APPENDIX 1 DETAILED DESCRIPTION OF METHODS

Protocol for scoring FISH analysis of subtelomeric regions

Two investigators each scored a minimum of five 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) or from sequences from mapped clones. The microsatellites used specifically for reconfirmation of the Xp deletion included markers DXS1060, DXS8051, DXS987, DXS7100, and 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, and 22 (pZ21, p14.1, D15Z1, p22.1A, and p41.1, respectively), and subsequently whole chromosome paints (Eurodiagnostics) for 13 and 21 were used to distinguish 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 SNRP probes (Vysis Inc), scoring 10 interphases and 10 metaphases for each of the two probes by two different technicians, applying as a definition for a duplication in interphase three or four signals (two of which are in close proximity) in 70% or more of scored interphases, and for duplication in metaphase the presence of a double (fig 1B) and/or large merged signal (fig 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 D15S10,102 D15S63,103 D15S113,104 D15S22,105 GABRB3,106 GABRA5,107 and ACTC.108

Real time PCR technique (primers and probes)

For locus D15S22 this technique was performed using AUT12 exon forward primer: GCCTGAGCTCGACATGAGTCATGA; AUT12 exon reverse primer: TTAGACAGGCTCTCGCTGTGT; AUT12 exon TaqMan probe (FAM labelled): AGGCCACCTGCTCACCACCTGTTGGGC. INSY ex03 forward primer: TGGGCCGTGTGTTCAACTTCA; INSR ex03 reverse primer: CTCGCCGAGTTTCCGAT; INSR exon3 TaqMan probe (VIC labelled): CTTCCTGCAAGGACCTGCACACA. For locus GABRB5 exon10 forward primer ACACCGGTCTGCATGACATAG; GABRB5 exon10 reverse primer AGGCCACTTTGCGGACAGA; GABRA5 exon10 TaqMan probe ACCTCCCATCAGGCACCAGGA. 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 Real time.109 Through measuring the PCR product accumulation through a dual labelled fluorogenic probe, it provides a highly accurate quantitation of gene copies.110 111

MECP2 mutation screening

This was performed as follows. The three coding exons of the MECP2 gene were amplified by PCR. Overlapping fragments of ± 300 bp were generated (a total of nine fragments) and analysed by denaturing high performance liquid chromatography (DHPLC)112 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, and 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.113 Gas chromatography of very long chain fatty acids (C24-C26) and phytic 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, for the Smith-Leimli-Opitz syndrome and related entities.114 115 Isoelectric focusing of serum transferrin was performed to search for a congenital disorder of glycosylation.116

References


**APPENDIX 2 CASE REPORTS**

**Case 1 (tables, No 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 the mother drank 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 orthopaedic therapy for bilateral pes planovalgus. Autism was diagnosed at 4 years of age and he was admitted to an institute for the mentally handicapped at 6 years. At the age of 17, his dyskinetic, explosive behaviour necessitated anti-psychotic medication, which had considerable side effects such as parkinsonism and slow mimicry. IQ was tested at 30 years, showing him to be moderately retarded. Physical investigation at 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 raised 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, No 11)**

The proband is the youngest of six children of healthy, non-consanguineous parents; the mother has borderline intelligence. Three sibs have mild mental retardation and there is a positive family history of deafness. During pregnancy, the mother smoked several cigarettes a day. The delivery was preterm (at 30 weeks) and birth weight was 1500 g. The neonatal period was complicated by rhesus incompatibility causing extremely high hyperbilirubinaemia (17 mg/100 ml), leading to choreoathetosis. Treatment was limited to supportive measures.

At the age of 2 years, 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 4 years, the diagnosis of autism was made. He was admitted to an institute for the mentally handicapped at 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 18, a pyeloplasty was performed for a subpelvic ureteric stenosis. Physical investigation at 24 years showed hypoplastic earlobes and a double folded helix as the only dysmorphic features, mild spasticity of the extremities, and choreoathetosis.

Complete additional investigations showed an asymmetrical EEG, sensorineural hearing loss (60 dB), an atrophic cerebellum, and enlarged ventricles, but otherwise normal results. All findings are compatible with kernicterus.

**Case 3 (tables, No 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, the mother drank moderate amounts of alcohol as well as taking phenobarbital in the first few months. The reason for the phenobarbital medication remained obscure. Two weeks before delivery, his 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 the age of 3 years, after which a special diet was introduced, which had to be stopped two years later because of constipation. During childhood, he had repeated infections as well as severe eczema. At 6 years he was admitted to an institute for the mentally retarded, where the diagnosis of autism was confirmed, and formal IQ testing showed severe mental retardation.

Physical examination at the age of 36 years showed deep set eyes, prominent zygoma, few molars, receding chin, and a decreased lumbar lordosis. Extremities showed a bilateral curvature of the second and fourth fingers and bilateral pes planus. Furthermore, his skin was dry and red with multiple scars and easy bruising. Neurological examination showed generalised muscle atrophy, brisk reflexes, and abnormal dysdiadochokinesis.

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

**Case 4 (tables, No 4)**

The proband is the only child of non-consanguineous parents both with borderline intelligence. The father has a psychiatric disorder and the mother has epilepsy. During pregnancy, the mother used anticonvulsants, but the exact medication is unknown. Birth weight was unknown and 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 fontanelle was still wide open and pneumencephalometry suggested the diagnosis of normal pressure hydrocephalus. At the age of 4, a urinary screen showed hypouria. He developed epilepsy at 8 years. Behaviour was characterised by self-mutilation. He developed severe acne conglobata. The diagnosis of autism was made at 16 years, and he was admitted to an institute for the mentally handicapped at 21 years. At 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 self-mutilation, synophrys, a small nose, long philtrum, thin upper lip, and macrostomia. Furthermore he had pectus excavatum, small nipples, and dry skin. He had excessive skin folds on the dorsal side of the hands and fingers, very small, flat feet, a sandal gap, and generalised hypermobility of the small joints. Neurological examination showed tremor and cog wheel rigidity. Karyotyping showed a 46,XY+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 showed densitometric abnormalities, suggesting a mosaic duplication pattern; this could, however, not be reproduced or reconfirmed by quantitative real time PCR techniques. Ophthalmological investigation showed multiple keratoconus, probably as a result of self-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, No 25)
The proband is the fourth child of healthy, non-consanguineous parents. There is a positive second degree family history of mental retardation. During pregnancy, the 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 characterised by self-mutilation, necessitating antipsychotics for several years with positive results. At 5 years, the diagnosis of autism was made. For surgical removal of a tumour, probably a gonadoblastoma, a hemicastration was performed at 14 years. He was admitted to an institute for the mentally retarded at 14.5 years. A malignant tumour of the bladder was diagnosed and extirpated at 33 years. During the same year spondylolisthesis of the lumbar spinal column was diagnosed. His IQ was not formally tested until the age of 40, which showed him to be severely retarded.

Physical examination at the age of 43 showed a small face, deep set eyes, small thumbs, and bilateral shortening of the fourth metatarsal bones, similar to Turner syndrome. His karyotype was a mosaic 45,X [75%]/46,XY [25%] configuration, ophthalmological investigation showed astigmatism as well as retinal abnormalities (fundus dextra, fibrae medulares) and a visual acuity of 0.2, and ENT investigation showed mild acute otitis with effusion as well as a perceptive hearing loss (left, 20-40 dB). The results of other studies were normal.

Case 6 (tables, No 22)
The 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 the 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 his first seizure. Despite anticonvulsant medication, he continued to have a tonic-clonic fit once a month. At the age of 9 years, he was admitted to an institute for the mentally retarded. At that time, the diagnosis of autism, first established at the age of 2.5 years, was reconfirmed. Formal IQ testing showed him to be severely 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 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, spondylolisthesis of the thoracic vertebral column, and a coarse structure of the bones of the hands and feet. The ophthalmologist diagnosed bilateral atrophy of the optic nerve with a visual acuity of 0.4. Other investigations were all with normal limits. 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 normal while those for Bloom syndrome are still pending.

Case 7 (tables, No 3)
The 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, the mother became icteric owing to a liver dysfunction. She denied the abuse of alcohol during pregnancy, although she had been known to have 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 10 years. At that time, her behaviour was compulsive and aggressive with frequent self-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, a 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 digits with hypoplastic terminal phalanges, bilateral splay gap, and small, hypoplastic nails of the toes and fingers, especially the fifth.

Additional investigations showed an abnormal EEG but otherwise normal results, including a normal CT scan.

Case 8 (tables, No 10)
The proband is the sixth born of nine children, two of whom died shortly after birth of unknown cause. The parents are distantly related (to the seventh degree) and there is a first degree history 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 as 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, deep 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, No 2)
The 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 spoken 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 mental retardation was graded as moderate by formal IQ testing. 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), deep set eyes, short philtrum, slight scoliosis, and hypermobility of small joints. All additional investigations gave normal results, except for mild OAE. Permission was not given for neuroradiological studies.