Mental retardation: diagnostic studies on aetiology
van Karnebeek, C.D.

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Chapter

An aetiological study of 25 mentally retarded adults with autism

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Background: The aetiology of autism, a chronic and severe neuropsychiatric disorder, is heterogeneous. Studying underlying mechanisms is important as it may offer new diagnostic and management possibilities, improve options in genetic counselling, and provide insights in pathogenetic pathways.

Objective: The objective of the present study was to search for somatic causes of autism in an unselected cohort of 25 consecutive adults (20 males) with autism (DSM-IV criteria), all residing in the same department of an institute for the mentally retarded.

Methods: The following data were retrospectively collected on each patient: pre-, peri-, and postnatal clinical history; family history; results of all previously performed investigations. Physical examination was performed including a general, anthropometric, dysmorphic, and neurologic screening. Additional investigations performed in all patients included: ophthalmologic and ENT investigations; EEG studies; karyotyping; FISH analysis of 22q11 region and all subtelomeric regions; molecular analysis for FraX gene expansions, MeCP2 gene mutations and duplications of 15q11-13 region; metabolic investigations including a general urine screen, search for peroxisomal and mitochondrial disturbances, and searches for glycosylation disturbances of N-glycans and for disturbed cholesterol metabolism. Neuroradiologic studies were only performed if neurologic symptoms were present and if no results from earlier studies were available. The study was approved by the local Medical Ethical Committee.

Results: In five (20%) patients an unequivocal aetiologica l diagnosis was identified: a prenatal factor (fetal alcohol syndrome), perinatal factor (kernicterus), a metabolic disturbance in two (phenylketonuria; disturbed cholesterol metabolism), and a chromosome abnormality in two (marker chromosome #13, mosaicism for XO/XY). In addition, a probable diagnosis was made in four (16%) other patients: Orstavik syndrome in two, a private syndrome in one; a teratogenic cause in one.

Conclusions: A complete work up of autistic mentally retarded adults yields a diagnosis responsible for autism in at least 20% and possibly up to 36%. If such studies are performed in cohorts of familial cases used for linkage analysis, such studies may well be more successful.
Autism is a chronic and severe neuropsychiatric disorder with an early onset, characterized by qualitative impairments of social interaction, deviant development of language and other communicative skills, delayed cognitive development, and restricted repetitive and stereotyped interests and behaviours. The prevalence in the general population was estimated at 5.5/10,000 but more recent investigations report higher rates. Males are affected more often than females, with a predominance of 3 to 1. Mental retardation is present in about 75 to 85% of patients with autism, and almost half of all autistic patients are functionally mute.

The causes and mechanisms underlying autism are heterogeneous, varying from genetic causes, i.e. chromosome abnormalities and conditions with Mendelian inheritance such as metabolic disturbances, to infectious causes and teratogenic influences. The prevailing view is that autism is caused by a pathophysiologic process arising from the interaction of an early environmental insult and a genetic predisposition. However, aetiology remains often unraveled and earlier studies have reported that causal medical conditions were detectable in a relatively small percentage of autistic patients. There is increasing evidence that genetic factors may well play a major role in the remaining idiopathic cases. In support of this hypothesis are the recent identification of deletions at the short arm of the X-chromosome, duplications of the Prader-Willi/Angelman critical region on chromosome 15q, linkage to 7q31 and 2q, as well as the high monozygotic twin concordance rates, and high recurrence risk among sibs of patients with idiopathic autism.

Autism poses an extremely heavy burden for affected individuals, their families and society. Research focussing on biological causes and on guidelines for these studies in each specific individual is important, both in diagnostics, management, as genetic counselling. Here we report on the results of a full diagnostic work up of 25 patients with autism, all residing in an institute for the mentally retarded.

Study subjects
In 1993, a behavioural study with regard to stereotypic movements in autism was initiated in 25 adults with autism residing in an institute for the mentally retarded ("Eemeroord", Baarn, The Netherlands). Inclusion criteria at that time were: confirmed diagnosis of autism using DSM-IV criteria, age>14 years, and residency in either of the two wards chosen for the study for practical reasons. The level of functioning was assessed in each patient using the SRZ scale and subsequently classified based on DSM-IV criteria. All subjects participated after written informed consent was obtained from parents or other legal caregivers. The study was approved by the Medical Ethical Committee of the Academic Medical Centre in Amsterdam.
Data collection
Archives were searched to retrospectively collect data on each patient.

Physical examination
All patients were examined by one physician for the mentally retarded and one clinical geneticist.

Chromosomes
Chromosome preparations from peripheral blood cultures and cytogenetic analyses were performed using standard techniques.

Fluorescence-in-situ-hybridisation (FISH)
FISH analysis of the minimal DiGeorge critical region was performed using the cosmid M51 probe. FISH analysis of the subtelomeric regions of all chromosomes was performed using the Cytocell Ltd Multiprobe technique, and scored by two investigators following the protocol described in Appendix 1. The FISH probes used to detect the origin and size of the marker chromosome identified in one of the patients are described in Appendix 1.

Screening for 15q11-13 interstitial duplications
The Prader-Willi/Angelman syndrome critical region (15q11-13) was screened for duplications by applying three different methods: 1) FISH analysis using the D15S10 and SNRPN probes (Vysis Inc), 2) densitometry using the microsatellite markers described in Appendix 1; 3) quantitative Real Time PCR of loci D15S122 and GABRA5 using probes and primers chosen with the assistance of the Primer Express software program (Applied Biosystems) and ordered from Applied Biosystems as well (for detailed description see Appendix 1).

Other molecular analyses
The presence of an FMR1 gene expansion was analysed using standard molecular PCR procedures. MECP2 gene mutation screening is described in detail in Appendix 1.

Neuro-imaging and EEG
Neuro-imaging (CT- and/or MRI scanning) of the brain was performed in all patients with either neurological signs at physical examination or in patients with microcephaly or macrocephaly (that is, an occipitofrontal head circumference below the 2nd percentile or above the 98th percentile, respectively) (n=10). Electro-encephalography was performed on all patients using standard methods.
Metabolic investigations

These included a general urinary screen as well as a search for peroxisomal, mitochondrial, glycosylation and cholesterol metabolism disturbances (for detailed description see Appendix 1).

Ophthalmologic and Ear-Nose-Throat (ENT) investigations

The investigations were performed according to international standards in all patients.
Other investigations

If clinical history, physical examination, or one of the other above mentioned studies produced clues for a specific diagnosis, further investigations in search for this diagnosis were initiated.

Patient characteristics

Detailed information on single patients is provided in Appendix 1. All patients are from Dutch extraction except for 1 Indonesian male and 1 Nigerian female. Age at physical examination varied from 22 to 45 years (mean 33.6 years). Intellectual abilities were limited in all 25 patients, the severity of mental retardation being mild (IQ 50/55-70) in 2, moderate (IQ 35/40-50/55) in 11, severe (IQ 20/25-35/40) in 11, and profound (IQ<20/25) in the remaining 2 patients. Six

Table 1. Patient characteristics of 25 mentally retarded adults with autism.

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|* mi=mild; mo=moderate; s=severe; p=profound
** only data pertaining first and second degree relatives
† degree of consanguinity
‡ c=cousins; g=g=grandfather; p=parents; s=siblings; u/a=uncle/aunt
§ rm=recurrent miscarriages; sb=sib with spina bifida;
‖ ++present, type unknown; a=alcohol; ac=anti-convulsants
patients were offspring of mothers, whose age at conception was 36 years or older. Mental retardation was present in 7 relatives; psychiatric disorders occurred in 5 relatives.

In 2 patients teratogenic influences were present during pregnancy: excessive maternal alcohol abuse in one and anti-convulsants use in the other. The patient born to the mother with alcohol abuse had multiple features fitting fetal alcohol syndrome; in the other patient no stigmata of fetal anti-convulsant syndrome were present. In a third patient phenotypic features were strongly suggestive of a teratogenic influence. However, mother denied use of medication or abuse of alcohol or other drugs during pregnancy, although she had been known with periods of alcohol abuse before.

At physical examination microcephaly or macrocephaly was diagnosed in 8 patients, and neurologic exam showed abnormalities (signs of a confirmed HNP L5-S1; drowsiness; tremor; and cogwheel rigidity) in 3 patients, while neurological side-effects of medication were present.

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++ = one or two; +++ = three or four; ++++ = multiple minor anomalies, strongly suggestive of a syndromal genesis.

malformations: + = one or two café au lait spots; ++ = three or more café au lait spots.
Table 2. Results of additional investigations and diagnoses in 25 mentally retarded adults with autism.

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<td>N</td>
<td>N</td>
<td>phi</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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</tr>
<tr>
<td>Diagnosis</td>
<td>I**</td>
<td>II**</td>
<td>III*</td>
<td>I**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=normal
m13=marker chromosome 13
mdup=mosaic pattern of duplication
pm=polymorphism
dcm=disturbed cholesterol metabolism; PKU=phenylketonuria
cacerebellar atrophy; ca=cerebral atrophy
kc=keratoconus; oo=optic atrophy; rv=reduced vision
chl=conductive hearing loss (as a result of radical eradication); phi=perceptive hearing loss
*unequivocal diagnosis; **=probable diagnosis
I=Orstavik syndrome; II=teratogenic influence; III=marker chromosome 13; IV=kernicterus;
V=phenylketonuria; VI=fetal alcohol syndrome/disturbed cholesterol metabolism; VII=private syndrome;
VIII=mosaicism for 45,XO/46,XY

In 2 patients. Minor congenital anomalies were present in 17 patients, varying from only one to multiple dysmorphic features. In 8 of them the features were suggestive for the presence of a multiple congenital anomalies syndrome. More expressed congenital anomalies (urethral stenosis; umbilical hernia) were found twice. Skin depigmentation disturbances, including café-au-lait spots and erythema, were found in 3 patients; none had symptoms of neurofibromatosis, tuberous sclerosis, or other neurocutaneous disorders.

**Additional investigations**
The results of all performed additional investigations are listed in Table 2. In this section only more specific details are provided.

**Cytogenetics**
The supernumerary marker chromosome detected in one patient was positive for FISH analysis with the centromere 13/21 probe (pZ21A), as well as for the whole paint chromosome 13.
Further studies showed the marker to be dicentric, without proximally located long arm material (13q11-12). In another patient a mosaic pattern of the sex chromosomes was found, indicating his karyotype to be [45X(75%)/46 XY(25%)]. In a third patient, subtelomeric FISH analysis yielded an Xpter deletion using cosmid probe CY29. However, this finding was not reproduced by the BAC probe 98C4 nor by molecular analysis. We concluded that the subtelomeric Xp abnormality was a polymorphism.

**Screening for 15q11-13 interstitial duplications**

The scoring results of *SNRPN* and D15S10 probes in interphase and metaphase showed a considerable intra- as well as interobserver variability (data not shown). Thus results were difficult to interpret: The number of scored interstitial duplications, applying the definitions described in the Methods section, for the *SNRPN* probe varied between 0 and 7(28%) patients and for the D15S10 probe between 1 (4.2%) and 11 (45.8%) patients. There was no patient in whom a duplication was scored in both interphase and metaphase by both technicians.

Densitometric studies did not reveal a true duplication in the patients of our cohort, but dosimetric abnormalities were found in 6 patients, indicating mosaic pattern of duplication in the region of marker D15S122 (n=4) and in marker region GABRA5 (n=2) (Fig 2). These results did not correspond to those of FISH analysis. Real-time quantitative PCR analysis of the D15S122 region in the former 4 patients did not show any abnormalities; nor did analysis of the GABRA5 region in the latter two. As a positive control, a patient known in our Department with a cytogenetically visible duplication of the region 15q11-13 was used, in whom the duplication was confirmed with Real-time quantitative PCR. Thus, by defining the latter technique as the gold standard, no individual in our cohort had an interstitial 15q duplication.
Figure 2.
Electropherograms for marker D15S122 in 4 patients:
A. Positive control with visible duplication of region #15q11-13 on karyogram. The amplitude ratio of the 154.8-mobility unit (mu) peak (**) vs the 148.9 mu peak (*) is 55.5 : 37.7 (=1.5).
B. Normal patient without a duplication. The amplitude of the 146.8 mu peak (**) vs the 144.9 mu peak (*) is 42.4 : 64.6 (=0.7).
C. Patient with a possible mosaic duplication of this locus. The blue (150.9) vs orange (146.8) peak ratio is 61.4 : 53.1 (=1.2), which is similar to, but smaller than golden standard A).
D. Another patient with a possible mosaic duplication of this locus. The blue (149) vs orange (144.9) mu peak ratio is 43.6 : 36.7 (=1.2), which is also similar to but smaller than A).

Molecular analysis
In one female analysis of MeCP2 showed a C to T transition at position 1125 (the C-terminal region) causing a substitution of serine for proline at amino-acid position 376. In the subsequent screening of 200 X-chromosomes of normal controls, a similar mutation was detected in one control, indicating it to be most probably a polymorphism.

Metabolic investigations
In one patient a previously established diagnosis of phenylketonuria (PKU) was reconfirmed by detection of elevated plasma concentration of phenylalanine. In another patient, in whom the clinical diagnosis fetal alcohol syndrome (FAS) was established, an abnormality of the distal cholesterol biosynthesis was detected: plasma concentrations of 5;7-dehydrocholesterol as well as 5;8-dehydrocholesterol were elevated, and plasma bile acid concentrations were low. Molecular analysis of the complete gene gave normal results however. It seems likely that the detected cholesterol abnormalities can be attributed to the patient's intake of haloperidol, as has been reported before. In 5 other patients in our cohort also using haloperidol, no abnormalities were detected in their cholesterol biosynthesis.

Neuro-imaging
In 10 patients neuro-imaging studies were performed. Cerebellar atrophy was found in one and in another possibly diffuse cerebral atrophy was present; in all others neuro-imaging did not show any abnormality.
Diagnosis

The individual case histories of the following 9 patients are described in detail in Appendix 1. In 5 (20%) patients the following unequivocal aetiological diagnoses were established: a prenatal factor in 1 (FAS; case #1), a perinatal factor in 1 (kernicterus; case #2), a metabolic disturbance in 1 (PKU; case #3); and a chromosome abnormality in 2 (marker chromosome 13; case #4) (mosaic karyotype [45X(75%)/46XY(25%)]; case #5). In 4 (16%) patients a diagnosis was probable but without firm proof: a private syndrome in 1 patient (case #6), a teratogenic factor in 1 (case #7), and Orstavik syndrome in 2 patients (cases #8 and #9). Of the latter 2, features are compared to literature cases in Table 3.

Table 3. Orstavik syndrome: comparison of our patients to the literature reports.

<table>
<thead>
<tr>
<th></th>
<th>Orstavik #1</th>
<th>Orstavik #2</th>
<th>Case #5</th>
<th>Case #13</th>
</tr>
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<tbody>
<tr>
<td>Gender</td>
<td>female</td>
<td>female</td>
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<tr>
<td>Age</td>
<td>14</td>
<td>&lt; 14</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>Family history:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental retardation</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>at term</td>
<td>at term</td>
<td>at term</td>
<td>at term</td>
</tr>
<tr>
<td>Weight at birth</td>
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<td>2660</td>
<td>3480</td>
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<tr>
<td>OFC at birth</td>
<td>36 cm (P75)</td>
<td>34 (P25)</td>
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<td>?</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Autism</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Seizures</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Dymorphic features</td>
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<tr>
<td>Macrocephaly</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>High, broad forehead</td>
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<td>-</td>
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<td>Deep set eyes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Short philtrum</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Laboratory investigations</td>
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<td>EEG abnormalities</td>
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<td>+</td>
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<td>Neuroradiologic abnormalities</td>
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<td>?</td>
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<td>Muscle biopsy</td>
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<td>?</td>
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<td>Spinal fluid (protein content &amp; electrophoresis)</td>
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<td>N</td>
<td>?</td>
<td>?</td>
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<tr>
<td>Urinary metabolic screening</td>
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<td>N</td>
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<td>N</td>
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<tr>
<td>Glutaryl CoA dehydrogenase</td>
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<td>46,XX</td>
<td>46, XY</td>
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<td>Molecular studies for fraX syndrome</td>
<td>N</td>
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</table>

Diagnostic yield of individual investigations

The diagnostic yield of each investigation differed widely (Table 4). A detailed clinical history identified abnormalities in 13 patients; in 5 of them, this investigation contributed significantly to the diagnosis. Family history gave abnormalities in 12 patients, which contributed significantly in 1 patient. Physical examination showed abnormalities in 20 patients, in 7 of whom these findings did contribute to establishing a diagnosis. Karyotyping lead to diagnosis in 2 patients, while the results of FISH screening for subtelomeric rearrangements as well as for 22q11 deletions
Table 4. Yield of investigations performed in all 25 mentally retarded adults with autism.

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Abnormalities identified (No patients)</th>
<th>Contributing to a diagnosis (No patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient clinical history</td>
<td>13</td>
<td>5</td>
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<tr>
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<td>Physical examination</td>
<td>20</td>
<td>7</td>
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<tr>
<td>Karyogram</td>
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<td>2</td>
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<td>FISH subtelomeric regions</td>
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<td>0</td>
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<tr>
<td>FISH DiGeorge region</td>
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<td>0</td>
</tr>
<tr>
<td>Densitometry 15q11-13 region</td>
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<td>0</td>
</tr>
<tr>
<td>Real Time PCR 15q11-13 region</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fragile X screening</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MECP2 mutation screening</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Metabolic screening</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>EEG</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Neuro-imaging</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ophthalmologic investigations</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>ENT investigations</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

were normal. Screening for duplications in the PW/AS region through densitometry were indicating possible mosaic duplications in 6 patients, but these could not be confirmed by the Real Time PCR technique. Molecular analysis for FMR1 gene expansions did not contribute to the diagnosis, nor did MECP2 gene mutation screening. Finally, EEG abnormalities were detected in 8 patients, contributing to a diagnosis in two. Based on neuro-imaging findings a diagnosis could be established in two individuals.

Remaining patients

Of the remaining 16 (64%) patients without any diagnosis, one had a major congenital malformation and 8 had minor anomalies on physical examination. Of the latter group, no individual had 4 or more anomalies, which would have been suggestive of a MR/MCA syndrome.

Since Kanner's description of autism as a developmental disorder characterized by “extreme autistic loneliness” and “an obsessional desire for the maintenance of sameness” autism has often been referred to as a diagnosis on its own. However, autism should be considered a symptom, caused by a variety of underlying disorders. Most investigators at present assume that the underlying pathophysiologic process causing autism arises from the interaction between a genetic predisposition and an early environmental insult.

Comparison to other studies

In the present study, a diagnosis was made in 9 (36%) patients, in 5 unequivocally, in 4 probably
These results fall in the middle range when compared to similar other studies: an organic aetiologic factor for autism was found to range from 10% to 60%. The variation in results of these studies may be explained by a difference in diagnostic criteria used to establish the diagnosis of autism, in patient selection and in diagnostic work up.

Present study: advantages and limitations
In the present study we analyzed a group of patients assembled previously for another study (with a different scope), residing all in the same institute for the mentally retarded, fulfilling all the DSM-IV criteria for autism, and with a standardized work up in all patients that was (almost) complete for present standards. The work up is limited only in the absence of screening of family members for components of the broad autism phenotype, as suggested by Piven and co-workers. However, this approach is especially important in linkage studies, and less so for finding organic causes as in the present study.

The fact that all patients were mentally retarded adults may have created a bias. However, as 75-85% of patients with autism is mentally retarded and as the distribution of the different degrees of delay within the present study group is similar to the distribution within persons with autism in general, the introduced bias is limited. For individuals with autism whose characteristics differ from those of our cohort, such as children and individuals without mental retardation, the results and conclusions of the present study are only partly applicable. Therefore the yield expected for a diagnostic work up should be adjusted to the age and developmental level of the autistic patient.

Values of each diagnostic investigation
Clinical history and physical examination
Clinical history and physical examination have proven to be the most rewarding parts of a diagnostic work up. A patient's history may provide clues for prenatal causes, i.e. fetal teratogens exposure such as thalidomide and alcohol. The exact teratogenic mechanism of alcohol is unknown, although a clue may be the disturbance in the migration of neuronal and glial cells, supported amongst others by the cerebellar anomalies present in both animal models of FAS as in individuals with autism. Perinatal history may provide clues for factors as neonatal jaundice. Before the advent of phototherapy and exchange blood transfusions neonatal jaundice often caused kernicterus, characterized by sensorineural hearing loss, mental retardation, and evidence on MRI of damaged basal ganglia, especially the globus pallidus. The latter characteristic may well explain the autistic behaviour reported in patients with kernicterus, as there is increasing evidence that volume and function of basal ganglia are different in autistic individuals compared to controls.

Detailed physical examination, including anthropometric, neurologic, and dysmorphologic exams, may provide clues to many possible etiologies. In general, the incidence of minor physical
anomalies in patients with autism is increased when compared to controls, indicating that in the former group structural development was disrupted during early embryogenesis as a result of underlying disorder. Abnormal cephalic measures occur among autistic persons in a significantly higher proportion than in the general population, and have been suggested the single most consistent physical characteristic of autistic individuals. In our cohort the frequency of macrocephaly (20%) and microcephaly (12%) is similar to the frequencies among the general autistic population (resp. 20% and 15%).

Many syndromes are known to have a distinct behavioural phenotype, indicating the potential for the causative genes to influence human cognitive development. Autism is considered such a behavioural phenotype associated with several well-described syndromes, such as Williams syndrome and Moebius syndrome. In our cohort a syndromic genesis was suspected for 2 individuals whose phenotype strongly resembled the autosomal recessive syndrome described by Orstavik et al, and which is characterized by epilepsy, mental retardation, facial dysmorphism, and macrocephaly (Table 3). This combination of features may have been described before in 1995 by Andermann in 4 patients. As neuro-imaging data is lacking in both our patients, an unequivocal diagnosis of Orstavik syndrome is currently not possible.

Karyotyping

A broad spectrum of chromosome anomalies in autism has been reported, involving almost all chromosomes and many types of rearrangements. Most frequently documented are (de novo) structural and numerical abnormalities of sex chromosomes and anomalies of chromosome 15. In our cohort 2 (8%) individuals were identified with numerical chromosome anomalies, a rate similar to the 5% reported by Bailey et al in 1996 and 6.3% by Konstantareas et al in 1999. In the literature 3 patients similar to our case with 45,X/46,XY mosaic and autistic behaviour have been described. The present patient with mosaic Turner karyotype showed bilateral shortening of the fourth metatarsal bones, a common feature in Turner syndrome. This is additional evidence that the chromosome anomaly exerts its effect on the phenotype. Furthermore the maternal origin of the remaining X in the present patient is in concert with the hypothesis of a parent-of-origin effect in the X-chromosome influencing social cognition. Although supernumerary marker chromosomes have been reported in individuals with autism, to our knowledge no other cases with a marker derived from chromosome #13 were reported to have autism. Interestingly, a chromosome 13q region was the most significant result in one collaborative linkage study (CLSA) genome scan. Other linkage studies also showed increased sharing of this locus, although weaker. It seems likely that the marker #13 chromosome has affected the phenotype, including the presence of autism.
Submicroscopic deletions

Patients with the velo-cardio-facial (VCF) syndrome have a distinct behavioural phenotype and often suffer psychiatric disorders, including autism. FISH analysis in all present patients was performed despite the absence of the dysmorphic features of VCF syndrome, and no deletion was identified. This concurs with a recent paper, in which no 22q11 deletions were not found in autistic patients without a phenotype suspect for VCF syndrome. Submicroscopic rearrangements involving (sub-)telomeric regions are emerging as important cause of mental retardation. In our cohort no subtelomeric rearrangements were found. Due to the limited number of patients in our cohort, however, no firm conclusions can be drawn. To ascertain presence and frequency of these cryptic rearrangements among autistic individuals, further similar and if possible larger studies are required.

15q11-13 abnormalities

Over the past decade numerous reports have mentioned abnormalities of chromosome 15 associated with autism, with or without mental retardation. Frequently it concerned supernumerary isodicentric chromosomes 15, less frequently interstitial duplications and rarely triplications of the 15q11-13 region were described, almost all maternally derived. The region may harbour potential susceptibility gene or genes for autism. In a large study performed by Schroer et al (1998), a cohort of 100 consecutive patients with autism was screened and abnormalities were identified in 4 individuals (4%): 2 with supernumerary bisatellited marker chromosomes #15, 1 with a 15q11-13 deletion and 1 with an interstitial duplication of region 15q11-13. In most cases from literature an interstitial duplication (or triplication) was already visible on G-banded chromosomes. However, there is as yet no generally accepted reconfirmation method, nor a method to screen for submicroscopic duplications in this region. FISH analysis, sometimes followed by PCR microsatellite analysis is the most widely applied method. There is only a single report of subtle 15q11-13 interstitial duplications, that according to the authors might have been missed in routine chromosome or FISH analysis: microsatellite densitometry analysis was used to detect this abnormality in 2 sibs with autism, which was also present in their unaffected mother. In our cohort, we found no patients with cytogenetically visible abnormalities of chromosome #15. We first performed FISH analysis, but this technique seems not suitable for detecting cryptic 15q11-13 duplications because: the company delivering the probes (Vysis, Inc.) states in their 'Quality Assurance Certificate' that “Occasionally, the LSI probes may appear as three or four signals, depending upon condensation of the DNA and the relative distances between chromatids. All probe signals may also appear diffuse or split”; no validated method has been published to quantify and qualify results of duplication screening of region 15q11-13 by FISH, and various subjective terms have been used, such as “a large merged signal”, “a double signal”, or “a split signal”; interobserver and intraobserver variability
studies are lacking. Subsequently, we have performed densitometric studies. This technique yielded possible interstitial duplications (in mosaic form) in several patients; these did however not coincide with those found by FISH. Furthermore, we doubted the validity, as the amplitude ratio of the electropherogram peaks in our 6 patients was less than the ratio in the positive control (Fig. 2). Therefore, quantitative Real Time PCR was performed, but no abnormalities were found, indicating that no individual in our cohort had such duplication. FISH analysis and densitometry would have lead to an overestimated frequency of interstitial 15q duplications in our cohort. Hence, this seems to be a less frequent cause of autism than suggested by previous clinical studies.15 72 Real Time PCR has proven to be a sensitive, specific, and reproducible method for diagnosing changes in gene dosage, especially in diagnostic cancer research.74 A recent exemplary study was performed by Aarskog and co-workers, who used Real Time PCR to detect cryptic PMP22 duplications and deletions in patients with Charcot-Marie-Tooth type 1A.75 For our study, its technical validity was proven by confirmation of a duplication in a cytogenetically positive control. Whether duplications in the PWAS region are really pathophysiologically significant in autism remains to be elucidated. Recently duplications of the GABRA5 gene were detected in unaffected individuals, suggesting that (some of) 15q11-13 duplications may be benign polymorphisms.76

**Fragile X syndrome**

No patients were identified with fraX. Indeed, the relationship between these 2 conditions remains uncertain. At first males and females with fraX were reported to display autistic behaviour frequently,77 but later on autism was proven to occur not more frequently in a fraX population than in the mentally retarded population in general.78 Also, no linkage with the FMR1 gene region was proven in a study of families multiplex for autism but without cytological evidence of fragile X expression.79

**MeCP2 gene screening**

The recent study by Lam et al reporting a MeCP2 gene mutation in one of the 21 screened (non-RTT) individuals with infantile autism and mental retardation, motivated us to perform a similar screening in our cohort.80 No true mutations were found, as the single point mutation we found is probably a polymorphism, as it has been reported before in unaffected family members.81 82

**DNA repair disorder**

One of the present patients (case report #6), showed many features compatible with a DNA repair disturbance, although firm proof at cellular level is still lacking. To our knowledge only one earlier report mentioned autism in a patient with a DNA repair disturbance.83 It remains uncertain how this relation, if any, might be explained. Well-known repair disturbances such as Bloom, Cockayne, Rothmund-Thomson, and Werner syndrome do not exhibit autism.86
Metabolic screening
Phenylketonuria, hypersuccinylpurinaemia, changes of aromatic acids and mono-amines, lactate acidosis, and cholesterol anomalies are metabolic disturbances reported in patients with autism. In our cohort extensive screening of multiple metabolic pathways resulted in the (re)confirmation of metabolic disturbances in two patients: phenylketonuria (PKU) in one and an abnormality in the distal cholesterol biosynthesis in another patient. The mechanism through which PKU causes autism remains uncertain, but has been suggested to involve dopaminergic pathways. The so-called Enzyme/Brain-Barrier has also been suggested to be involved. In our cohort, no proof of a mitochondrial dysfunction was present. A disturbance in brain energy metabolism due to mitochondrial dysfunction has been proposed as a cause of autism, supported by reports of lactate acidosis and urinary excretion of Krebs cycle metabolites in autistic patients. It has been hypothesized that mitochondrial dysfunction may act through an excessive nitric oxide production.

Finally, abnormalities in the glycosylation process cause the CDG syndrome phenotypes. As these are still in the process of delineation, we investigated all patients, all with negative results.

EEG
The recognition of a high incidence of EEG abnormalities and of seizure disorders involving all areas of the cerebral cortex was among the earliest evidence of an organic basis of autism. In autistic individuals with a single EEG, abnormalities are present in about 40% versus 65% with multiple EEGs. In our cohort single EEG’s identified abnormalities suggestive of epilepsy in about one third of patients (n=8). However, epilepsy should not be considered a separate aetiological entity but a symptom.

Neuro-anatomic imaging
Neuro-anatomical abnormalities are frequently found in autistic individuals, affecting the cerebral cortex, thalamus, brainstem and most often the cerebellum. It has been difficult to integrate these findings in an explanatory model. Of the present 10 patients in whom neuro-imaging was performed, findings concuring with the diagnosis kernicterus were identified in one patient, and in the patient with a possible DNA repair disorder signs of cerebral atrophy were present. Otherwise all neuroradiologic studies gave normal results. Thus in our cohort neuro-imaging provided few clues for prior unsuspected diagnoses.

ENT and ophthalmologic investigation
Investigation of both vision and hearing is important in any individual with a developmental delay for adequate support and care and to detect specific causes for the mental handicap. Although in our cohort the ENT and ophthalmologic investigations did not provide new
aaetiological insights, a considerable number of individuals were identified with insufficient sensory function, with therapeutical consequences.

In autism, a specific aetiological diagnosis is of considerable value, both to establish prognosis and to provide adequate care, as well as for genetic counselling and for unraveling its pathogenetic mechanisms. The present study demonstrates that an extensive, structured work up yields a diagnosis in at least 20%, and possibly up to 36% of adult autistic individuals with mental retardation. As these underlying medical conditions encompass teratogenic, metabolic, as well as syndromic influences, prerequisites for such a yield are a multidisciplinary approach, of which clinical history taking and physical examination form the basis. In the future similar studies diagnostic data should be gathered on a larger number of patients, allowing more firm conclusions. Linkage studies, used to screen multiplex families in order to identify autism susceptible loci, will profit from an initial diagnostic work up of all individuals prior to inclusion as all other causation of autism will be ruled out. The diagnostic yield of linkage studies will undoubtedly increase if the cohorts comprise only these remaining cases with truely idiopathic autism.

This project was financially supported by grants from the ‘Stichting tot Steun van het Emma Kinderziekenhuis’ and the ‘Stichting Klinische Genetica Amsterdam’. We are grateful to the patients and their parents for their cooperation in this study project. We thank M Roelink-Reynhoudt for performing venapunctures; E de Boer and C Koevoets for performing cytogenetic analyses; R Rust, Drs R van den Boogaard, Dr M Alders and Dr M Mannens for molecular analyses; Dr V Kalscheuer for providing YAC clones; Dr H Waterham for metabolic studies; N Briare, Drs W de Bruin, M Dudok van Heel, A Kraak and JAPM de Laat for audiometric/ENT studies; and Dr W Dorsman, F Gunther, and G Kinds and for ophthalmologic/optometric investigations.
Detailed description of methods

(published electronically: www.jmedgenet.com)

Protocol for scoring FISH analysis of subtelomeric regions

Two investigators each scored a minimum of 5 metaphases per probe focusing on deletion, duplication and balanced translocation events involving the subtelomeric region of every chromosome. Metaphases were accepted for analysis if all 46 chromosomes were present and if both the p-arm and the q-arm of both chromosome homologues could be scored. Results were considered conclusive if at least the first five metaphases were 100% concordant. If an abnormality was suspected, parents were investigated for the specific telomeres using the same technique. To reconfirm the presence of the subtelomeric rearrangement microsatellite PCR analysis was performed using standard molecular procedures. Microsatellites were obtained from genome databases (http://www.genome.wi.mit.edu) (http://www.gdb.org/) or from sequences from mapped clones. The microsatellites used specifically for reconfirmation of the Xp deletion included markers DXS1060, DXS8051, DXS987, DXS7100, DYS402.

FISH analysis of marker chromosome

To detect the origin of the marker chromosome that was found in one of the patients, FISH analysis was performed using probes for centromeric regions of the acrocentric chromosomes 13, 14, 15, 21, 22 (respectively pZ21A, p14.1, D15Z1, pZ21A, p41.1), and subsequently whole chromosome paints (Eurodiagnostics) for 13 and 21 were used to discern between chromosomes 13 and 21. To assess the size of the marker chromosome, FISH analysis was performed with the following probes: YAC 911h08 (location 13q11) and YAC 748f02 (location 13q11-12).

Analysis of the 15q11-13 region

Protocol for scoring FISH

The Prader-Willi/Angelman syndrome critical region (15q11-13) was screened for duplications by FISH analysis using the D15S10 and SNRPN probes (Vysis Inc), scoring 10 interphases and 10 metaphases for each of the 2 probes by two different technicians, applying as a definition for a duplication in interphase 3 or 4 signals (two of which in close proximity) in 70% or more of scored interphases, and for duplication in metaphase the presence of a double (Figure 1B) and/or large merged signal (Figure 1C) on one of the chromosomes 15 in at least 70% of scored metaphases.

Densitometry (markers)

The 15q11-13 region was screened by densitometry using microsatellite markers D15S10, D15S63, D15S113, D15S122, GABRB3, GABRA5 and ACTC.
Real Time PCR technique (primers and probes)

Of locus D15S122 this technique was performed using AUT122 exon forward primer: GCCTGAGACTGCAATGAGTTATGA; AUT122 exon reverse primer: TTAGAGACAGGTCTCGCTGTGT; AUT122 exon TaqMan probe (FAM labelled): AGCACCACTGCACTCCAACTTGGGC; INSR exon3 forward primer: TGGCGCTGTGAAGTCTCA; INSR exon3 reverse primer: CCTCCCGAGTTCTTTGACAT; INSR exon3 TaqMan probe (VIC labelled): CTTCTGACGCAGCTGCACACAA.

Of locus GABRA5 this technique was performed using GABRA5 exon10 forward primer ACCACGGTGCTGACCATA; GABRA5 exon10 reverse primer AGGCCACTTGGGCAGAGA; GABRA5 exon10 Taqman probe ACCCTCATCAGCGCCAGGA. The quantitative Real Time PCR technique uses standard PCR in conjunction with a fluorescent TaqMan method and an ABI prism 7700 sequence detector, which is capable of measuring fluorescence in realtime. Through measuring the PCR product accumulation through a dual-labeled fluorogenic probe, it provides an highly accurate quantitation of gene copies.

MeCP2 mutation screening

This was performed as follows: The three coding exons of the MeCP2 gene were amplified by PCR. Overlapping fragments of +/- 300 base-pairs were generated (a total of 9 fragments) and analysed by denaturing high performance liquid chromatography (DHPLC) or single stranded conformational polymorphism (SSC) (genephor system AmershamPharmaciaBiotech).

Metabolic investigations

Metabolic screening for inborn errors of metabolism was performed in all patients including a urine screen for amino acids, organic acids, oligosaccharides, acid mucopolysaccharides, and uric acid; a blood screen for lactic acid, pyruvic acid and ketone bodies, serum free fatty acids, copper and caeruloplasmin. Tandem mass spectrometry of plasma acylcarnitines was performed for screening of mitochondrial fatty oxidation defects and defects in the catabolism of branched chain amino acids. Gas chromatography of very long chain fatty acids (C24–C26) and phytanic acid in plasma was applied for detection of peroxisomal disorders and an extensive analysis of phospholipid metabolism was performed as well. The distal cholesterol pathway was investigated by determining the plasma level of the total cholesterol as well as of the diene sterols of 7-and 8-dehydrocholesterol, to search for the Smith-Lemli-Opitcz syndrome and related entities. Isoelectric focussing of serum transferrin was performed to search for a congenital disorder of glycosylation.
Case Reports
(published electronically: www.jmedgenet.com)

Case 1 (tables:#14)
The proband is the second child born to healthy non-consanguineous parents. There is no known family history of psychiatric disorders, mental retardation, or congenital anomalies. During pregnancy mother used moderate amounts of alcohol. The delivery was at term, birth weight and OFC are unknown. During childhood he had recurrent upper airway infections and received orthopedic therapy for pedes plani valgi. Autism was diagnosed at the age of 4 years and he was admitted to an institute for the mentally handicapped at age 6 years. At the age of 17 years, his disharmonic, explosive behaviour necessitated anti-psychotic medication, which had considerable side effects such as parkinsonism and slow mimicry. IQ was tested at the age of 30 years, showing him to be moderately retarded. Physical investigation at the age of 34 years showed macrocephaly, receding forehead, deep set eyes, blepharophimosis, high nasal bridge, large nose, hypoplastic malae, thin upper vermilion border, prominent antihelices of the ears, mild pectus excavatum, prominent ribs, and inverted nipples.
Metabolic investigations showed elevated levels of cholesterol precursors, and ENT studies confirmed a left-sided hearing loss as a result of a total lumen eradication. All other investigations were normal.

Case 2 (tables:#11)
The proband is the youngest of six children of healthy, non-consanguineous parents of whom the mother has a borderline intelligence. Three sibs have mild mental retardation and there is a positive family history of deafness. During pregnancy, mother smoked several cigarettes a day. The delivery was preterm (at 30 weeks), birth weight was 1500 g. The neonatal period was complicated by rhesus antagonism causing extremely high hyperbilirubinaemia (17 mg/100ml), leading to choreoathetosis. Treatment was limited to supportive measures.
At the age of 2 yrs, a delay in psychomotor development was noted. He walked at 3.5 years. During early childhood bilateral sensorineural hearing loss was diagnosed necessitating a hearing aid. At age the age of 4 years, the diagnosis autism was made. He was admitted to an institute for the mentally handicapped at the age of 16 years; at that age mental retardation was graded as moderate, which was reconfirmed at the age of 29 years by formal IQ testing. At the age of 18 years, a pyelumplasty was placed for a subpelvine ureter stenosis. Physical investigation at the age of 24 years showed hypoplastic earlobes and a double folded helix as only dysmorphic features, mild spasticity of the extremities, and choreoathetosis. Complete additional investigations showed an asymmetric EEG, sensorineural hearing loss (60dB), an atrophic cerebellum, and enlarged ventricles, but otherwise normal results. All findings are compatible with kernicterus.
Case 3 (tables:#12)
The proband is the first-born child of healthy, non-consanguineous parents. There is no family history of mental retardation, psychiatric disorders or congenital anomalies. During pregnancy, mother used moderate amounts of alcohol as well as phenobarbital in the first few months. The reason for the phenobarbital medication remained obscure. Two weeks prior to delivery, mother was diagnosed with hypertension. Delivery was uncomplicated and at term with a birth weight of about 3000 g. During the neonatal period he suffered convulsions several times while feeding. A delay in psychomotor development was noted when he was 9 months old. Phenylketonuria was finally diagnosed at age 3yrs, after which a special diet was implicated, which had to be stopped 2 years later because of constipation. During childhood, he had repeated infections as well as severe eczema. At the age of 6 years he was admitted to an institute for the mentally retarded, where the diagnosis autism was confirmed and formal IQ testing showed severe mental retardation.

Physical examination at the age of 36 years showed deep set eyes, prominent zygomatae, few molars, receding chin, and a decreased lumbar lordosis. Extremities showed a bilateral curvature of 2nd and 4th fingers and bilateral pes planus. Furthermore his skin was dry and red with multiple scars and easy susceptibility for bruising. Neurological examination showed generalized muscle atrophy, abnormal reflexes, and abnormal dysdiachokinesis.

Urinary metabolic screen at the age of 24 years reconfirmed the diagnosis PKU. All other additional investigations gave normal results, except for mild OAE.

Case 4 (tables:#4)
The proband is the only child of non-consanguineous parents both with borderline intelligence. Father has a psychiatric disorder, mother is known with epilepsy. During pregnancy, mother used anti-convulsants, but the exact medication remains unknown. Birth weight was unknown, there were no neonatal problems. Delay in psychomotor development was noticed during the first year of life. At the age of 1.4 years the anterior fontanel was still widely open and pneumencephalometry suggested the diagnosis normal pressure hydrocephaly. At the age of 4 years, a urinary screening showed lysinuria. He developed epilepsy at the age of 8 years. Behaviour was characterized by auto-mutilation. He developed severe acne conglobata. The diagnosis of autism was made at the age of 16 years, and he was admitted to an institute for the mentally handicapped at the age of 21 years. At the age of 24 years, formal IQ testing was performed and his mental retardation was graded as severe.

Physical examination at the age of 28 years showed facial features marked by acne and auto-mutilation, synophrys, small nose, long philtrum, thin upper lip, and macrostomia. Furthermore he had a pectus excavatum, small nipples and dry skin. He had excessive skin folds on the dorsal side of hands and fingers, very small, flat feet, sandal gap, and generalized hypermobility.
of the small joints. Neurological examination showed tremor and cog-wheel rigidity. Karyotyping revealed a 46XY + marker configuration. Further cytogenetic work up showed the origin of the marker to be the q arm of chromosome 13. Molecular analysis of the 15q11-13 region with marker D15S122 revealed densitometric abnormalities, suggesting a mosaic duplication pattern; this could however not be reproduced nor reconfirmed by quantitative Real Time PCR techniques. Ophthalmologic investigation showed multiple keratoconus, probably as a result of auto-mutilation (visual acuity of 0.1). Also, a bilateral perceptive hearing loss (20-40 dB) was diagnosed. All other studies gave normal results.

Case 5 (tables:#25)
The proband is the fourth child of healthy non-consanguineous parents. There is a positive 2nd degree family history for mental retardation. During pregnancy, mother developed hypertension but no medication was used. Delivery at term was prolonged, birth weight was 3200 g. At 9 months of age a delay in psychomotor development was noted. During childhood behaviour was destructive, chaotic and characterized by auto-mutilation, necessitating anti-psychotics during several years with positive results. At the age of 5 years the diagnosis autism was made. For surgical removal of a tumour, probably a gonadoblastoma, a hemicastration was performed at the age of 14 years. He was admitted to an institute for the mentally retarded at the age of 14.5 years. A malignant tumor of the bladder was diagnosed and extirpated at age 33 years. During the same year spondylolisthesis of the lumbal spinal column was diagnosed. His IQ was not formally tested until the age of 40 years, which showed him to be severely retarded. Physical examination at age 43 showed a small face, fluent eyebrows, deep set eyes, small thumbs and bilateral shortening of the fourth metatarsal bones, similar to the Turner syndrome. His karyotype was a mosaic 45,X\[75\%\] / 46,XY\[25\%\] configuration, ophthalmologic investigation showed astigmatism as well as retina abnormalities (fundus dextra: fibrae medulares) and a visual acuity of 0.2, and ENT investigation revealed mild acute otitis with effusion as well as a perceptive hearing loss (left, 20-40 dB). The results of other studies were without abnormalities.

Case 6 (tables:#22)
Proband is the youngest child of healthy, non-consanguineous parents; his two healthy female sibs are of normal intelligence. Family history is unremarkable. After an uncomplicated, term pregnancy, he was born weighing 3650 g. Delivery and neonatal period were unremarkable. His motor development was normal, but a delay in speech and cognitive development soon became apparent as did destructive behaviour. At the age of 1 year, he had a first seizure. Despite anti-convulsant medications he continued to have a tonic-clonic insult once a month. At the age of 9 years, he was admitted to an institute for the mentally retarded. At that time the diagnosis autism, first established at the age of 2.5 years, was reconfirmed. Formal IQ testing showed him to be profoundly retarded.
Physical examination at the age of 36 years showed thick, dark blond hair, thin eyebrows with lateral fanning, and notable patches of red-brown hyperpigmentation on the cheeks. He had a mild pectus excavatum, tapering fingers, and pes cavus. No other pigmentation defects were present. The EEG indicated diffuse cortical dysfunction; a CT scan showed cortical atrophy. Radiography showed an abnormal shape of the aortic arch, spondylosis of the thoracic vertebral column, and a coarse structure of bones of hands and feet. The ophthalmologist diagnosed a bilateral atrophy of the nervus opticus with a visual acuity of 0.4. Other investigations were all with normal results. No DNA-repair disturbance was found after ultra-red radiation of fibroblasts, making a breakage syndrome less likely. Results of molecular studies for Cockayne syndrome were with normal results while those for Bloom syndrome are still pending.

Case 7 (tables:#3)
Proband is the second-born child of healthy, non-consanguineous parents. There is no family history of mental retardation, psychiatric disorders or congenital anomalies. During the sixth month of pregnancy mother was icteric due to a liver dysfunction. Mother denied the abuse of alcohol during pregnancy, although she had been known with periods of excessive alcohol intake before. At birth the proband weighed 3300 g and was found to be microcephalic. A delay in psychomotor development was noted in her first year of life. She experienced no specific health problems in infancy or childhood. Autism was diagnosed at the age of 8.2 years and she was admitted to an institute for the mentally retarded at the age of 10 years. At that time her behaviour was compulsive and aggressive with frequent auto-mutilation. Formal IQ testing at 25 years showed her to be severely retarded. Physical investigation at the age of 27 years showed microcephaly, a small face, blepharophimosis, short and hypoplastic philtrum, thin upper vermilion border, small ears with dysplastic helices and absent lobules, kyphoscoliosis, small hands with broadening of the proximal interphalangeal joints, short fifth digiti with hypoplastic terminal phalanges, bilateral sandal gap and small, hypoplastic nails of toes and fingers, especially of the fifth. Additional investigations showed an abnormal EEG but otherwise normal results, including a normal CT scan.

Case 8 (tables:#10)
Proband is the sixth born of 9 children, two of whom died shortly after birth of unknown cause. Parents are distantly related (to the 7th degree) and there is a first degree of mental retardation and behavioural problems. Pregnancy and delivery were uncomplicated, weight at birth and head circumference are unknown. Motor development was normal but speech as well as social skills were severely delayed from early on. Behavioural problems included hyperactivity, temper tantrums, and autism. Genuine autism was diagnosed based on DSM-IV criteria, and mental
retardation was graded moderate. He was admitted to an institute for the mentally retarded at the age of 15 years.

At 33 years, physical investigation showed macrocephaly (OFC 60.0 cm, >98th centile), a long and narrow face, high prominent forehead, deeply set eyes, short philtrum, prominent lips, angular shape of the mandible, and inverted nipples.

All additional investigations gave normal results. MRI scanning of the brain is pending.

**Case 9 (tables:#2)**

Proband is the second-born child of healthy non-consanguineous parents. There is no family history of mental retardation, psychiatric disorders or congenital anomalies. The pregnancy was uncomplicated, delivery was at term, birth weight 3500 g, and head circumference 54 cm. Psychomotor development was at first considered normal, and it was not until the age of 2 years that the delay and autistic behaviour became apparent. First words were used at the age of 24 months. At this age he developed seizures confirmed by EEG. Autism was diagnosed by an experienced psychologist based on DSM-IV criteria and by formal IQ testing mental retardation was graded moderate. He was admitted to an institute for the mentally handicapped at the age of 7.5 years. Physical investigation at 24 years of age showed macrocephaly (OFC 60.0 cm, >98th centile), deeply set eyes, short philtrum, slight scoliosis and hypermobility of small joints.

All additional investigations gave normal results, except for mild OAE. No permission was given for neuroradiologic studies.

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Aetiological study of 25 mentally retarded adults with autism


Riordan D, Dawson AJ. The evaluation of 15q proximal duplications by FISH. *Clin Genet* 1998;54:517-21


Doctors may be able to find a cause for autism in more than a third of cases, researchers in the Netherlands suggest.

Autism is not fully understood, and it is thought a range of factors may cause the disorder.

It is a developmental disability that affects the way a person communicates and interacts with other people.

It can mean severely impaired language, social skills, delayed development, and repetitive behaviour.

The disorder affects three times as many boys as girls, and three quarters of those with autism will have a mental disability.

It has been suggested that a combination of a genetic predisposition and a harmful external factor early in infancy could explain its development.

Symptom

The 25 autistic adults in the Netherlands research all had a mental disability.

They were given a full medical examination, including eye, ear and nose investigations, brain scans, tests for metabolic and blood abnormalities and full genetic analysis.

Definite causes of autism were found in five of the adults. These included fetal alcohol syndrome where the mother drinks too much during pregnancy, thereby affecting the baby, maternal infections, problems with the processing of cholesterol and genetic defects.

Probable causes were found for another four. In one, the characteristics of damage by an external agent were seen, though the mother denied using medication, alcohol or drugs during pregnancy.

The researchers, from the Department of Paediatrics at the Emma Children’s Hospital, Amsterdam, suggest more research should be carried out looking at genetic factors in the development of autism, with large studies of families needed for more conclusive evidence.

Dr Claartje van Karnebeek, who led the research, told BBC News Online the number of causes for which they had suggested a link was important.

"It’s significant because it's a relatively high number. One in every three patients -- that's an important finding."

"It’s important for doctors and medical workers to know that there are actually causes of autism."

"Autism is no more a symptom of an underlying illness than it is the dramatic event."

Small and selective

But experts in the UK say the Netherlands study’s findings cannot be extrapolated across the autism population because it concentrated on such a small number of severely affected autistic adults.

"Small and selective"

David Potter, head of policy for the National Autistic Society, said improved knowledge about the causes of autism could lead to better treatments and care for people with autism, and better advice for parents of the possible risks of autism in further children they might be planning or in the offspring of their own children.

But he said the Netherlands study was small and selective, and did not add any new knowledge.

"The problem is that the abnormalities found are still inconsistent and do not apply across the population of those affected by autism."

"It may be premature to extrapolate the results from this study to other populations of people with autism and learning disability. However, further studies of this nature would be welcome."

But Dr Anthony Bailey, Medical Research Council clinical scientist at the Institute of Psychiatry, London, said most scientists estimated a cause could be found in only around 10% of cases of autism, and that the Netherlands research may have reported a higher figure because it looked at a particularly handicapped group.

The research is published in the Journal of Medical Genetics.

Oorzaak van autisme vaak te achterhalen

AMSTERDAM — De oorzaak van autisme is bij ruim een op de drie patiënten te achterhalen, waardoor iets te zeggen is over de kans op herhaling bij ouders met een autistisch kind. Het gaat om genetische afwijkingen in een chromosoom of in een enkel gene, of om blootstelling aan schadelijke stoffen tijdens de zwangerschap. Dat schrijven AMC-onderzoekers in het Journal of Medical Genetics dat vandaag verschijnt. (ANP)

Spits 14-03-2002

Oorzaken van autisme vaak goed te achterhalen

De oorzaak van autisme is bij meer dan een op de drie patiënten te achterhalen.

Bij een bekende oorzaak is het mogelijk uitspraken te doen over de kans op herhaling voor ouders van een autistisch kind. Dat schrijven onderzoekers van het AMC in het Journal of Medical Genetics, dat vandaag verschijnt.

De AMC-onderzoekers onderwierpen 25 autistische volwassenen aan een onderzoek en ontdekten bij negen van hen een oorzaak. "De oorzaken van autisme zijn erg divers", stelt C. van Karnebeek van de afdeling Kindergeneeskunde en Klinische Genetica, die het artikel schreef. Wellicht dat het onderzoek ooit aanknopingspunten biedt voor behandeling. (ANP)

International Herald Tribune 15-03-2002
Oorzaak autisme vaak te achterhalen

AMSTERDAM (ANP) - De oorzaak van autisme is bij meer dan een derd delen van de drie patiënten te achterhalen. Wanneer de oorzaak bekend is, wordt het mogelijk uit te zoeken of de oorzaak welke het risico oplevert van de stoornis op te sporen. Dat biedt mogelijkheid uitspraken te doen over de kans op herhaling voor ouders van een autistisch kind. Ongeveer zes op de 10.000 Nederlanders leiden aan autisme. De AMC-onderzoekers onderwierpen 25 autistische volwassenen aan een uitgebreid onderzoek. Alle tests die momenteel bekend zijn om een mogelijke oorzaak vast te stellen, werden uit de kast gehaald. Er werd onder meer gekkeken naar genetische fouten, stofwisselingsziekten, hersenafwijkingen en bloedwaarden. Op basis van deze onderzoeken kon bij negen patiënten de oorzaak van hun afwijking worden vastgesteld. Ongeveer zestien op de 10.000 Nederlanders lenen aan autisme. Van Karnebeek stelde dat kennis over de oorzaken van autisme wellicht in de toekomst ook aanknopingspunten voor behandeling kan bieden, maar zover is het nog niet. "Wij richten ons nu voor al op het uitzoeken van de grote variëteit aan oorzaken." Ongeveer zestien op de 10.000 Nederlanders leiden aan autisme. De patiënten hebben moeite contact te maken, met taalontwikkeling en vertrouwen vaak stereotype gedrag. Zeker driekwart van de autisten is verstandelijk gehandicapt. Autisme komt vaker voor bij mannen dan bij vrouwen.

De oorzaak van autisme is vaak te achterhalen

De oorzaak van autisme is bij meer dan een op drie patiënten te achterhalen. Bij een bekende oorzaak is het mogelijk uitspraken te doen over de kans op herhaling voor ouders van een autistisch kind. Ongeveer zestien op de 10.000 Nederlanders leiden aan autisme. De oorzaak van autisme is genetisch, zoals een afwijking in een chromosoom of een aanlopende schadelijke stoffen tijdens de zwangerschap. Zo had één van de patiënten in de baarmoeder blootgestaan aan veel alcohol en leed aan een zogeheten foetaal alcohol syndroom. Van Karnebeek stelde dat kennis over de oorzaken van autisme wellicht in de toekomst ook aanknopingspunten voor behandeling kan bieden, maar zover is het nog niet. "Wij richten ons nu voor al op het uitzoeken van de grote variëteit aan oorzaken."