Mental retardation: diagnostic studies on aetiology
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Summary

...the diversity in causes of mental retardation and autism resembles the fan shape of a peacock’s plumage...
In Chapter 1, an exemplary story of a boy with mental retardation (MR) demonstrates the fundamental importance of understanding the pathogenesis of MR in a patient, both for optimal patient management as for care of patients and family, including genetic counselling. A general introduction on MR is given. Relatively little is known of its causes: aetiological diagnoses are usually identified in less than half of the patients, and frequencies of the two major causal groups are reported with a marked variability. Exogeneous causes vary from 18.6% to 44.5%, genetic causes from 17.4% to 47 1%. The lack of consensus in the medical literature on the way a patient with a developmental delay should be evaluated and on the yield of the different diagnostic investigations, combined with the aetiological heterogeneity of MR make proper diagnostic evaluation of individuals with MR a major challenge for most clinicians. The work-up therefore often becomes unnecessarily complex, invasive, and expensive, constituting a large burden for the affected person, their parents, and society.

The major goals of the thesis are described: To optimize the diagnostic evaluation of an individual with MR, a systematic literature review was performed to collect the evidence available on the yield of the major diagnostic investigations in MR, as well as an aetiological study of a consecutive group of children referred to our paediatric outward clinic with unexplained MR. In addition a similar but more extensive study was performed in a group of young adults with a specific symptom next to their delay, i.e. autism. The cytogenetic studies screening for subtelomeric rearrangements are described separately, including a pilot study in a biased group, and a large prospective study in children in whom aetiology of MR remains unknown, after a complete work-up conform present day standards. A case report of a female with a subtelomeric deletion of chromosome 14qter, detected in the pilot study, is presented and compared to cases previously described in the literature. Finally, to enhance uniformity in the scoring and classification of abnormal physical features, a classification system is proposed based on a review of the present nomenclature for errors of morphogenesis detectable on surface examination.

In Chapter 2 a systematic review is presented on the yield of cytogenetic investigations (chromosome studies and subtelomeric FISH analysis) in well-defined groups of MR individuals, and the relation between the yield and setting, MR severity, gender is analysed. This chapter is the first part of a larger review that will encompass also 5 other major diagnostic investigations (dysmorphologic examination, neurologic examination, neuroradiology, metabolic studies, and molecular/cytogenetic studies for the fragile X syndrome). As the methodology of the whole review was applied for all 6 investigations, this part is presented in its complete form in this Chapter. In the first phase of the study, definitions were set for all concepts involved. Diagnostic yield of the investigations was expressed as detection frequency of (significant) anomalies in the MR group under study. Publications were retrieved by a computerised search in several databases, and by handsearching personal files and bibliographies of included articles, for all studies published in peer-reviewed medical journals during the period January 1966-July 2002.
Publications should report on the application and yield of at least one of the above mentioned diagnostic investigation techniques in groups of minimally 25 well-defined individuals with previously unexplained MR. Of the in total 8,152 citations yielded by the database searches and handsearching of references, 219 (2.7%) potentially appropriate MR articles were selected by 2 independent reviewers for the last phase of the review. Of these, 117 reported on cytogenetic investigations. Following a pilot study more strict criteria were formulated. Methods for data extraction, quality assessment, and tabulations are listed. Finally, 38 publications were included in the review, of which 33 report the results of chromosome studies only (20 reliably numerical and structural anomalies; 13 numerical only), 4 of subtelomeric FISH investigations, and 1 study reports results of both cytogenetic investigations. All previous steps were performed by two investigators independently. The quality and quantity of the data yielded by the included articles were sufficient to reliably draw conclusions regarding the yield of cytogenetic investigations in MR patients. Chromosome anomalies have been detected in all MR study groups, and with considerable frequency, the median frequency was about 1 in 10. General cytogenetic studies were found, also in this evidence based study, to be a valuable diagnostic technique in studying individuals with MR. The variation in yield was considerable (2-50%), especially for numerical anomalies. Although the detection frequency of chromosome anomalies is higher in patients with a moderate to profound MR than those with a borderline to mild degree, the yield in the latter is still sufficient to advocate investigations in all patients without other obvious causes for their retardation. As most studies were performed in either an institution or an outpatient clinic, reliable analyses of the relation between yield and setting was hampered. Structural chromosome anomalies are reported more frequently in females. There is no simple explanation for this, but possibilities are discussed. For subtelomeric FISH studies, the results of the review were based on only a relatively small number of articles. However, the review does show that the frequency of subtelomeric rearrangements in unselected MR patients (median frequency 4.4%) may be lower than previously reported. Explanations for the variation in detection frequency (20-fold) are extensively discussed in Chapter 5. Recommendations for study design and reporting of results are outlined, as well as possibilities for collecting the yielded evidence in the future.

In Chapter 3 a prospective study is presented of 281 children with unexplained MR or borderline cognitive delay (IQ<85) consecutively referred to a the specialised outpatient clinic of a tertiary care centre for diagnostic evaluation during the period November 1998 – November 2000. All patients underwent a standard assessment including a complete clinical history, a three-generation pedigree, physical examination, and behavioural assessment. If clinical work-up findings suggested a particular diagnosis, appropriate investigations to confirm this diagnosis were performed. If no diagnosis was evident, cytogenetic studies and metabolic investigations (urinary analysis; screening for glycosylation and cholesterol metabolism defects) were performed. If normal,
FISH analysis of all subtelomeric regions was performed. If normal, further additional investigations were performed. Of the 281 children (162 male) with a mean age of 7.4 years, MR was graded borderline to mild in 55.2% and moderate to profound in 44.8%. In 150 patients (54%), a diagnosis aetiologic for MR was established. Diagnosis was not dependent on the severity of MR. In this academic setting, perinatal factors were an infrequent cause of MR; teratogenic factors constitute 1 of every 20 aetiologic diagnoses; cytogenetic anomalies 1 of 5 diagnoses; and monogenic entities were responsible for MR in almost half of previously unexplained cases. The likelihood of detecting a cytogenetic imbalance was higher in a patient with more and more widespread minor anomalies ("dysmorphic features"). A high number of minor anomalies and malformations did not exclude a metabolic disorder. One-third of diagnoses was established on findings of clinical history and physical exam only; for another third clinical history and physical exam provided essential clues to direct additional investigations which were needed to confirm the diagnosis; the remaining one-third of diagnoses were established through additional investigations only. Diagnostic procedures in patients with unexplained MR were often extremely complex and required almost always a multidisciplinary approach.

In Chapter 4.1 a pilot study is reported on the presence of subtelomeric rearrangements in a highly biased group of 30 paediatric and adult cases with MR (17m:13f), all with normal karyotype at a 550 G-band level, but suspected to have a chromosome anomaly based on one or more the following characteristics: suspect dysmorphic features or "chromosomal phenotype"; family history positive for MR; or multiple unexplained miscarriages within the same sibship. All subtelomeric regions were screened by standard FISH analysis using different commercial and home-made probes. A cryptic rearrangement was detected in 5 patients (16.7%): del2q; tris 2q/del6p;; del 4p; del 4q; and del 14q. We concluded that, also in our hands, FISH analysis is a valid technique for detecting subtelomeric rearrangements.

In Chapter 4.2 the female from the pilot study with a small subtelomeric deletion of the long arm of chromosome 14(q32.31-qter) is presented. She showed typical dysmorphic features, hypotonia, and mild developmental delay. In comparing her phenotype with previously reported patients with similar 14q deletions, due to either a linear deletion or to a ring chromosome 14, a clinically recognisable terminal 14q microdeletion syndrome was evident. Due to the limited number of cases reported it appeared not possible to assign specific features to specific regions of terminal 14q. The comparison of features in cases with a linear deletion of 14qter (n=19) to cases with a deletion due to a ring chromosome 14 (n=23), with the same breakpoint in 14q, showed that seizures and retinitis pigmentosa have only been found in patients with ring chromosomes. Several hypotheses are put forward to explain this difference: mitotic instability of ring chromosomes; a telomere position effect in ring chromosomes (the 14p telomere silences nearby gene(s) on the q-arm); and dose dependent gene(s) involved in seizures and retinitis pigmentosa located on the short arm of chromosome 14.
In Chapter 5 the prospective screening for cytogenetic anomalies in general and subtelomeric rearrangements in particular, is reported for the 266 children with MR belonging to the cohort described in Chapter 3. Karyotyping (High Resolution Banding techniques) detected anomalies in 20 children (7.5%, 7 numerical, 13 structural); 39 children were analysed by FISH for specific interstitial microdeletions, and anomalies were found in 9 (23%). FISH analyses for subtelomeric rearrangements (using the Chromoprobe™ Multiprobe kit) were performed in 184 children (44% moderate-profound MR; 51% familial MR), and 1 rearrangement (0.5%) was identified in a girl with mild non-familial MR (de novo deletion 12q24.33-qter). The number of (probable) polymorphisms was considerable: 2qter (n=7); Xpter (n=3); Ypter (n=1). We concluded that the total frequency of cytogenetic anomalies in the study was high, but the frequency of subtelomeric rearrangements was low. The most likely explanations for the latter are the high quality of the cytogenetic studies and (especially) the lack of clinical selection bias.

In Chapter 6 an aetiologic study on a specific subgroup of patients with MR is presented: 25 consecutive mentally retarded adults (20 males) with autism (DSM-IV criteria). Each patient underwent: physical exam including detailed dysmorphic exam; ophthalmologic and ENT investigations; EEG studies; karyotyping; FISH analysis of 22q11 region and all subtelomeric regions; molecular analysis for FMR1 gene expansions, MeCP2 gene mutations and duplications of 15q11-13 region; metabolic investigations including a general urinary screen, search for peroxisomal, mitochondrial, glycosylation and cholesterol metabolism disturbances; and neuroradiologic studies (if neurologic symptoms were present). In 5 patients an unequivocal diagnosis was established. A probable diagnosis was made in 4 other patients. We concluded that a complete work-up of mentally retarded adults with autism yields a diagnosis in at least 20% and possibly up to 36%. If such studies will be performed in cohorts of familial cases used for linkage analysis, such studies may well be more successful.

In Chapter 7 an adapted nomenclature for errors of morphogenesis detectable on surface examination (as performed in the study of the cohort of children with MR) is proposed as well as a uniform classification into categories: I) Abnormalities, further subdivided in 1) malformations, defined as defects of organogenesis, resulting from events during the first three months of gestation, often oligogenic, and frequently involving the threshold principle; and 2) major abnormalities secondary to a dysplasia, disruption, deformity, neurologic dysfunction, other abnormally functioning structure, and disorder/dysfunction not otherwise specified. II) Minor variants, defined as defects of phenogenesis, arising during fetal or early postnatal life, and specified as polygenic events. Phenogenesis represents a process of developmental fine-tuning, and defects lead to quantitatively differences between individuals involving only shades of differences. This category is further subdivided in 1) minor anomalies, which are defects of phenogenesis occurring in 4% or less of the normal population; and 2) spectrum variants, which have an expected prevalence of more than 4%.
This classification system proved feasible in a pilot study comprising patients who suffered cancer as a child. Its application allows systematic evaluation and weighing of the presence of minor variants and abnormalities, a useful tool in the aetiological evaluation of disorders with a suspected developmental aetiology. Once normal values of abnormal physical features are available, it will be better possible to discern specific patterns of anomalies in specific patient groups, which in turn will help to identify new candidate genes in disorders showing only well after birth, such as MR and autism.

In the closing Chapter 8 results of the studies on the aetiology of MR presented in this thesis, are further discussed. The essential first step in a diagnostic work-up of a child with MR is knowing what establishing a diagnosis means for a parent, as only then can their specific concerns be adequately addressed. Physicians involved in the diagnostic evaluation of MR should always stay critical, towards themselves, towards results yielded by all their clinical and laboratory investigations, and towards data from literature. The term 'aetiologic diagnosis' should be applied with care, and a physician should be sure that the disorder is highly likely to be causative of MR and not only an associated symptom of another underlying disorder. Our studies stress the general assumption that most of the causes of what currently is called idiopathic MR will be genetic, either multifactorial in origin or the cumulative or interactive effect of more than one factor. Novel techniques that will be further developed and applied in the near future, and may lead to the detection of previously unrecognised or unknown causes of MR, possibly providing more insight into mechanisms leading from the disorder to developmental delay, are discussed. Prevention of MR through avoidance of environmental or neonatal factors, or through prenatal diagnostic studies was found possible in about 1 in every 10 patients of the study group described in Chapter 3. This indicates that still more effort should be invested in broadening of the scope of preventive measures as well as increasing their application. Possibilities for treatment, or at least reduction of symptoms, are slowly emerging for disorders associated with MR, especially for metabolic disturbances.

Suggestions are provided how to perform future studies on the yield of diagnostic investigations in persons with MR. To accurately ascertain the relation between yield and variables such as phenotype, gender, severity of MR, and setting, a firm and meticulously described study design is essential, as well strict as definitions for all used terms, and the application of a uniform scoring and classification system of abnormal physical features. The data yielded by such studies should allow re-evaluation and updating of existing guidelines for the evaluation of the individual with MR. A Consensus Meeting may well be the best additional instrument to reach this. Subsequently, studies applying these guidelines should be performed, and critical evaluation of their yield and usefulness should lead once again to the necessary adaptations and improvements.