine programming of adult disease. *Mol Med Today.* 1995;1:418-423.

Barker DJ. *Mothers, babies and health in later life, 2nd edition.* Edinburgh, UK: Churchill Livingstone; 1998.

Barker DJ. The origins of the developmental origins theory. *J Intern Med.* 2007;261:412-417.

Barr CS, Newman TK, Lindell S, et al. Interaction between serotonin transporter gene variation and rearing condition in alcohol preference and consumption in female primates. *Arch Gen Psychiatry.* 2004a;61:1146-1152.

Barr CS, Newman TK, Shannon C, et al. Rearing condition and rh5-HTTLPR interact to influence limbic-hypothalamic-pituitary-adrenal axis response to stress in infant macaques. *Biol Psychiatry.* 2004b;55:733-738.

Barratt ES. The Relationship of the Progressive Matrices (1938) and the Columbia Mental Maturity Scale to the WISC. *J Consult Psychol.* 1956;20:294-296.

Bartels M, de Geus EJ, Kirschbaum C, Sluyter F, Boomsma DI. Heritability of daytime cortisol levels in children. *Behav Genet.* 2003;33:421-433.

Bateson P, Barker D, Clutton-Brock T, et al. Developmental plasticity and human health. *Nature.* 2004;430:419-421.

Batty GD, Deary IJ. Early life intelligence and adult health. *BMJ.* 2004;329:585-586.

Batty GD, Deary IJ, Gottfredson LS. Premorbid (early life) IQ and later mortality risk: systematic review. *Ann Epidemiol.* 2007;17:278-288.

Beauchaine T. Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. *Dev Psychopathol.* 2001;13:183-214.

Belsky J, de Haan M. Annual Research Review: Parenting and children's brain development: the end of the beginning. *J Child Psychol Psychiatry.* 2011;52:409-428.

Belsky J, Pluess M. Beyond diathesis stress: differential susceptibility to environmental influences. *Psychol Bull.* 2009;135:885-908.

Bennett AJ, Lesch KP, Heils A, et al. Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Mol Psychiatry.* 2002;7:118-122.

Bergvall N, Iliadou A, Johansson S, Tuvemo T, Cnattingius S. Risks for low intellectual performance related to being born small for gestational age are modified by gestational age. *Pediatrics.* 2006;117:e460-467.

Bhutta ZA, Ahmed T, Black RE, et al. Maternal and Child Undernutrition Study Group. What works? Interventions for maternal and child undernutrition and survival. *Lancet.* 2008;371:417-440.

Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *JAMA.* 2002;288:728-737.

Bicanic IA, Meijer M, Sinnema G, van de Putte EM, Olff M. Neuroendocrine dysregulations in sexually abused children and adolescents: a systematic review. *Prog Brain Res.* 2008;167:303-306.

Birmes P, Brunet A, Carreras D, et al. The predictive power of peritraumatic dissociation and acute stress symptoms for posttraumatic stress symptoms: a three-month prospective study. *Am J Psychiatry.* 2003;160:1337-1339.

Birmes P, Carreras D, Charlet JP, Warner BA, Lauque D, Schmidt L. Peritraumatic dissociation and posttraumatic stress disorder in victims of violent assault. *J Nerv Ment Dis.* 2001;189:796-798.

Björntorp P. Alterations in the ageing corticotrophin stressresponse axis. *Novartis Foundation Symp.* 2002;242:46-58.

Black RE, Allen LH, Bhutta ZA, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet.* 2008;371:243-260.

Blair C, Peters R, Granger D. Physiological and neuropsychological correlates of approach/withdrawal tendencies in preschool: further examination of the behavioral inhibition system/behavioral activation system scales for young children. *Dev Psychobiol.* 2004;45:113-124.

Boer F. Stress early in life; a developmental perspective. *Tijdschr Psychiatr.* 2009;51:579-586.

Boer F, Smit C, Morren M, Roorda J, Yzermans J. Impact of a technological disaster on young children: a five-year postdisaster multiinformant study. *J Traum Stress.* 2009;22:516-524.

Boer F, Westenberg PM. The factor structure of the buss and plomin EAS temperament survey (parental ratings) in a Dutch sample of elementary school children. *J Pers Assess.* 1994;62:537-551.

Bohnert KM, Breslau N. Stability of psychiatric outcomes of low birth weight: a longitudinal investigation. *Arch Gen Psychiatry.* 2008;65:1080-1086.

Bonanno GA, Galea S, Bucciarelli A, Vlahov D. Psychological resilience after disaster: New York City in the aftermath of the September 11th terrorist attack. *Psychol Sci.* 2006;17:181-186.

Bonanno GA, Ho SM, Chan JC, et al. Psychological resilience and dysfunction among hospitalized survivors of the SARS epidemic in Hong Kong: a latent class approach. *Health Psychol.* 2008;27:659-667.

Bondy B, Kuznik J, Baghai T, et al. Lack of association of serotonin-2A receptor gene polymorphism (T102C) with suicidal ideation and suicide. *Am J Med Genet.* 2000;96:831-835.

Bonne O, Bain F, Neumeister A, et al. No change in serotonin type 1A receptor binding in patients with posttraumatic stress disorder. *Am J Psychiatry.* 2005;162:383-385.

Bonnier C. Evaluation of early stimulation programs for enhancing brain development. *Acta Paediatr.* 2008;97:853-858.

Boomsma DI, van Beijsterveldt CE, Rietveld MJ, Bartels M, van Baal GC. Genetics mediate relation of birth weight to childhood IQ. *BMJ.* 2001;323:1426-1427.

Boyce WT, Ellis BJ. Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Dev Psychopathol.* 2005;17:271-301.

Boyle MH, Offord DR, Hofmann HG, et al. Ontario Child Health Study: I. Methodology. *Arch Gen Psychiatry.* 1987;44:826-831.

Brand SR, Engel SM, Canfield RL, Yehuda R. The effect of maternal PTSD following in utero trauma exposure on behaviour and temperament in the 9-month-old infant. *Ann N Y Acad Sci.* 2006;1071:454-458.

Braun P, Greenberg D, Dasberg H, Lerer B. Core symptoms of posttraumatic stress disorder unimproved by alprazolam treatment. *J Clin Psychiatry.* 1990;51:236-238.

Bredy TW, Zhang TY, Grant RJ, Diorio J, Meaney MJ. Peripubertal environmental enrichment reverses the effects of maternal care on hippocampal development and glutamate receptor subunit expression. *Eur J Neurosci.* 2004;20:1355-1362.

Breslau N. The epidemiology of posttraumatic stress disorder: what is the extent of the problem? *J Clin Psychiatry.* 2001;62:16-22.

Breslau N, Davis GC. Risk factors for PTSD-related traumatic events. *Am J Psychiatry.* 1995;152:529-535.

Breslau N, Kessler RC, Chilcoat HD, Schultz LR, Davis GC, Andreski P. Trauma and posttraumatic stress disorder in the community: the 1996 Detroit Area Survey of Trauma. *Arch Gen Psychiatry.* 1998;55:626-632.

Breslau J, Miller E, Breslau N, Bohnert K, Lucia V, Schweitzer J. The impact of early behavior disturbances on academic achievement in high school. *Pediatrics.* 2009;123:1472-1476.

Brewin CR, Andrews B, Valentine JD. Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. *J Consult Clin Psychol.* 2000;68:748-766.

Brezo J, Bureau A, Merette C, et al. Differences and similarities in the serotonergic diathesis for suicide attempts and mood disorders: a 22-year longitudinal gene-environment study. *Mol Psychiatry.* 2009;8:831-843.

Broekman BFP, Chan YH, Chong YS, et al. The influence of birth size on intelligence in healthy children. *Pediatrics.* 2009;123:e1011-1016.

Broekman BFP, Olff M, Boer F. The genetic background to PTSD. *Neurosc and Biobeh Reviews.* 2007;31:348-362.

Buckner JC, Mezzacappa E, Beardslee WR. Characteristics of resilient youths living in poverty: the role of self-regulating processes. *Dev Psychopathol.* 2003;15:139-162.

Buemann B, Vohl MC, Chagnon M, et al. Abdominal visceral fat is associated with a BclI restriction fragment length polymorphism at the glucocorticoid receptor gene locus. *Obes Res.* 1997;5:186-192.

Burack J, Blidner A, Flores H, Fitch T. Constructions and deconstructions of risk, resilience and wellbeing: a model for understanding the development of Aboriginal adolescents. *Australas Psychiatry.* 2007;15:S18-23.

Burchard EG, Ziv E, Coyle N, et al. The importance of race and ethnic background in biomedical research and clinical practice. *N Engl J Med.* 2003;348:1170-1175.

Buss C, Lord C, Wadiwalla M, et al. Maternal care modulates the relationship between prenatal risk and hippocampal volume in women but not in men. *J Neurosci.* 2007;27:2592-2595.

Buss AH, Plomin R. The EAS approach to temperament. In: Plomin R, Dunn J. (Eds.). *The study of temperament: changes, continuities and challenges.* Hillsdale, NJ: Lawrence Erlbaum Associates; 1986.

Cameron CA, Ungar M, Liebenberg L. Cultural understandings of resilience: roots for wings in the development of affective resources for resilience. *Child Adolesc Psychiatr Clin N Am.* 2007;16:285-301.

Campbell-Sills L, Cohan SL, Stein MB. Relationship of resilience to personality, coping, and psychiatric symptoms in young adults. *Behav Res Ther.* 2006;44:585-599.

Canino G, Shrout PE, Rubio-Stipec M, et al. The DSM-IV rates of child and adolescent disorders in Puerto Rico: prevalence, correlates, service use and effects of impairment. *Arch Gen Psychiatry.* 2004;61:85-93.

Carlson SR, Iacono WG, McGue M. P300 amplitude in nonalcoholic adolescent twin pairs who become discordant for alcoholism as adults. *Psychophysiology.* 2004;41:841-844.

Carson DK, Swanson DM, Cooney MH, Gillum BJ, Cunningham D. Stress and coping as predictors of young children's development and psychosocial adjustment. *Child Study J.* 1992;22:273-302.

Cartwright-Hatton S, McNally D, White C, Verduyn C. Parenting skills training: an effective intervention for internalizing symptoms in younger children? *J Child Adolesc Psychiatr Nurs.* 2005;18:45-52.

Caspi A, McClay J, Moffitt TE, et al. Role of genotype in the cycle of violence in maltreated children. *Science.* 2002;297:851-854.

Caspi A, Moffitt TE, Newman DL, Silva PA. Behavioral observations at age 3 years predict adult psychiatric disorders. Longitudinal evidence from a birth cohort. *Arch Gen Psychiatry.* 1996;53:1033-1039.

Caspi A, Sudgen K, Moffitt TE, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science.* 2003;301:386-389.

Castro AJ, Kelly J, Shih M. Resilience strategies for new teachers in high-needs areas. *Teaching Teacher Edu.* 2010;26:622-629.

Cedar H. DNA methylation and gene activity. *Cell.* 1998;8:3-4.

Cederblad M, Dahlin L, Hagnell O, Hansson K. Intelligence and temperament as protective factors for mental health. A cross-sectional and prospective epidemiological study. *Eur Arch Psychiatry Clin Neurosci.* 1995;245:11-19.

Challis JR, Sloboda D, Matthews SG, et al. The fetal placental hypothalamic-pituitary-adrenal (HPA) axis, parturition and post natal health. *Mol Cell Endocrinol.* 2001;185:135-144.

Chantarujikapong SI, Scherrer JF, Xian H, et al. A twin study of generalized anxiety disorder symptoms, panic disorder symptoms and post-traumatic stress disorder in men. *Psychiatry Res.* 2001;103:133-145.

Chao RK. Beyond parental control and authoritarian parenting style: understanding Chinese parenting through the cultural notion of training. *Child Dev.* 1994;65:1111-1119.

Chao HM, Ma LY, McEwen BS, Sakai RR. Regulation of glucocorticoid receptor and mineralocorticoid receptor messenger ribonucleic acids by selective agonists in the rat hippocampus. *Endocrinology.* 1998;139:1810-1814.

Charney DS. Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. *Am J Psychiatry.* 2004;161:195-216.

Chen AJ, Ling SL. Birth weight of newborns and maternal health. *Singapore Med J.* 1984;6:400-403.

Chen GL, Novak MA, Meyer JS, Kelly BJ, Vallender EJ, Miller GM. TPH2 5'- and 3'-regulatory polymorphisms are differentially associated with HPA axis function and self-injurious behavior in rhesus monkeys. *Genes Brain Behav.* 2010;9:335-347.

Chen K, Wu C, Yao G. Applicability of the WHOQOL-BREF on early adolescence. *Soc Indic Res.* 2006;79:215-234.

Chen X, Hastings PD, Rubin KH, Chen H, Cen G, Stewart SL. Child-rearing attitudes and behavioral inhibition in Chinese and Canadian toddlers: a cross-cultural study. *Dev Psychol.* 1998;34:677-686.

Chia SE, Lee J, Chia KS, Chan OY. Low birth weight in relation to parental occupations – a population-based registry in Singapore (1994-1998). *Neurotoxicol Teratol.* 2004;26:285-290.

Christensen K, Petersen I, Skytthe A, Herskind AM, MdGue M, Bingley P. Comparison of academic performance of twins and singletons in adolescence: follow-up study. *BMJ.* 2006;333:1095.

Christopher M. A broader view of trauma: a biopsychosocial-evolutionary view of the role of the traumatic stress response in the emergence of pathology and/or growth. *Clin Psychol Rev.* 2004;24:75-98.

Chung B, Kim H, Lee S, Kwon K, Lee J. *Restoring Korean education from the bandage of entrance examination education*. Seoul: Nanam Publication; 1993.

Ciarrochi J, Heaven PCL, Davies F. The impact of hope, selfesteem, and attributional style on adolescents' school grades and emotional well-being: a longitudinal study. *J Res Pers.* 2007;41:1161-1178.

Clark LA, Watson D, Mineka D. Temperament, personality, and the mood and anxiety disorders. *J Abnorm Psychol.* 1994;103:103-116.

Clayton D, MdKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. *Lancet.* 2001;358:1356-1360.

Cohen RM, Small C, Lalonde F. Effect of apolipoprotein E genotype on hippocampal volume loss in aging healthy women. *Neurology.* 2001;57:2223-2228.

Collishaw S, Pickles A, Messer J, Rutter M, Shearer C, Maughan B. Resilience to adult psychopathology following childhood maltreatment: evidence from a community sample. *Child Abuse Ngl.* 2007;31:211-229.

Comings DE, Blum K. Reward deficiency syndrome: genetic aspects of behavioral disorders. *Prog Brain Res.* 2000;126:325-341.

Comings DE, Muhleman D, Gysin R. Dopamine D2 Receptor (DRD2) gene and susceptibility to posttraumatic stress disorder: a study and replication. *Biol Psychiatry.* 1996;40:368-372.

Conger RD, Ge X, Elder GH, Lorenz FO, Simons RL. Economic stress, coercive family process, and developmental problems of adolescents. *Child Dev.* 1994;65:541-561.

Connor KM, Davidson JR. Development of a new resilience scale: the Connor-Davidson Resilience Scale (CD-RISC). *Depress Anxiety.* 2003;18:76-82.

Copping VE, Warling DL, Benner DG, Woodside DW. A child trauma treatment pilot study. *J Child Fam Stud.* 2001;10:467-475.

Costello EJ, Compton FN, Keeler G, Angold A. Relationships between poverty and psychopathology: a natural experiment. *JAMA.* 2003a;290:2023-2029.

Costello EJ, Foley DL, Angold A. 10-year research update review: the epidemiology of child and adolescent psychiatric disorders. *J Am Acad Child Adolesc Psychiatry.* 2006;45:8-25.

Costello JE, Mustillo S, Erkanli A, Keeler G, Angold A. Prevalence and development of psychiatric disorders in childhood and adolescence. *Arch Gen Psychiatry.* 2003b;60:837-844.

Costello EJ, Pine DS, Hammen C, March JS, et al. Development and natural history of mood disorders. *Biol Psychiatry.* 2002;52:529-542.

Costello EJ, Worthman C, Erkanli A, Angod A. Prediction from low birth weight to female adolescent depression: a test of competing hypotheses. *Arch Gen Psychiatry.* 2007;64:338-344.

Cunha F, Heckman JJ, Lochner LJ, Masterov DV. Interpreting the evidence on life cycle skill formation. In: Hanushek EA, Welch F. (Eds). *Handbook of the economics of education*, Amsterdam: North–Holland; 2006.

Cunha F, Heckman JJ. The technology of skill formation. *Am Econ Rev.* 2007;97:31-47.

Dahl RE. Adolescent brain development: a period of vulnerabilities and opportunities. *Ann N Y Acad Sci.* 2004;1021:1-22.

Davey M, Eaker DG, Walters LH. Resilience processes in adolescents: personality profiles, self-worth, and coping. *J Adolesc Res.* 2003;18:357-362.

Davis EP, Sandman CA. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Dev.* 2010;81:131-148.

Davis LL, Suris A, Lambert MT, Heimberg C, Petty F. Posttraumatic stress disorder and serotonin: new directions for research and treatment. *J Psychiatry Neurosci.* 1997;22:318-326.

Davydov DM, Stewart R, Ritchie K, Chaudieu I. Resilience and mental health. *Clin Psychol Rev.* 2010;30:479-495.

Deater-Deckard K, Dodge KA, Bates JE, Pettit GS. Multiple risk factors in the development of externalizing behaviour problems: group and individual differences. *Dev Psychopathol.* 1998;10:469-493.

Depape AR, Hakim-Larson J, Voelker S, Page S, Jackson DL. Self-talk and emotional intelligence in University Students. *Can J Behav Sci.* 2006;38:250-260.

Singapore Population: Census. Singapore: Department of Statistics; 2000. http://www.singstat.gov.sg/pubn/papers/people/c2000adr-religion.pdf

Di Blasio A, van Rossum EFC, Maestrini S, et al. The relation between two polymorphisms in the glucocorticoid receptor gene and body mass index, blood pressure and cholesterol in obese patients. *Clin Endocrinol.* 2003;59:68-74.

D'Imperio RL, Dubow EF, Ippolito MF. Resilient and stress affected adolescents in an urban setting. *J Clin Child Psychol.* 2000;29:129-142.

Dobson MG, Redfern CP, Unwin N, Weaver JU. The N363S polymorphism of the glucocorticoid receptor: potential contribution to central obesity in men and lack of association with other risk factors for coronary heart disease and diabetes mellitus. *J Clin Endocrinol Metab.* 2001;86:2270-2274.

Doussard-Roosevelt JA, Montgomery LA, Porges SW. Short-term stability of physiological measures in kindergarten children: respiratory sinus arrhythmia, heart period, and cortisol. *Dev Psychobiol.* 2003;43:230-242.

Drago A, Liappas I, Petio C, et al. Epistasis between IL1A, IL1B, TNF, HTR2A, 5-HTTLPR and TPH2 variations does not impact alcohol dependence disorder features. *Int J Environ Res Public Health.* 2009;6:1980-1990.

Du L, Bakish D, Lapierre YD, Ravindran AV, Hrdina PD. Association of polymorphism of serotonin 2A receptor gene with suicidal ideation in major depressive disorder. *Am J Med Genet.* 2000;96:56-60.

Durlak JA, Wells AM. Primary prevention mental health programs for children and adolescents: a meta-analytic review. *Am J Community Psychol.* 1997;25:115-152.

Dwyer SB, Nicholson JM, Battistutta D. Population level assessment of the family risk factors related to the onset of children's mental health problems. *J Child Psychol Psychiatry.* 2003;44:699-711.

van Dyke CJ, Elias MJ. How forgiveness, purpose, and religiosity are related to the mental health and well-being of youth: a review of the literature. *MHRC.* 2007;10:395-415.

Eide MG, Oyen N, Skjaerven R, Bjerkedal T. Associations of birth size, gestational age, and adult size with intellectual performance: evidence from a cohort of Norwegian men. *Pediatr Res.* 2007;62:636-642.

Eley TC, Sugden K, Corsico A, et al. Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Mol Psychiatry.* 2004;9:908-915.

van der Ende J. Personal communication and provided data. Rotterdam, The Netherlands: Erasmus University; 2009.

Eng E. Locke's tabula rasa and Freud's mystic writing pad. *J Hist Ideas.* 1980;41:133-140.

Engelmann M, Landgraf R, Wotjak C. The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. *Front Neuroendocrinol.* 2004;25:132-149.

Engle PL, Castle S, Menon P. Child development: vulnerability and resilience. *Soc Sci Med.* 1996;43:621-635.

Evers HD. Southeast Asia: sociocultural aspects. *Int Encyclopedia Soc Behav Sci.* 2004:14661-14666.

Feusner J, Ritchie T, Lawford B, Young RM, Kann B, Noble EP. GABA (A) receptor beta 3 subunit gene and psychiatric morbidity in a post-traumatic stress disorder population. *Psychiatry Res.* 2001;104:109-117.

Figee M, van Amelsvoort T, de Haan L, Linszen DH. Genetic research into schizophrenia. *Tijdschr Psychiatr.* 2004;46:15-25.

Fish EW, Shahrokh D, Bagot R, et al. Epigenetic programming of stress responses through variations in maternal care. *Ann N Y Acad Sci.* 2004;1036:167-180.

Fisher PA, Gunnar MR, Chamberlain P, Reid JB. Preventive intervention for maltreated preschool children: impact on children's behaviour, neuroendocrine activity, and foster parent functioning. *J Am Acad Child Adolesc Psychiatry.* 2000;39:1356-1364.

Flisher AJ, Gerein N. Adolescents. In: Heggenhogen HK (Eds.). *Encyclopedia of public health.* San Diego, CA: Elsevier; 2008.

Flory JD, Manuck SB, Ferrell RE. Memory performance and the apolipoprotein E polymorphism in a community sample of middleaged adults. *Am J Med Gen.* 2000;96:707-711.

Fogel RW. *The escape from hunger and premature death, 1700-2100: Europe, America and the Third World.* Cambridge, UK: Cambridge Univ Press; 2003.

Fones CS, Kua EH, Ng TP, Ko SM. Studying the mental health of a nation: a preliminary report on a population survey in Singapore. *Singapore Med J.* 1998;39:251-255.

Ford T, Goodman R, Meltzer H. The relative importance of child, family, school and neighbourhood correlates of childhood psychiatric disorder. *Soc Psychiatry Psychiatr Epidemiol.* 2004;39:487-496.

Fossion P, Linkowski P. The relevance of the concept of resiliency in the field of psychiatry. *Rev Med Brux.* 2007;28:33-38.

Francis DD, Caldji C, Champagne F, Plotsky PM, Meaney MJ. The role of corticotropin-releasing factor--norepinephrine systems in mediating the effects of early experience on the development of behavioral and endocrine responses to stress. *Biol Psychiatry*. 1999;46:1153-1166.

Franić S, Middeldorp CM, Dolan CV, Ligthart L, Boomsma DI. Childhood and adolescent anxiety and depression: beyond heritability. *J Am Acad Child Adolesc Psychiatry*. 2010;49:820-829.

Freeman T, Roca V, Guggenheim F, Kimbrell T, Griffin WST. Neuropsychiatric associations of apolipoprotein E alleles in subjects with combat-related posttraumatic stress disorder. *J Neuropsychiatry Clin Neurosci*. 2005;17:541-543.

Freisling H, Elmadfa I, Gall I. The effect of socioeconomic status on dietary intake, physical activity and Body Mass Index in Austrian pregnant women. *J Hum Nutr Diet*. 2006;19:437-445.

Friedman HL. The health of adolescents: beliefs and behaviour. *Soc Sci Med*. 1989;29:309-315.

Frisch A, Postilnick D, Rockah R, et al. Association of unipolar major depressive disorder with genes of the serotonergic and dopaminergic pathways. *Mol Psychiatry*. 1999;4:389-392.

Fukuchi I, Morato MM, Rodriques RE, et al. Pre and postoperative psychological profile of children submitted to adenoidectomy and/or tonsillectomy. *Rev Bras de Otorrinolaringol*. 2005;71:521-525.

Fullerton CS, Ursano RJ, Epstein RS, et al. Peritraumatic dissociation following motor vehicle accidents: relationship to prior trauma and prior major depression. *J Nerv Ment Dis*. 2000;188:267-272.

Fullerton CS, Ursano RJ, Epstein RS, et al. Gender differences in posttraumatic stress disorder after motor vehicle accidents. *Am J Psychiatry*. 2001;158:1468-1491.

Gale CR, Martyn CN. Birth weight and later risk of depression in a national birth cohort. *Br J Psychiatry*. 2004;184:28-33.

Gale CR, O'Callaghan FJ, Bredow M, Martyn CN. Avon Longitudinal Study of Parents and Children Study Team.The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. *Pediatrics*. 2006;118:1486-1492.

Gale CR, O'Callaghan FJ, Godfrey KM, Law CM, Martyn CN. Critical periods of brain growth and cognitive function in children. *Brain*. 2004;127:321-329.

Gallagher-Thompson D, O'Hara R, Simmons A. Apolipoprotein E epsilon4 allele affects the relationship between stress and depression in caregivers of patients with Alzheimer's disease. *J Geriatr Psychiatry Neurol*. 2001;14:115-119.

Gallinat J, Strohle A, Lang UE, et al. Association of human hippocampal neurochemistry, serotonin transporter genetic variation, and anxiety. *Neuroimage*. 2005;26:123-131.

Garetz SL. Behaviour, cognition, and quality of life after adenotonsillectomy for pediatric sleep-disordered breathing: summary of the literature. *Arch Otolaryngol Head Neck Surg*. 2008;138:S19-26.

Garmezy N. Stressors of childhood. In: Garmezy N, Rutter M. (Eds.). *Stress, coping, and development in children*. New York, US: McGraw-Hill; 1983.

Garmezy N, Masten AS, Tellegen A. The study of stress and competence in children: a building block for developmental psychopathology. *Child Dev*. 1984;55:97-111.

Gaspar P, Cases O, Maroteaux L. The developmental role of serotonin: news from mouse molecular genetics. *Nat Rev Neurosci*. 2003;4:1002-1012.

Gass P, Reichardt HM, Strekalova T, Henn F, Tronche F. Mice with targeted mutations of glucocorticoid and mineralocorticoid receptors: models for depression and anxiety? *Physiol Behav*. 2001;73:811-825.

Gau SS, Chong MY, Chen TH, Cheng AT. A 3-year panel study of mental disorders among adolescents in Taiwan. *Am J Psychiatry*. 2005;162:1344-1350.

Gelernter J, Southwick S, Goodson S, Morgan A, Nagy L, Charney DS. No association between D2 dopamine receptor (DRD2) 'A' system alleles, or DRD2 haplotypes, and posttraumatic stress disorder. *Biol Psychiatry*. 1999;45:620-625.

Gilbertson MW, Shenton ME, Ciszewski A, et al. Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat Neurosci*. 2002;5:1242-1247.

Gillespie NA, Whitfield JB, Williams B, Heath AC, Martin NG. The relationship between stressful life events, the serotonin transporter (5 HTTLPR) genotype and major depression. *Psychol Med*. 2005;35:101-111.

Glatz K, Mossner R, Heils A, Lesch KP. Glucocorticoidregulated human serotonin transporter (5-HTT) expression is modulated by the 5-HTT gene-promoter-linked polymorphic region. *J Neurochem*. 2003;86:1072-1078.

Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science.* 2004;305:1733-1736.

Gluckman PD, Hanson MA. *The fetal matrix: evolution, development, and disease.* Cambridge, UK: Cambridge Univ Press; 2005.

Gluckman PD, Hanson MA. *Developmental origins of health and disease.* Cambridge, UK: Cambridge University Press; 2006a.

Gluckman PD, Hanson MA. *Mismatch: why our world no longer fits our bodies.* Oxford, UK: Oxford Univ Press; 2006b.

Gluckman PD, Hanson MA, Beedle AS. Early life events and their consequences for later disease: a life history and evolutionary perspective. *Am J Hum Biol.* 2007;19:1-19.

Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med.* 2008;359:61-73.

Gluckman PD, Hanson MA, Morton SM, Pinal CS. Life-long echoes--a critical analysis of the developmental origins of adult disease model. *Biol Neonate.* 2005;87:127-139.

Goland RS, Jozak S, Warren WB, Conwell IM, Stark RI, Tropper PJ. Elevated levels of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses. *J Clin Endocrinol Metab.* 1993;77:1174-1179.

Goldberg DP, Hillier VF. A scaled version of the General health Questionnaire. *Psychol Med.* 1979;9:139-145.

Golding J, Rogers IS, Emmett PM. Association between breast feeding, child development and behaviour. *Early Hum Dev.* 1997;49:S175-184.

Goldstein NA, Fatima M, Campbell TF, Rosenfeld RM. Child behaviour and quality of life before and after tonsillectomy and adenoidectomy. *Arch Otolaryngol Head Neck Surg.* 2002;128:770-775.

Goldstein LE, Rasmusson AM, Bunney BS, Roth RH. Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *J Neurosci.* 1996;16:4787-4798.

Gómez F, De Kloet ER, Armario A. Glucocorticoid negative feedback on the HPA axis in five inbred rat strains. *Am J Physiol.* 1998;274:R420-R427.

Goodlin-Jones BL, Sitnick SL, Tang K, Liu J, Anders TF. The children's sleep habits questionnaire in toddlers and preschool children. *J Dev Behav Pediatr.* 2008;29:82-88.

Gordon J, Barnes NM. Lymphocytes transport serotonin and dopamine: agony or ecstasy? *Trends Immunol.* 2003;24:438-443.

Gordon KA. The self-concept and motivational patterns of resilient African American high school students. *J Black Psychol.* 1995;21:239-255.

Gotlib IH, Joormann J, Minor KL, Hallmayer J. HPA axis reactivity: a mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biol Psychiatry.* 2008;63:847-851.

Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry.* 2003;160:636-645.

Graeff FG, Guimaraes FS, De Andrade TG, Deakin JF. Role of 5-HT in stress, anxiety and depression. *Pharmacol Biochem Behav.* 1996;54:129-141.

Greben SE. *The place of psychotherapy in medicine and the related professions.* Jerusalem, Israel: Latner Institute for Research in Social Psychiatry and Psychotherapy; 1992.

Groot P, de Geus E, de Vries J. *Ambulatory Monitoring System.* Amsterdam, The Netherlands: Department of Psychophysiology, Vrije University; 1998a.

Groot P, de Geus E, de Vries J. *AMRES Manual: Scoring the Respiration Signal.* Amsterdam, The Netherlands: Department of Psychophysiology, Vrije University; 1998b.

Groschl M, Rauh M, Dorr H. Circadian rhythm of salivary cortisol, 17alphahydroxyprogesterone, and progesterone in healthy children. *Clin Chem.* 2003;49:1688-1691.

Gross C, Hen R. Genetic and environmental factors interact to influence anxiety. *Neurotox Res.* 2004a;6:493-501.

Gross C, Hen R. The developmental origins of anxiety. *Nat Rev Neurosci.* 2004b;5:545-552.

Grossman R, Buchsbaum MS, Yehuda R. Neuroimaging studies in post-traumatic stress disorder. *Psychiatr Clin North Am.* 2002;25:317-340.

Gunnar M, Quevedo K. The neurobiology of stress and development. *Annu Rev Psychol.* 2007;58:145-173.

Gutknecht L, Jacob C, Strobel A, et al. Tryptophan hydroxylase-2 gene variation influences personality traits and disorders related to emotional dysregulation. *Int J Neuropsychopharmacol.* 2007;10:309-320.

Hackman DA, Farah MJ, Meaney MJ. Socioeconomic status and the brain: mechanistic insights from human and animal research. *Nat Rev Neurosci.* 2010;11:651-656.

Haddadi P, Besharat MA. Resilience, vulnerability and mental health. *Proc Soc Behav Sci.* 2010;5:639-642.

Hamet P, Tremblay J. Genetics and genomics of depression. *Metabolism.* 2005;54:10-15.

Hamner MB, Diamond BI. Elevated plasma dopamine in posttraumatic stress disorder: a preliminary report. *Biol Psychiatry.* 1993;33:304-306.

Harbuz MS, Lightman SL. Stress and the hypothalamo-pituitary-adrenal axis: acute, chronic and immunological activation. *J Endocrinol.* 1992;134:327-329.

Harden BJ. Safety and stability of foster children: a developmental perspective. *Future Child.* 2004;14:30-47.

Hardt J, Rutter M. Validity of adult retrospective reports of adverse childhood experiences: review of the evidence. *J Child Psychol Psychiatry.* 2004;45:260-273.

Hardy J, Singleton A. The HapMap: charting a course for genetic discovery in neurological diseases. *Arch Neurol.* 2008;65:319-321.

Hariri AR, Drabant EM, Munoz KE, et al. A susceptibility gene for affective disorders and the response of the human amygdala. *Arch Gen Psychiatry.* 2005;62:146-152.

Hariri AR, Mattay VS, Tessitore A, et al. Serotonin transporter genetic variation and the response of the human amygdala. *Science.* 2002;297:400-403.

Hawk LW, Dougall AL, Ursamno RJ, Baum A. Urinary catecholamines and cortisol in recent-onset posttraumatic stress disorder after motor vehicle accidents. *Psychosom Med.* 2000;62:423-434.

Heaven PC, Ciarrochi J. Personality and religious values among adolescents: a three-wave longitudinal analysis. *Br J Psychol.* 2007;98:93-99.

Hefner J, Eisenberg D. Social support and mental health among college students. *Am J Orthopsychiatry.* 2009;79:491-499.

Heim C, Nemeroff CB. The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. *Biol Psychiatry.* 1999;46:1509-1522.

Heim C, Nemeroff CB. Neurobiology of early life stress: clinical studies. *Semin Clin Neuropsychiatry.* 2002;7:147-159.

van Helvoort K, Clarijs Y. *Handboek Marietje Kessels Project. Weerbaarheidsvergroting bij kinderen van 10 tot 13 jaar.* Amsterdam, The Netherlands: SWP; 2005

Henry JP. Biological basis of the stress response. *Integr Physiol Behav Sci.* 1992;27:66-83.

Herba CM, Roza SJ, Govaert P, et al. Infant brain development and vulnerability to later internalizing difficulties: the Generation R study. *J Am Acad Child Adolesc Psychiatry.* 2010;49:1053-1063.

Hertzman C, Wiens M. Child development and long-term outcomes: a population health perspective and summary of successful interventions. *Soc Sci Med.* 1996;43:1083-1095.

Hesselbrock V, Begleiter H, Porjesz B, O'Connor S, Bauer L. P300 event-related potential amplitude as an endophenotype of alcoholism- evidence from the collaborative study on the genetics of alcoholism. *J Biomed Sci.* 2001;8:77-82.

Ho KC, Yip J. *YOUTH.sg: The state of youth in Singapore.* Singapore: National Youth Council; 2003.

Hobfoll SE. *The ecology of stress.* USA: Hemisphere Publishing Corporation; 1988.

Hoffman-Goetz L, Pedersen BK. Exercise and the immune system: a model of the stress response? *Immunol Today.* 1994;15:382-287.

Hollo O, Rautava P, Korhonen T, Helenius H, Kero P, Sillanpää M. Academic achievement of small-for-gestational-age children at age 10 years. *Arch Pediatr Adolesc Med.* 2002;156:179-187.

Holsboer F, Barden N. Antidepressants and hypothalamicpituitary-adrenocortical regulation. *Endocr Rev.* 1996;17:187-205.

van den Hove DL, Kenis G, Steinbusch HW, Blanco CE, Prickaerts J. Maternal stress-induced reduction in birth weight as a marker for adult affective state. *Front Biosci.* 2010;2:43-46

Hu YQ, Gan YQ. Development and psychometric validity of the Resilience Scale for Chinese Adolescents. *Act Psychol Sin.* 2008;40:902-912.

Huang RC, Burke V, Newnham JP, et al. Perinatal and childhood origins of cardiovascular disease. *Int J Obes.* 2007;31:236-244.

Huizenga NA, Koper JW, de Lange P, et al. A polymorphism in the glucocorticoid receptor gene may be associated with an increased sensitivity to glucocorticoids in vivo. *J Clin Endocrinol Metab.* 1998;83:144-151.

Illi A, Setälä-Soikkeli E, Viikki M, et al. 5-HTR1A, 5-HTR2A, 5-HTR6, TPH1 and TPH2 polymorphisms and major depression. *Neuroreport.* 2009;20:1125-1128.

Inglis FM, Moghaddam B. Dopaminergic innervation of the amygdala is highly responsive to stress. *J Neurochem.* 1999;72:1088-1094.

Isralowitz RE, Hong OT. Singapore youth: the impact of social status on perceptions of adolescent problems. *Adolescence.* 1990;25:358-362.

Jaenisch R, Bird A. Epigenetic regulation of gene expression; how the genome integrates intrinsic and environmental signals. *Nat Genet.* 2003;33:245-254.

Janicke DM, Harman JS, Kelleher KJ, Zhang J. Psychiatric diagnosis in children and adolescents with obesity-related health conditions. *J Dev Behav Pediatr.* 2008;29:276-284.

Johnson, M. Approaching the salutogenesis of sense of coherence: the role of „active" self-esteem and coping. *Br J Health Psychol.* 2004;9:419-432.

Jokela M, Keltikangas-Järvinen L, Kivimäki M, et al. Serotonin receptor 2A gene and the influence of childhood maternal nurturance on adulthood depressive symptoms. *Arch Gen Psychiatry.* 2007;64:356-360.

Jolly R. Early childhood development: the global challenge. *Lancet.* 2007;369:8-9.

Joseph J. Twin studies in psychiatry and psychology: science or pseudoscience? *Psychiatr Q.* 2002;73:71-82.

Kagan JJ, Reznick S, Snidman N. Biological bases of childhood shyness. *Science.* 1988;240:167-171.

Kajantie E, Räikkönen K. Early life predictors of the physiological stress response later in life. *Neurosci Biobehav Rev.* 2010;35:23-32.

Kalia, M. Neurobiological basis of depression: an update. *Metabolism.* 2005;54:24-27.

Kallio J, Pesonen U, Kaipio K, et al. Altered intracellular processing and release of neuropeptide Y due to Leucine 7 to Proline 7 polymorphism in the signal peptide of preproneuropeptide Y in humans. *FASEB J.* 2001;15:1242-1244.

Kamin LJ, Goldberger AS. Twin studies in behavioral research: a skeptical view. *Theor Popul Biol.* 2002;61:83-95.

Kassel JD, Bornovalova M, Mehta N. Generalized expectancies for negative mood regulation predict change in anxiety and depression among college students. *Behav Res Ther.* 2006;45:939-950.

Kebir O, Tabbane K, Sengupta S, Joober R. Candidate genes and neuropsychological phenotypes in children with ADHD: a review of association studies. *J Psychiatry Neurosci.* 2009;34:88-101.

Keck ME, Holsboer F, Muller MB. Mouse mutants for the study of corticotropin-releasing hormone receptor function: development of novel treatment strategies for mood disorders. *Ann N Y Acad Sci.* 2004;1018:445-457.

Keiley MK, Howe TR, Dodge KA, Bates JE, Pettit GS. The timing of child physical maltreatment: a cross-domain growth analysis of impact on adolescent externalizing and internalizing problems. *Dev Psychopathol.* 2001;13:891-912.

Keiley MK, Lofthouse N, Bates JE, Dodge KA, Pettit GS. Differential risks of co-varying and pure components in mother and teacher reports of externalising and internalising behaviour across ages 5 to 14. *J Abnorm Child Psychol.* 2003;31:267-283.

Kellner M, Baker DG, Yassouridis A, et al. Mineralocorticoid receptor function in patients with posttraumatic stress disorder. *Am J Psychiatry.* 2002;159:1938-1940.

Kelly YJ, Nazroo JY, McMunn A, Boreham R, Marmot M. Birthweight and behavioural problems in children: a modifiable effect? *Int J Epidemiol.* 2001;30:88-94.

Kendler KS, Karkowski-Schuman L. Stressful life events and genetic liability to major depression: genetic control of exposure to the environment? *Psychol Med.* 1997;27:539-547.

Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch Gen Psychiatry.* 2005;62:529-535.

Kerr M, Tremblay RE, Pagani L, Vitaro F. Boys' behavioral inhibition and the risk of later delinquency. *Arch Gen Psychiatry*. 1997;54:809-816.

Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 2005;62:617-627.

Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB. Posttraumatic Stress Disorder in the National Comorbidity Survey. *Arch Gen Psychiatry*. 1995;52:1048-1060.

Kierans WJ, Joseph KS, Luo ZC, Platt R, Wilkins R, Kramer MS. Does one size fit all? The case for ethnic-specific standards of fetal growth. *BMC Pregnancy Childbirth*. 2008;8:1

Kim DY, Rah YC, Kim DW, et al. Impact of tonsillectomy on pediatric psychological status. *Int J Pediatr Otorhinolaryngol*. 2008;72:1359-1363.

de Kloet ER, Derijk R. Signaling pathways in brain involved in predisposition and pathogenesis of stress-related disease: genetic and kinetic factors affecting the MR/GR balance. *Ann N Y Acad Sci*. 2004;1032:14-34.

de Kloet ER, Sibug RM, Helmerhorst FM, Schmidt M. Stress, genes and the mechanism of programming the brain for later life. *Neurosci Biobehav Rev*. 2005;29:271-281.

de Kloet ER, Vreugdenhil E, Oitzl AS, Joels M. Brain corticosteroid receptor balance in health and disease. *Endocr Rev*. 1998;19:269-301.

Knudson EI, Heckman JJ, Cameron JL, Shonkoff J. Economic, neurobiological, and behavioral perspectives on building America's future workforce. *PNAS*. 2006;103:10155-10162.

Koenen KC, Lyons MJ, Goldberg J, et al. A high risk twin study of combat-related PTSD comorbidity. *Twin Res*. 2003;6:218-226.

Kong DS, Wong ST, Goh SW, Lam SL, Chua KL, Kok LP. Child-rearing practices of Chinese parents and their relationship to behavioural problems in toddlers. *Acta Psychiatr Scand*. 1988;S344:127-132.

Koopman C, Classen C, Spiegel D. Predictors of posttraumatic stress symptoms among survivors of the Oakland/Berkeley, California, firestorm. *Am J Psychiatry*. 1994;151:888-894.

Kostovic I, Rakic P. Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J Comp Neurol*. 1990;297:441-470.

Kotinimie LH, Ryhanen PT, Moilanen IK. Behavioural changes following routine ENT operations in two-to-ten-year-old children. *Paediatr Anaesth*. 1996;6:45-49.

Kramer MS, Aboud F, Mironova E, et al. Promotion of Breastfeeding Intervention Trial (PROBIT) Study Group. Breastfeeding and child cognitive development: new evidence from a large randomized trial. *Arch Gen Psychiatry*. 2008;65:578-584.

Kua J, Wong KE, Kua EH, Tsoi WF. A 20-year follow-up study on schizophrenia in Singapore. *Acta Psychiatr Scand*. 2003;108:118-125.

Kuller LH, Shemanski L, Manolio T. Relationship between APOE, MRI findings, and cognitive function in the Cardiovascular Health Study. *Stroke*. 1998;29:388-389.

Lahti J, Räikkönen K, Heinonen K, et al. Body size at birth and socio-economic status in childhood: implications for Cloninger's psychobiological model of temperament at age 60. *Psychiatry Res*. 2008;160:167-174.

Langeland W, Olff M. Psychobiology of posttraumatic stress disorder in pediatric injury patients: a review of the literature. *Neurosci Biobehav Rev*. 2008;32:161-174.

Lappalainen J, Kranzler HR, Malison R, et al. A functional neuropeptide Y Leu7Pro polymorphism associated with alcohol dependence in a large population sample from the United States. *Arch Gen Psychiatry*. 2002;59:825-831.

Larroque B, Ancel PY, Marret S, et al. Neurodevelopmental disabilities and special care of 5-year-old children born before 33 weeks of gestation (the EPIPAGE study): a longitudinal cohort study. *Lancet*. 2008;371:813-820.

Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT. Metaanalysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. *Am J Med Genet B Neuropsychiatr Genet*. 2005;133:110-115.

Lauder JM. Hormonal and humoral influences on brain development. *Psychoneuroendocinology*. 1983;8:121-155.

Lawford BR, Young RMcD, Noble EP, et al. D2 dopamine receptor gene polymorphism: paroxetine and social functioning in posttraumatic stress disorder. *Eur Neuropsychopharmacol*. 2003;13:313-320.

Lawlor DA, Clark H, Smith GD, Leon DA. Intrauterine growth and intelligence within sibling pairs: findings from the Aberdeen children of the 1950s cohort. *Pediatrics.* 2006;117:e894-902.

Lazarus RS. From psychological stress to the emotions: a history of changing outlooks. *Annu Rev Psychol.* 1993;44:1-22.

Lazarus RS, Folkman S. *Stress, appraisal and coping*. New York, USA: Springer; 1984.

Lazary J. Serotonin transporter gene and negative life change events are associated with depressive phenotype. *Neuropsychopharmacol Hung.* 2010;12:347-354.

Lazary J, Lazary A, Gonda X, et al. New evidence for the association of the serotonin transporter gene (SLC6A4) haplotypes, threatening life events, and depressive phenotype. *Biol Psychiatry.* 2008;64:498-504.

Lee M, Larson R. The Korean "examination hell": long hours of studying, distress and depression. *J Youth Adolesc.* 2002;29:249-272.

Lee HJ, Kang RH, Lim SW, Paik JW, Choi MJ, Lee MS. Short communication: no association between the brain-derived neurotrophic factor gene Val66Met polymorphism and post-traumatic stress disorder. *Stress Health.* 2006;22:115-119.

Lee HJ, Lee MS, Kang RH, et al. Influence of the serotonin transporter promoter gene polymorphism on susceptibility to posttraumatic stress disorder. *Depress Anxiety.* 2005;21:135-139.

Lee TY. Resilience as a positive youth development construct: conceptual bases and implications for curriculum development. *Int J Adolesc Med Health.* 2006;18:475-482.

Lee TY, Kwong WM, Cheung CK, Ungar M, Cheung MYL. Children's resilience-related beliefs as a predictor of positive child development in the face of adversities: implications for interventions to enhance children's quality of life. *Soc Indic Res.* 2010;95:437-453.

Lee TY, Shek DT, Kwong WM. Chinese approaches to understanding and building resilience in at-risk children and adolescents. *Child Adolesc Psychiatr Clin N Am.* 2007;16:377-392.

Lerner RM, East PL. The role of temperament in stress, coping and socioemotional functioning in early development. *IMHJ.* 1984;5:148-159.

Lesch KP. Gene transfer to the brain: emerging therapeutic strategy in psychiatry? *Biol Psychiatry.* 1999;45:247-253.

Leung PW, Hung SF, Ho TP, et al. Prevalence of DSM-IV disorders in Chinese adolescents and the effects of an impairment criterion: a pilot community study in Hong Kong. *Eur Child Adolesc Psychiatry.* 2008;17:452-461.

Levenson JM, Sweatt JD. Epigenetic mechanisms in memory formation. *Nat Rev Neurosci.* 2005;6:108-118.

Levinson DF. The genetics of depression: a review. *Biol Psychiatry.* 2006;60:84-92.

Li YJ, Goh, L, Khor CC, et al. Genome-wide association studies reveal genetic variants in CTNND2 for high myopia in Singapore Chinese. *Opthalmology.* 2010;2:368-375.

Liberzon I, Lopez JF, Flagel SB, et al. Differential regulation of hippocampal glucocorticoid receptors mRNA and fast feedback: relevance to post traumatic stress disorder. *J Neuroendocrinol.* 1999;11:11-17.

van Lieshout RJ, Boylan K. Increased depressive symptoms in female but not male adolescents born at low birth weight in the offspring of a national cohort. *Can J Psychiatry.* 2010;55:422-430.

Lim JE, Pinsonneault J, Sadee W, Saffen D. Tryptophan hydroxylase 2 (TPH2) haplotypes predict levels of TPH2 mRNA expression in human pons. *Mol Psychiatry.* 2007;12:491-501.

Lin CC, Fu V. A comparison of child-rearing practices among Chinese, immigrant Chinese, and Caucasian-American parents. *Child Dev.* 1990;61:429-433.

Lipman EL, Boyle MH, Dooley MD, Offord DR. Child well-being in single-mother families. *J Am Acad Child Adolesc Psychiatry.* 2002;41:75-82.

Lippa CF, Smith TW, Saunders WB. Apolipoprotein E-epsilon 2 and Alzheimer's disease: genotype influences pathologic phenotype. *Neurology.* 1997;48:515-519.

Lipton SD. On the psychology of childhood tonsillectomy. In: Eissler RS, Kris M, Hartmann H, Freud A (Eds.). *The psychoanalytic study of the child, vol. XVIII.* New York, USA: International University Press; 1962.

Liu X, Kurita H, Guo C, Miyake Y, Ze J, Cao H. Prevalence and risk factors of behavioural and emotional problems among Chinese children aged 6 through 11 years. *J Am Acad Child Adolesc Psychiatry.* 1999;38:708-715.

Lopez VA, Detera-Wadleigh S, Cardona I, National Institute of Mental Health Genetics Initiative Bipolar Disorder Consortium, Kassem L, McMahon FJ. Nested association between genetic variation in tryptophan hydroxylase II, bipolar affective disorder, and suicide attempts. *Biol Psychiatry.* 2007;61:181-186.

Lupien SJ, De Leon M, De Santi S, et al. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci.* 1998;1:69-73.

Lupien SJ, Gaudreau S, Tschiteya BM, et al. Stress-induced declarative memory impairment in healthy elderly subjects: relationship to cortisol reactivity. *J Clin Endocrinol Metab.* 1997;82:2070-2075.

Lupien SJ, King S, Meaney MJ, McEwen BS. Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. *Biol Psychiatry.* 2000;48:976-980.

Luthar SS. Vulnerability and resilience: a study of high-risk adolescents. *Child Dev.* 1991;62:600-616.

Luthar SS, Cicchetti D, Becker B. The construct of resilience: a critical evaluation and guidelines for future work. *Child Dev.* 2000;71:543-562.

Luthar SS, Zigler E. Vulnerability and competence: a review of research on resilience in childhood. *Am J Orthopsychiatry.* 1992;61:6-22.

Maccari S, Darnaudery M, Morley-Fletcher S, Zuena AR, Cinque C, Van Reeth O. Prenatal stress and long-term consequences: implications of glucocorticoid hormones. *Neurosci Biobehav Rev.* 2003;27:119-127.

Mahley RW. Apolipoprotin E: cholesterol transport protein with expanding role in cell biology. *Science.* 1988;240:622-630.

Mäkikangas A, Kinnunen U, Feld T. Self-esteem, dispositional optimism, and health: evidence from cross-lagged data on employees. *J Res Pers.* 2000;38:556-575.

Martin RA, Puhlik-Doris P, Larsen G, Gray JWK. Individual differences in uses of humour and their relation to psychological well-being: development of the Humour Styles Questionnaire. *J Res Pers.* 2003;37:48-75.

Martinez D, Gelernter J, Abi-Dargham A, et al. The variable number of tandem repeats polymorphism of the dopamine transporter gene is not associated with significant change in dopamine transporter phenotype in humans. *Neuropsychopharmacology.* 2001;24:553-560.

Mason JW, Wang S, Yehuda R, Riney S, Charney DS, Southwick SM. Psychogenic lowering of urinary cortisol levels linked to increased emotional numbing and a shame-depressive syndrome in combat-related posttraumatic stress disorder. *Psychosom Med.* 2001;63:387-401.

Masten AS. Resilience in individual development: successful adaptation despite risk and adversity. In: Wang MC and Gordon EW (Eds.). *Educational resilience in inner-city America, challenges and prospects.* Hillsdale, New Jersey, USA: Lawrence Erlbaum Associated Inc.; 1994.

Masten AS, Best K, Garmezy N. Resilience and development: contributions from the study of children who overcome adversity. *Dev Psychopathol.* 1990;2:425-444.

Masten AS, Hubbard JJ, Gest SD, Tellegen A, Garmezy N, Ramirez M. Competence in the context of adversity: pathways to resilience and maladaptation from childhood to late adolescence. *Dev Psychopathol.* 1999;11:143-169.

Matte TD, Bresnahan M, Begg MD, Susser E. Influence of variation in birth weight within normal range and within sibships on IQ at age 7 years: cohort study. *BMJ.* 2001;323:310-314.

May FS, Chen QC, Gilbertson MW, Shenton ME, Pitman RK. Cavum septum pellucidum in monozygotic twins discordant for combat exposure: relationship to posttraumatic stress disorder. *Biol Psychiatry.* 2004;55:656-658.

McAuley EZ, Fullerton JM, Blair IP, Donald JA, Mitchell PB, Schofield PR. Association between the serotonin 2A receptor gene and bipolar affective disorder in an Australian cohort. *Psychiatr Genet.* 2009;19:244-252.

McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med.* 1998;338:171-179.

McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.* 2000;886:172-189.

McEwen BS. Protective and damaging effects of stress mediators: central role of the brain. *Dialogues Clin Neurosci.* 2006;8:367-381.

McEwen BS. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol.* 2008;583:174-85.

McGowan PO, Sasaki A, D'Alessio AC, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci.* 2009;12:342-348.

McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, Kessler RC. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication II. *Arch Gen Psychiatry.* 2010;67:124-132.

McMahon FJ, Buervenich S, Charney D, et al. Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *Am J Hum Genet.* 2006;78:804-814.

Meaney MJ. Epigenetics and the biological definition of gene x environment interactions. *Child Dev.* 2010;81:41-79.

Meaney MJ, Szyf M. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin Neurosci.* 2005;7:103-123.

Meaney MJ, Szyf M, Seckl JR. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends Mol Med.* 2007;13:269-277.

Meewisse ML, Reitsma JB, de Vries GJ, Gersons BP, Olff M. Cortisol and post-traumatic stress disorder in adults: systematic review and meta-analysis. *Br J Psychiatry.* 2007;191:387-92.

Meloni EG, Davis M. Enhancement of the acoustic startle response in rats by the dopamine D1 receptor agonist SKF 82958. *Psychopharmacology.* 1999;144:373-380.

Mendlewicz J, Massat I, Souery D, et al. Neuroendocrine aspects of the response to stress. *Metabolism.* 2002;51:5-10.

Milanova V, van Broeckhoven C. Serotonin transporter 5HTTLPR polymorphism and affective disorders: no evidence of association in a large European multicenter study. *Eur J Hum Genet.* 2004;12:377-382.

Miller DM, O'Callaghan JP. Neuroendocrine aspects of the response to stress. *Metabolism.* 2002;51:5-10.

Monroe SM, Simons AD, Thase ME. Onset of depression and time to treatment entry: roles of life stress. *J Consult Clin Psychol.* 1991;59:566-573.

Mordkowitz ER, Ginsberg HP. Early academic socialization of successful Asian American college students. *LCHC.* 1987;9:85-91.

Morgan III CA, Wang S, Southwick SM, et al. Plasma neuropeptide-Y concentrations in humans exposed to military survival training. *Biol Psychiatry.* 2000;47:902-909.

Munroe-Blum H, Boyle M, Offord DR. Single parent families: child psychiatric disorder and school performance. *J Am Acad Child Adolesc Psychiatry.* 1988;27:214-219.

Murphy DL, Li Q, Engel S, et al. Genetic perspectives on the serotonin transporter. *Brain Res Bull.* 2001;56:487-494.

Nakaya M, Oshio A, Kaneko H. Correlations for adolescent resilience scale with big five personality traits. *Psychol Rep.* 2006;98:927-930.

Noble EP. The DRD2 gene in psychiatric and neurological disorders and its phenotypes. *Pharmacogenomics.* 2000;1:309-333.

Nomura Y, Wickramaratne PJ, Pilowsky DJ, et al. Low birth weight and risk of affective disorders and selected medical illness in offspring at high and low risk for depression. *Compr Psychiatry.* 2007;48:470-478.

Obradović J, Boyce WT. Individual differences in behavioral, physiological, and genetic sensitivities to contexts: implications for development and adaptation. *Dev Neurosci.* 2009;31:300-308.

Obradović J, Bush NR, Stamperdahl J, Adler NE, Boyce WT. Biological sensitivity to context: the interactive effects of stress reactivity and family adversity on socioemotional behavior and school readiness. *Child Dev.* 2010;81:270-289.

O'Connor TG, Rutter M, Beckett C, Keaveney L, Kreppner JM. The effects of global severe privation on cognitive competence: extension and longitudinal follow-up. English and Romanian Adoptees Study Team. *Child Dev.* 2000;71:376-390.

Offord DR, Boyle MH, Szatmari P, et al. Ontario Child Health Study II. Six-month prevalence of disorder and rates of service utilization. *Arch Gen Psychiatry.* 1987;44:832-836.

Ohan JL, Myers K, Collet BR. Ten-year review of rating scales. IV. Sales assessing trauma and its effect. *J Am Acad Child Adoles Psychiatry.* 2002;41:1404-1422.

Olds DL, Robinson J, O'Brien R, et al. Home visiting by nurses and by paraprofessionals: a randomized controlled trial. *Pediatrics.* 2002;110:486-496.

Olff M. Stress, depression and immunity: the role of defense and coping styles. *Psychiatry Res.* 1999;85:7-15.

Olff M, Langeland W, Draijer N, Gersons BP. Gender differences in posttraumatic stress disorder. *Psychol Bull.* 2007;133:183-204.

Olff M, Langeland W, Gersons BP. Effects of appraisal and coping on the neuroendocrine response to extreme stress. *Neurosci Biobehav Rev.* 2005a;29:457-467.

Olff M, Langeland W, Gersons BP. The psychobiology of PTSD: coping with trauma. *Psychoneuroendocrinology.* 2005b;30:974-982.

Olff M, Güzelcan Y, de Vries GJ, Assies J, Gersons BP. HPA- and HPT-axis alterations in chronic posttraumatic stress disorder. *Psychoneuroendocrinology.* 2006;31:1220-1230.

Olff M, Langeland W, Witteveen A, Denys D. A psychobiological rationale for oxytocin in the treatment of posttraumatic stress disorder. *CNS Spectr.* 2010;15:522-530.

Olff M, de Vries GJ. *Prevalence of trauma and PTSD in the Netherlands.* Toronto: 21st Annual Meeting of International Society for Traumatic Stress Studies; 2005.

Oshio A, Kaneko H, Nagamine S, Nakaya M. Construct validity of the Adolescent Resilience Scale. *Psychol Rep.* 2003;93:1217-1222.

Owens JA, Spirito A, McGuinn M. The children's sleep habits questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. *Sleep.* 2000;23:1043-1051.

Ozer EJ, Best SR, Lipsey TL, Weiss DS. Predictors of posttraumatic stress disorder and symptoms in adults: a meta-analysis. *Psychol Bull.* 2003;129:52-73.

Panarelli M, Holloway CD, Fraser R, et al. Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. *J Clin Endocrinol Metab.* 1998;83:1846-1852.

Papakostas, Moraitis D, Lancaster J, McCormick MS. Depressive symptoms in children after tonsillectomy. *Int J Pediatr Otorhinolaryngol.* 2003;67:127-132.

Pasick RJ, Burke NJ, Barker JC, et al. Behavioral theory in a diverse society: like a compass on Mars. *Health Educ Behav.* 2009;36:11S-35S.

Peacock EJ, Wong PTP. The stress appraisal measure (SAM): a multidimensional approach to cognitive appraisal. *Stress Med.* 1990;6:227-236.

Pearce MS, Deary IJ, Young AH, Parker L. Growth in early life and childhood IQ at age 11 years: the Newcastle Thousand Families Study. *Int J Epidemiol.* 2005;34:673-677.

Peleg T, Shalev AY. Longitudinal studies of PTSD: overview of findings and methods. *CNS Spectr.* 2006;11:589-602.

Perkonigg A, Pfister H, Stein MB, et al. Longitudinal course of posttraumatic stress disorder and posttraumatic stress disorder symptoms in a community sample of adolescents and young adults. *Am J Psychiatry.* 2005;162:1320-1327.

Perrin S, Meiser-Stedman R, Smith P. The children's revised impact of event scale (CRIES): validity as a screening instrument for PTSD. *Behav Cogn Psychother.* 2005;33:487-489.

Perrin S, Smith P, Yule W. Practitioner review: the assessment and treatment of post-traumatic stress disorder in children and adolescents. *J Child Psychol Psychiatry.* 2000;41:277-289.

Peskind ER, Wilkinson CW, Petrie EC. Increased CSF cortisol in AD is a function of APOE genotype. *Neurology.* 2001;56:1094-1108.

Pesonen AK, Räikkönen K, Kajantie E, Heinonen K, Strandberg TE, Järvenpää AL. Fetal programming of temperamental negative affectivity among children born healthy at term. *Dev Psychobiol.* 2006;48:633-643.

Peters EJ, Slager SL, McGrath PJ, Knowles JA, Hamilton SP. Investigation of serotonin-related genes in antidepressant response. *Mol Psychiatry.* 2004;9:879-889.

Phillips DI. Birth weight and the future development of diabetes. a review of the evidence. *Diabetes Care.* 1998;21:B150-155.

Plomin R. *Genetics and experience: the interplay between nature and nurture.* Thousand Oaks, CA: Sage; 1994.

Plomin R, Bergeman CS. The nature of nurture: genetic influence on "environmental" measures. *Behav Brain Sci.* 1991;14:373-427.

Plomin R, Haworth CM, Davis OS. Common disorders are quantitative traits. *Nat Rev Genet.* 2009;10:872-878.

Porges SW. Vagal tone: an autonomic mediator of affect. In: Garber J, Dodge KA (Eds.). *The development of emotion regulation and dysregulation.* Cambridge, UK: University Press; 1991.

Porges SW. Vagal tone: a physiological marker of stress vulnerability. *Pediatrics.* 1992;90:498-504.

Pratchett LC, Pelcovitz MR, Yehuda R. Trauma and violence: are women the weaker sex? *Psychiatr Clin North Am*. 2010;33:465-474.

Prince-Embury S. *Resiliency scales for children and adolescents: profiles of personal strength*. San Antonio, TX: Harcourt Assessment Inc; 2007.

Prins PJM. Anxiety in medical settings. In: Ollendick TH, King NJ, Yule W (Eds.). *International handbook of phobic and anxiety disorders in children and adolescents*. New York, USA: Plenus Press; 1994.

Prior M, Virasinghe S, Smart D. Behavioural problems in Sri Lankan schoolchildren: associations with socio-economic status, age, gender, academic progress, ethnicity and religion. *Soc Psychiatry Psychiatr Epidemiol*. 2005;40:654-662.

Puglisi-Allegra S, Cabib S. Psychopharmacology of dopamine: the contribution of comparative studies in inbred strains of mice. *Prog Neurobiol*. 1997;51:637-661.

Pungello EP, Kainz K, Burchinal M, et al. Early educational intervention, early cumulative risk, and the early home environment as predictors of young adult outcomes within a high-risk sample. *Child Dev*. 2010;81:410-426.

Raber J, Akana SF, Bhatnagar S, Dallman MF, Wong D, Mucke L. Hypothalamic-pituitary-adrenal function in Apoe-/- mice: possible role in behavioral and metabolic alterations. *J Neurosci*. 2000;20:2064-2071.

Radant A, Tsuang D, Peskind ER, McFall M, Raskind W. Biological markers and diagnostic accuracy in the genetics of posttraumatic stress disorder. *Psychiatry Res*. 2001;102:203-215.

Rahu K, Rahu M, Pullmann H, Allik J. Effect of birth weight, maternal education and prenatal smoking on offspring intelligence at school age. *Early Hum Dev*. 2010;86:493-497.

Raison CL, Miller AH.When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am J Psychiatry*. 2003;160:1554-1565.

Ramey CT, Farran DC, Campbell FA. Predicting IQ from mother-infant interactions. *Child Dev*. 1979;50:804-814.

Rasmusson AM, Shi L, Duman R. Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. *Neuropsychopharmacology*. 2002;27:133-142.

Raven J, Raven JC, Court JH. *Manual for Raven's Standard Progressive Matrices*. Oxford, UK: Oxford Psychologists Press; 1998.

Raven J, Raven JC, Court JH. *Standard Progressive Matrices*. Oxford, UK: Oxford Psychologists Press; 2000.

Reinherz HZ, Giaconia RM, Hauf AM, Wasserman MS, Paradis AD. General and specific childhood risk factors for depression and drug disorders by early adulthood. *J Am Acad Child Adolesc Psychiatry*. 2000;39:223-231.

Ressler KJ, Mayberg HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nat Neurosci*. 2007;10:1116-1124.

Rew L, Horner SD. Youth resilience framework for reducing health-risk behaviors in adolescents. *J Pediatr Nurs*. 2003;18:379-388.

Rew L, Horner SD, Fouladi RT. Factors associated with health behaviors in middle childhood. *J Pediatr Nurs*. 2010;25:157-166.

Ricciardi C, Guastadisegni C. Environmental inequities and low birth weight. *Ann Ist Super Sanita*. 2003;39:229-234.

Rice F, Harold GT, Boivin J, van den Bree M, Hay DF, Thapar A. The links between prenatal stress and offspring development and psychopathology: disentangling environmental and inherited influences. *Psychol Med*. 2010;40:335-345.

Rice F, Jones I, Thapar A. The impact of gestational stress and prenatal growth on emotional problems in offspring: a review. *Acta Psychiatr Scand*. 2007;115:171-183.

Richardson GE. The meta theory of resilience and resiliency. *J Clin Psychol*. 2002;58:307-321.

Richardson GE, Waite PJ. Mental health promotion through resilience and resiliency education. *Int J Emerg Ment Health*. 2002;4:65-75.

Richter LM. Stuying adolescence. *Science*. 2006;312:1902-1905.

Risch N, Herrell R, Lehner T, et al. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA*. 2009;301:2462-2471.

Robert JS. Schizophrenia epigenesis? *Theor Med Bioeth*. 2000;21:191-215.

Robertson J, Curley J, Kaye J, Quinn J, Pfankuch T, Raber J. ApoE isoforms and measures of anxiety in probable AD patients and Apoe-/- mice. *Neurobiol Aging*. 2005;26:637-643.

Roche S, McKeon P. Support for tryptophan hydroxylase-2 as a susceptibility gene for bipolar affective disorder. *Psychiatr Genet*. 2009;19:142-146.

Rosmond R. The glucocorticoid receptor gene and its association to metabolic syndrome. *Obes Res*. 2002;10:1078-1086.

Rosmond R, Bouchard C, Bjorntorp P. Allelic variants in the GABA(A)alpha6 receptor subunit gene (GABRA6) is associated with abdominal obesity and cortisol secretion. *Int J Obes Relat Metab Disord*. 2002a;26:938-941.

Rosmond R, Bouchard C, Bjorntorp P. Association between a variant at the GABA(A)alpha6 receptor subunit gene, abdominal obesity, and cortisol secretion. *Ann N Y Acad Sci*. 2002b;967:566-570.

van Rossum EF, Koper JW, van den Beld AW, et al. Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. *Clin Endocrinol*. 2003;59:585-592.

van Rossum EF, Russcher H, Lamberts SWJ. Genetic polymorphisms and multifactorial diseases: facts and fallacies revealed by the glucocorticoid receptor gene. *Trends Endocrinol Metab*. 2005;16:445-450.

Rowe DC. *The limits of family influence: genes, experience, and behavior*. New York, USA: Guilford; 1994.

Rutter M. Resilience in the face of adversity. Protective factors and resistance to psychiatric disorder. *Br J Psychiatry*. 1985;147:598-611.

Rutter M. *Psychosocial resilience and protective mechanisms*. New York, USA: Cambridge University Press; 1990.

Rutter M. Resilience, some conceptual considerations. *J Adolesc Health*. 1993;14:626-631, 690-696.

Rutter M. Resilience concepts and findings: implications for family therapy. *J Fam Ther*. 1999;21:119-144.

Rutter M. The interplay of nature, nurture, and developmental influences: the challenge ahead for mental health. *Arch Gen Psychiatry*. 2002;59:996-1000.

Rutter M. Implications of resilience concepts for scientific understanding. *Ann N Y Acad Sci*. 2006a;1094:1-12.

Rutter M. *Genes and behavior: nature–nurture interplay explained*. Oxford, UK: Blackwell; 2006b.

Rutter M. Child and adolescent psychiatry: past scientific achievements and challenges for the future. *Eur Child Adolesc Psychiatry*. 2010;19:689-703.

Rutter M, Dunn J, Plomin R, et al. Integrating nature and nurture: implications of person-environment correlations and interactions for developmental psychopathology. *Dev Psychopathol*. 1997;9:335-364.

Rutter M, Moffitt TE, Caspi A. Gene-environment interplay and psychopathology: multiple varieties but real effects. *J Child Psychol Psychiatry*. 2006;47:226-261.

Rutter M, Silberg J. Gene-environment interplay in relation to emotional and behavioral disturbance. *Annu Rev Psychol*. 2002;53:463-490.

Rutter M, Smith D. *Psychosocial disorders in young people: time trends and their causes*. Chichester, UK: Wiley; 1995.

Rutter M, Thapar A, Pickles A. Gene-environment interactions: biologically valid pathway or artifact? *Arch Gen Psychiatry*. 2009;66:1287-1289.

Rydell AM. Family factors and children's disruptive behaviour: an investigation of links between demographic characteristics, negative life events and symptoms of ODD and ADHD. *Soc Psychiatry Psychiatr Epidemiol*. 2010;45:233-244.

Samchit S, Sriyaporn P. The relationship among resilience factors, perception of adversities, negative behavior, and academic achievement of 4th- to 6th-grade children in Thad-Thong, Chonburi, Thailand. *J Pediatr Nurs*. 2004;19:294-303.

Sameroff A. A unified theory of development: a dialectic integration of nature and nurture. *Child Dev*. 2010;81:6-22.

Saw SM, Hong CY, Chia KS, Stone RS, Tan D. Nearwork and myopia in young children. *Lancet*. 2001;357:390.

Saw SM, Tan SB, Fung D, et al. IQ and the association with myopia in children. *Invest Ophthalmol Vis Sci*. 2004;45:2943-2948.

Saxena S, Carlson D, Billington R. The WHO quality of life assessment instrument (WHOQOL-Bref): the importance of its items for cross-cultural research. *Qual Life Res*. 2001;10:711-721.

Scarr S. Developmental theories for the 1990s: development and individual differences. *Child Dev*. 1992;63:1-19.

Schell TL, Marshall GN, Jaycox LH. All symptoms are not created equal: the prominent role of hyperarousal in the natural course of posttraumatic psychological distress. *J Abnorm Psychol.* 2004;113:189-197.

Schinka JA, Busch RM, Robichaux-Keene N. A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. *Mol Psychiatry.* 2004;9:197-202.

Schlotz W, Phillips DI. Fetal origins of mental health: evidence and mechanisms. *Brain Behav Immun.* 2009;23:905-916.

Schmidt LA, Miskovic V, Boyle M, Saigal S. Frontal electroencephalogram asymmetry, salivary cortisol, and internalizing behavior problems in young adults who were born at extremely low birth weight. *Child Dev.* 2010;81:183-199.

Schneider ML, Moore CF, Kraemer GW, Roberts AD, DeJesus OT. The impact of prenatal stress, fetal alcohol exposure, or both on development: perspectives from a primate model. *Psychoneuroendocrinology.* 2002;27:285-298.

Schreiber W, Lauer CJ, Krumrey K, Holsboer F, Krieg JC. Dysregulation of the hypothalamic-pituitary-adrenocortical system in panic disorder. *Neuropsychopharmacology.* 1996;15:7-15.

Schuerger JM, Witt AC. The temporal stability of individually tested intelligence. *J Clin Psychol.* 1989;45:294-302.

Schweinhart LJ, Montie J, Xiang Z, Barnett WS, Belfield CR, Nores M. *Lifetime effects: the high/scope perry preschool study through age 40*. Ypsilanti, USA: High/Scope Press; 2005.

Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. *Nat Clin Pract Endocrinol Metab.* 2007;3:479-488.

Seckl JR, Meaney MJ. Glucocorticoid programming. *Ann N Y Acad Sci.* 2004;1032:63-84.

Seckl JR, Meaney MJ. Glucocorticoid "programming" and PTSD risk. *Ann N Y Acad Sci.* 2006;1071:351-378.

Seedat S, Scott KM, Angermeyer MC, et al. Cross-national associations between gender and mental disorders in the world health organization world mental health surveys. *Arch Gen Psychiatry.* 2009;66:785-795.

Segman RH, Cooper-Kazaz R, Macciardi F, et al. Association between the dopamine transporter gene and posttraumatic stress disorder. *Mol Psychiatry.* 2002;7:903-907.

Segman RH, Shalev AY. Genetics of posttraumatic stress disorder. *CNS Spectr.* 2003;8:693-698.

Segman RH, Shefi N, Goltser-Dubner T, Friedman N, Kaminski N, Shalev AY. Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. *Mol Psychiatry.* 2005;10:500-513,425.

Selye H. Stress and distress. *Compr Ther.* 1975;1:9-13.

Selye H. History of the stress concept. In: Goldberger L, Breznitz S. (Eds). *Handbook of stress: theoretical and clinical aspects.* New York, USA: The Free Press; 1982.

Shalev AY, Freedman S, Peri T, et al. Prospective study of posttraumatic stress disorder and depression following trauma. *Am J Psychiatry.* 1998;155:630-637.

Shapiro VB, Lebuffe PA. Using protective factors in practice: lessons learned about resilience from a study of children aged five to thirteen. *Ann N Y Acad Sci.* 2006;1094:350-353.

Shaw DS, Emery RE. Parental conflicts and other correlates of the adjustment of school-age children whose parents have separated. *J Abnorm Child Psychol.* 1987;15:269-281.

Shek DT. Conceptual framework underlying the development of a positive youth development program in Hong Kong. *Int J Adolesc Med Health.* 2006;18:303-314.

Shek DT, Siu AM, Lee TY, et al. Development and validation of a positive youth development scale in Hong Kong. *Int J Adolesc Med Health.* 2006;18:547-558.

Shekar A, McCann UD, Meaney MJ, et al. Summary of a national institute of mental health workshop: developing animal models of anxiety disorders. *Psychopharmacology.* 2001;157:327-339.

Shenkin SD, Starr JM, Deary IJ. Birth weight and cognitive ability in childhood: a systematic review. *Psychol Bull.* 2004;130:989-1013.

Siegel LJ. Measuring children's adjustment to hospitalization and to medical procedures. In: Karoly P (Eds.). *Handbook of child health assessment: biopsychological perspectives*. New York, USA: JohnWiley; 1988.

Silberg J, Pickles A, Rutter M, et al. The influence of genetic factors and life stress on depression among adolescent girls. *Arch Gen Psychiatry*. 1999;56:225-232.

Silva ML, Mallozi MC, Ferrari GF. Salivary cortisol to assess the hypothalamic–pituitary–adrenal axis in healthy children under 3 years old. *J Pediatr.* 2007;83:121-126.

Skinner EA, Zimmer-Gembeck MJ. The development of coping. *Annu Rev Psychol.* 2007;58:119-144.

Skre I, Onstad S, Torgersen S, Lygren S, Kringlen E. A twin study of DSM-III-R anxiety disorders. *Acta Psychiatr Scand.* 1993;88:85-92.

Smith DJ, Kyle S, Forty L, et al. Differences in depressive symptom profile between males and females. *J Affect Disord.* 2008;108:279-284

Smokowski PR, Reynolds AJ, Bezruczko N. Resilience and protective factors in adolescence: an autobiographical perspective from disadvantaged youth. *J School Psychol.* 1999;37:425-448.

Smoller JW, Tsuang MT. Panic and phobic anxiety: defining phenotypes for genetic studies. *Am J Psychiatry.* 1998;155:1152-1162.

Sonuga-Barke EJS, Mistry M. The effect of extended family living on the mental health of three generations within two Asian communities. *Br J Clin Psychol.* 2000;39:129-141.

Stahl SM. Is psychopharmacologic 'inoculation' effective in preventing posttraumatic stress disorder? *J Clin Psychiatry.* 2005;66:5-6.

Stein MB, Jang KL, Taylor S, Vernon PA, Livesley WJ. Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. *Am J Psychiatry.* 2002;159:1675-1681.

Steinberg L, Dornbusch SM, Brown BB. Ethnic differences in adolescent achievement: an ecological perspective. *Am Psychol.* 1992;47:723-729.

Stradling JR, Thomas G, Warley AR, Williams P, Freeland A. Effect of adenotonsillectomy on nocturnal hypoxaemia, sleep disturbance, and symptoms in snoring children. *Lancet.* 1990;335:249-253.

Strug LJ, Suresh R, Fyer AJ, et al. Panic disorder is associated with the serotonin transporter gene (SCL6A4) but not the promoter region (5-HTTLRP). *Mol Psychiatry.* 2010;15:166-176.

Su S, Zhao J, Bremner JD, Miller AH, Tang W, Bouzyk M. Serotonin transporter gene, depressive symptoms, and interleukin-6. *Circ Cardiovasc Genet.* 2009;2:614-620.

Sullivan PF, Neale MC, Silverman MA, et al. An association study of DRD5 with smoking initiation and progression to nicotine dependence. *Am J Med Genet.* 2001;105:259-265.

Suomi SJ. Risk, resilience, and gene x environment interactions in rhesus monkeys. *Ann N Y Acad Sci.* 2006;1094:52-62.

Talbot PS. The molecular neuroimaging of anxiety disorders. *Curr Psychiatry Rep.* 2004;6:274-279.

Tedeschi G, Calhoun G. The posttraumatic growth inventory: measuring the positive legacy of trauma. *J Trauma Stress.* 1996;9:455-471.

Tiet QQ, Bird HR, Davies M, et al. Adverse life events and resilience. *J Am Acad Child Adolesc Psychiatry.* 1998;37:1191-1200.

Thomas DC, Greenland S. The efficiency of matching in case-control studies of risk-factor interactions. *J Chronic Dis.* 1985;38:569-574.

Thompson RG Jr, Lizardi D, Keyes KM, Hasin DS. Childhood or adolescent parental divorce/separation, parental history of alcohol problems, and offspring lifetime alcohol dependence. *Drug Alcohol Depend.* 2008;98:264-269.

Tong S, Baghurst P, McMichael A. Birthweight and cognitive development during childhood. *J Paediatr Child Health.* 2006;42:98-103.

Tornhage CJ. Reference values for morning salivary cortisol concentrations in healthy school-aged children. *J Pediatr Endocrinol Metab.* 2002;15:197-204.

True WR, Rice J, Eisen SA, et al. A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. *Arch Gen Psychiatry.* 1993;50:257-264.

Tsai SJ, Hong CJ, Liou YJ, et al. Tryptophan hydroxylase 2 gene is associated with major depression and antidepressant treatment response. *Prog Neuropsychopharmacol Biol Psychiatry.* 2009;33:637-641.

Tschann JM, Kaiser P, Chesney MA, Alkon A, Boyce WT. Resilience and vulnerability among preschool children: family functioning, temperament, and behaviour problems. *J Am Acad Child Adolesc Psychiatry.* 1996;35:184-192.

Tully LA, Arseneault L, Caspi A, Moffitt TE, Morgan J. Does maternal warmth moderate the effects of birth weight on twins' attention-deficit/hyperactivity disorder (ADHD) symptoms and low IQ? *J Consult Clin Psychol.* 2004;72:218-226.

Turecki G, Brière R, Dewar K, et al. Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide. *Am J Psychiatry.* 1999;156:1456-1458.

Tyrka AR, Mello AF, Mello M, et al. Temperament and hypothalamic–pituitary–adrenal axis function in healthy adults. *Psychoneuroendocrinology.* 2006;31:1036-1045.

Ungar M. Nurturing hidden resilience in at-risk youth in different cultures. *J Can Acad Child Adolesc Psychiatry.* 2006;15:53-58.

Ungar M. Resilience across cultures. *Br J Soc Work.* 2008;38:218-235.

UNICEF 2007. *Child poverty in perspective: an overview of child well-being in rich countries, Innocenti Report Card 7*. Florence, Italy: UNICEF Innocenti Research Centre; 2007.

Unschuld PG, Ising M, Erhardt A, et al. Polymorphisms in the serotonin receptor gene HTR2A are associated with quantitative traits in panic disorder. *Am J Med Genet B Neuropsychiatr Genet.* 2007;144B:424-429.

Ursin H, Olff M. Psychobiology of coping and defence strategies. *Neuropsychobiology.* 1993;28:66-71.

van der Vegt AL, Diepeveen M, Klerks M, Voorpostel M, de Weerd M. *Je verweren kun je leren*. Amsterdam, the Netherlands: Regioplan; 2001

Veldhuis HD, van Koppen C, van Ittersum M, de Kloet ER. Specificity of the adrenal steroid receptor system in the rat hippocampus. *Endocrinology.* 1982;110:2044-2051.

de Vente W, Olff M, van Amsterdam JG, Kamphuis JH, Emmelkamp PM. Physiological differences between burnout patients and healthy controls: blood pressure, heart rate, and cortisol responses. *Occup Environm Med.* 2003;60:i54-61.

Victora CG, Adair L, Fall C, et al. Maternal and Child Undernutrition Study Group. Maternal and child undernutrition: consequences for adult health and human capital. *Lancet.* 2008;371:340-357.

Villar J, Belizan JM. The timing factor in the pathophysiology of the intrauterine growth retardation syndrome. *Obstet Gynecol Surv.* 1982;37:499-506.

Vonk R, van de Wetering BJM, Niermeijer MF. The genetics of psychiatric disorders. *Tijdschr Psychiatr.* 1998;40:86-94.

Vostanis P, Graves A, Meltzer H, Goodman R, Jenkins R, Brugha T. Relationship between parental psychopathology, parenting strategies and child mental health: findings from the GB national study. *Soc Psychiatry Psychiatr Epidemiol.* 2006;41:509-514.

de Vries GJ, Olff M. The lifetime prevalence of traumatic events and posttraumatic stress disorder in the Netherlands. *J Trauma Stress.* 2009;22:259-267.

Vujkovic M, de Vries JH, Dohle GR, et al. Associations between dietary patterns and semen quality in men undergoing IVF/ICSI treatment. *Hum Reprod.* 2009;24:1304-1312.

van Waarde JA, de Rijk RH, Zitman FG. Genomics and psychiatry: not an easy marriage. *Tijdschr Psychiatr.* 2002;44:551-560.

Wagnild GM, Young HM. Development and psychometric evaluation of the Resilience Scale. *J Nurs Meas.* 1993;1:165-178.

Wahlsten D. Insensitivity of the analysis of variance to heredity–environment interaction. *Behav Brain Sci.* 1990;13:109-161.

Waite PJ, Richardson GE. Determining the efficacy of resiliency training in the work site. *J Allied Health.* 2004;33:178-183.

Wang S, Zhang K, Xu Y, Sun N, Shen Y, Xu Q. An association study of the serotonin transporter and receptor genes with the suicidal ideation of major depression in a Chinese Han population. *Psychiatry Res.* 2009;170:204-207.

Walker SP, Thame MM, Chang SM, Bennett F, Forrester TE. Association of growth in utero with cognitive function at age 6-8 years. *Early Hum Dev.* 2007;83:355-360.

Watson PE, McDonald BW. The association of maternal diet and dietary supplement intake in pregnant New Zealand women with infant birthweight. *Eur J Clin Nutr.* 2010;64:184-193.

Wei JL, Mayo MS, Smith HJ, Reese M, Weatherly RA. Improved behavior and sleep after adenotonsillectomy in children with sleep-disordered breathing. *Arch Otolaryngol Head Neck Surg.* 2007;133:974-979.

Weinstock M. Intrauterine factors as determinants of depressive disorder. *Isr J Psychiatry Relat Sci.* 2010;47:36-45.

Weizman A, Weizman R, Kook KA, Vocci F, Deutsch SI, Paul SM. Adrenalectomy prevents the stress-induced decrease in in vivo [3H]Ro15-1788 binding to GABAA benzodiazepine receptors in the mouse. *Brain Res.* 1990;519:347-350.

Westphal LM, Polan ML, Trant AS, Mooney SB. A nutritional supplement for improving fertility in women: a pilot study. *J Reprod Med.* 2004;49:289-293.

WHOQOL-Group. Development of the World Health Organization WHOQOL-BREF Quality of Life Assessment. *Psychol Med.* 1998;28:551-558.

Wiles NJ, Peters TJ, Leon DA, Lewis G. Birth weight and psychological distress at age 45-51 years: results from the Aberdeen Children of the 1950s cohort study. *Br J Psychiatry.* 2005;187:21-28.

Woo BS, Ng TP, Fung DS, et al. Emotional and behavioural problems in Singaporean children based on parent, teacher and child reports. *Singapore Med J.* 2007;48:1100-1106.

Woods SJ, Wineman NM. Trauma, posttraumatic stress disorder symptom clusters, and physical health symptoms in postabused women. *Arch Psychiatr Nurs.* 2004;18:26-34.

Wray NR, James MR, Gordon SD, et al. Accurate, large scale genotyping of 5-HTTLRP and flanking single nucleotide polymorphisms in an association study of depression, anxiety, and personality measures. *Biol Psychiatry.* 2009;66:468-476.

Wurtman RJ. Genes, stress, and depression. *Metabolism.* 2005;54:16-19.

Wyman PA, Cowen EL, Work WC, Hoyt-Meyers L, Magnus KB, Fagen DB. Care-giving and developmental factors differentiating young at-risk urban children showing resilient versus stress-affected outcomes: a replication and extension. *Child Dev.* 1999;70:645-659.

Xian H, Chantarujikapong SI, Scherrer JF, et al. Genetic and environmental influences on posttraumatic stress disorder, alcohol and drug dependence in twin pairs. *Drug Alcohol Depend.* 2000;61:95-102.

Yamada K, Mizuno M, Nabeshima T. Role for brain-derived neurotrophic factor in learning and memory. *Life Sci.* 2002;70:735-744.

Yeh CJ, Huang K. The collectivistic nature of ethnic identity development among Asian-American college students. *Adolescence.* 1996;31:645-662.

Yehuda R. Post-traumatic stress disorder. *N Engl J Med.* 2002;346:108-114.

Yehuda R, Bierer LM, Schmeidler J, Aferiat DH, Breslau I, Dolan S. Low cortisol and risk for PTSD in adult offspring of holocaust survivors. *Am J Psychiatry.* 2000;157:1252-1259.

Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS. Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the World Trade Center attacks during pregnancy. *J Clin Endocr Metab.* 2005;90:4115-4118.

Yehuda R, Golier JA, Yang RK, Tischler L. Enhanced sensitivity to glucocorticoids in peripheral mononuclear leucocytes in posttraumatic stress disorder. *Biol Psychiatry.* 2004;55:1110-1116.

Yehuda R, Halligan SL, Bierer LM. Relationships of parental trauma exposure and PTSD to PTSD, depressive and anxiety disorders in offspring. *J Psychiatr Res.* 2001;35:261-270.

Yehuda R, Halligan SL, Bierer LM. Cortisol levels in adult offspring of Holocaust survivors: relation to PTSD symptom severity in the parent and child. *Psychoneuroendocrinology.* 2002;27:171-180.

Yehuda R, Schmeidler J, Giller Jr. EL, Siever LJ, Binder-Brynes K. Relationship between posttraumatic stress disorder characteristics of Holocaust survivors and their adult offspring. *Am J Psychiatry.* 1998;155:841-843.

Yehuda R, Southwick S, Giller EL, Ma X, Mason JW. Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans. *J Nerv Ment Dis.* 1992;180:321-325.

Young EA, Lopez JF, Murphy-Weinberg V, Watson SJ, Akil H. The role of mineralocorticoid receptors in hypothalamic–pituitary–adrenal axis regulation in humans. *J Clin Endocrinol Metab.* 1998;83:3339-3345.

Young RM, Lawford BR, Noble EP, et al. Harmful drinking in military veterans with post-traumatic stress disorder: association with the D2 dopamine receptor A1 allele. *Alcohol Alcohol.* 2002;37:451-456.

Yu X, Zhang JX. Factor analysis and psychometric evaluation of the Connor-Davidson Resilience Scale (CD-RISC) with Chinese people. *SPB.* 2007;35:19-30.

Yule W, Williams RM. Posttraumatic stress reactions in children. *J Trauma Stress.* 1990;3:279-295.

Zahn-Waxler C, Shirtcliff EA, Marceau K. Disorders of childhood and adolescence: gender and psychopathology. *Annu Rev Clin Psychol.* 2008;4:275-303.

Zhang J, Shen Y, He G, et al. Lack of association between three serotonin genes and suicidal behavior in Chinese psychiatric patients. *Prog Neuropsychopharmacol Biol Psychiatry.* 2008;32:467-471.

Zhang H, Zhou R, Li X, Ursano RJ, Li H. Stress-induced change of mitochondria membrane potential regulated by genomic and non-genomic GR signaling: a possible mechanism for hippocampus atrophy in PTSD. *Med Hypotheses.* 2006a;66:1205-1208.

Zhang H, Ozbay F, Lappalainen J, et al. Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. *Am J Med Genet B Neuropsychiatr Genet.* 2006b;141:387-393.

Zhang Y, Zhang C, Yuan G, et al. Effect of tryptophan hydroxylase-2 rs7305115 SNP on suicide attempts risk in major depression. *Behav Brain Funct.* 2010;25:49.

Zhou Z, Roy A, Lipsky R, et al. Haplotype-based linkage of tryptophan hydroxylase 2 to suicide attempt, major depression, and cerebrospinal fluid 5-hydroxyindoleacetic acid in 4 populations. *Arch Gen Psychiatry.* 2005;62:1109-1118.

Zill P, Baghai TC, Zwanzger P, et al. SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. *Mol Psychiatry.* 2004;11:1030-1036.

Zobel AW, Yassouridis A, Frieboes RM, Holsboer F. Prediction of medium-term outcome by cortisol response to the combined dexamethasone-CRH test in patients with remitted depression. *Am J Psychiatry.* 1999;156:949-951

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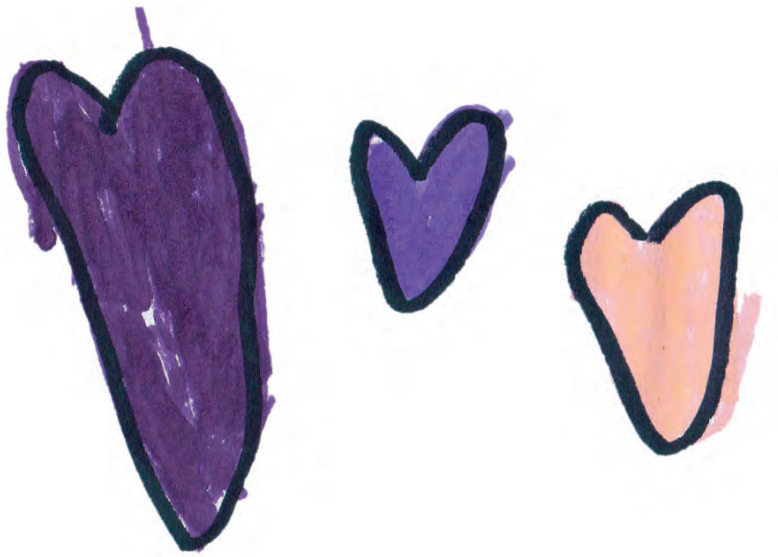

"Hearts" by Fleur van Velthoven and Tess van den Bichelaer, 4 years old

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Living abroad has even brought us closer. Of course it is a wonderful experience to take on parenthood of our lovely children together! I love you!

List of publications

Broekman BFP, Chan YH, Goh L, Fung D, Gluckman PD, Saw SM, Meaney MJ. Serotonergic function genes modulate the influence of birth weight on internalizing behaviour. *Pediatrics.* Accepted July 2011.

Broekman BFP. Stress, vulnerability and resilience, a developmental approach. PhD Summary. *EJPT***.** Accepted May 2011.

Broekman BFP, Niti M, Nyunt SZ, Jin AZ, Ko SM, Kumar R, Ng TP. Validation of a brief seven items response bias free geriatric depression scale. *Am J Geriatr Psychiatry.* 2011;19:589-596.

Lim ML, **Broekman BFP**, Wong JCM, Wong ST, Ng TP. The development and validation of the Singapore Youth Resilience Scale (SYRESS). *TIJEPA.* 2011;8:16-30.

Broekman BFP, Olff M, Tan FM, Schreuder BJ, Fokkens W, Boer F. The psychological impact of an adenoidectomy and adenotonsillectomy on young children. *Int J Pediatr Otorhinolaryngol.* 2010;74:37-42.

Broekman BFP, Chan YH, Chong YS, Quek SC, Fung D, Low YL, Ooi YP, Gluckman PD, Meaney MJ, Wong TY, Saw SM. The influence of birth size on intelligence in healthy children. *Pediatrics.* 2009;123:1011-1016.

Ng TP, **Broekman BFP**, Niti M, Gwee X, Kua EH. Determinants of successful aging using a multidimensional definition among Chinese elderly in Singapore. *Am J Geriatr Psychiatry.* 2009;17:407-416.

Ho RCM, Chen KY, **Broekman BFP**, Mak A. Buprenorphine prescription, misuse and service provision: a global perspective. *Adv Psychiatr.* 2009;15:354-363.

de Jongh A, Olff M, van Hoolwerff H, Aartman IH, **Broekman BFP**, Lindauer R, Boer F. Anxiety and post-traumatic stress symptoms following wisdom tooth removal. *Behav Res Ther.* 2008;46:1305-1310.

Broekman BFP, Kumar R, Toh AYP, Kua EH. Recruiting Psychiatrists-the Singapore experience. *The Psychiatrist.* 2008;32:275.

Broekman BFP, Nyunt SZ, Niti M, Jin AZ, Ko SM, Kumar R, Fones CS, Ng TP. Differential item functioning of the Geriatric Depression Scale in an Asian population. *J Affect Disord.* 2008;108:285-290.

Broekman BFP, Olff M, Boer F. The genetic background to PTSD. *Neurosci Biobehav Rev.* 2007;31:348-362.

Broekman BFP, De Vos R, Waalewijn RA. Obstacles to the routine discussion with hospital patients of possible cardiopulmonary resuscitation. *Ned Tijdschr Geneesked.* 2002;146:2374-2377.

Submitted/under review:

Broekman BFP, Woo B, Chan YH, Fung D, Ng TP. Risks and protective factors associated with mental wellbeing and academic performance in primary school aged children in Asia. *Under review.*

Low YL, Ooi YP, Fung D, **Broekman BFP**, Chong YS, Wong TY, Gluckman PD, Levitan R, Meaney MJ, Saw SM. Emotional and behavioural traits associate with diet in children. *Submitted.*

Curriculum Vitae

Birit Froukje Philippien Leutscher-Broekman was born on 2th March 1974 in The Hague, the Netherlands. After finishing her secondary education (Gymnasium, 1st VCL in The Hague), she studied Psychology at the University of Groningen from 1992 to 1996. After completing her MA in Psychology, she started the study Medicine at the University of Amsterdam in 1996, and finished her MSc degree in Medicine cum laude in 2000, with a Major in Child Neurology. Directly after completing her MD in 2002, again cum laude, she followed the formal training in General Practice at the University of Leiden for one year. At that time she decided to specialize in psychiatry, and started her formal training in Psychiatry in 2003. She finished the major part of her formal training in 2007. She did her postings mostly in the Academic Medical Centre (University of Amsterdam) and partly in 'De Meren'. In 2007 she was appointed as a Visiting Fellow at the National University of Singapore, where she finished her official training in Psychiatry with an elective in research. She was fully registered as a Psychiatrist by the 'Medisch Specialisten Registratie Commissie' of the Netherlands in February 2008, and by the Specialist Accreditation Board of Singapore in 2009.

Since 2008 she has been working as an academic Psychiatrist at the Department of Psychological Medicine, National University Hospital of Singapore, specializing in anxiety disorders, perinatal psychiatry and child psychiatry. Additionally she has been appointed as an Assistant Professor for teaching and research at the National University of Singapore.