**Rett syndrome** is an X linked mental retardation syndrome almost exclusively affecting girls, and has long been regarded as an X linked dominant condition lethal in hemizygous males. Mutations in the gene encoding the methyl-CpG binding protein 2 (MECP2) were demonstrated as the cause of Rett syndrome, and confirmed by a number of studies. The vast majority (95%) of MECP2 mutations occur de novo. Girls affected by “classic” Rett syndrome show mental retardation and regression, with a typical pattern of symptoms including initially normal development, stagnation, loss of acquired abilities, stereotypic hand movements, regression of speech, profound psychomotor retardation, epilepsy, and autism, although molecular diagnostics has proven that variant clinical forms exist.

It has recently been shown that missense mutations in MECP2 can cause severe neonatal encephalopathy in boys. Classic Rett phenotypes in boys have so far only been reported in rare cases of somatic mosaicism or XXY karyotypes. In girls, larger intragenic deletions are responsible for about 11–16% of typical Rett syndrome without point mutations in the coding exons. Larger deletions have not yet been found in boys, and duplications of MECP2 have not yet been reported as a cause for typical Rett syndrome at all. We have established quantitative PCR for diagnosis of deletions affecting MECP2, and in this paper, we report a boy manifesting clinical features of Rett syndrome and a submicroscopic duplication within the cytotegal band Xq28 encompassing the entire MECP2 gene.

**CLINICAL FEATURES**

The boy is the second child of healthy, unrelated parents, whose older brother had developed normally. There is no family history of mental retardation or developmental disorders. The patient was born in the 41st week after an uneventful pregnancy. Birth was spontaneous but with protracted labour (birth weight 3940 g, length 54 cm, head circumference 36.2 cm). Head growth was normal and did not decelerate (head circumference 54.5 cm at 7.5 years), but growth of length was retarded (length 117 cm at 7.5 years). Psychomotor development was retarded from birth. Initially, reduced movements, muscular hypotonia, and insufficient weight gain were noticed. The patient could not turn at the age of 15 months, and could not sit up until the age of 4 years. The first stereotypic hand movements were noticed at the age of 4 years. At the age of 6 years, he was crawling and able to walk a few steps with assistance. He was able to hold things and play with toys, but showed gradual loss of purposeful hand use around the same age. There was no spasticity or fixed scoliosis. The patient never learned to speak, but made babbling sounds to communicate basic needs.

During the first hospitalisation at the age of 10 months, magnetic resonance imaging (MRI) showed mildly enlarged inner and outer liquor spaces, but no retardation of myelination. In the following years, thorough screening for metabolic disorders including liquor tests revealed normal results. No signs of energy metabolism disorders, organoacidopathies, aminoacidopathies, peroxisomal or lysosomal disorders, or storage disorders were detected. There was no organomegaly. Ophthalmological examination showed mild...
hyperorpa, but no retinopathy or optic atrophy. Nerve conduction velocities and electromyogram were normal. Owing to insufficient weight gain, a gluten enteropathy was suspected at the age of 1 year. A gluten free diet has been followed since then, although gliadin antibodies had not been found and enteroscopy had never been carried out to verify the diagnosis. Weight normalised gradually, and was in the normal range at the age of 6½ years.

No seizures were noted in the first years. EEG showed signs of unspecific encephalopathy, with theta waves, but no epileptogenic potentials between the age of 3 and 4½ years. At 6 years, the parents observed phases of white matter surrounding the trigonum (probably enlarged Virchow-Robin spaces). Diagnostic evaluation confirmed generalised epilepsy with absences, and myotonic-atatic and tonic seizures. EEG was markedly abnormal, with hypersynchronous activity (spike and sharp wave complexes, multifocal but most pronounced in the right hemisphere) and a very slow basal rhythm in the awake EEG. Antiepileptic therapy was induced, but treatment of epilepsy proved to be difficult. Monotherapy with different drugs (topiramate, clobazame, lamotrigine) was unsatisfactory. Current medication with lamotrigine and sulthiamine has led to a decrease in seizure frequency, but the patient still has at least one seizure daily.

MRI showed unspecific white matter changes, which may also be secondary and caused by epilepsy, including small cystic structures in the supratentorial white matter surrounding the trigonum (probably enlarged Virchow-Robin spaces). Cortex relief, and size of inner and outer liquor spaces, brain stem, and cerebellum were interpreted as normal.

At the age of 6½ years, the boy showed severe global retardation and autistic features. The parents described regression of motor abilities over 6 months with loss of crawling and assisted walking, and suggested coincidence with onset of epilepsy. However, motor abilities did not recover significantly after onset of medication. Speech development was still not recognisable. Stereotypic hand movements and teeth grinding were observed. Dysmorphological evaluation showed only mild dysmorphic features such as a narrow midface and undescended testis. The extremities were frequently pink and cold, indicating signs of unspecific encephalopathy, with theta waves, but no breathing irregularities have been observed as yet. Further regression of basic communication skills and loss of purposeful hand use and interest in toys has been noted.

MATERIALS AND METHODS

Molecular genetic analysis

Mutation screening in MECP2 was performed by PCR amplification of coding exons in overlapping fragments, followed by single strand conformational polymorphism (SSCP) analysis. The analysis included all parts of the open reading frame in exons 2–4 of the full length transcript MECP2A, and also the part of exon 1 coding in the alternative transcript MECP2B recently reported.14 Band shifts indicative of small intragenic aberrations were not detected (not shown).

Quantification of MECP2 gene dosage, characterisation of the duplicated region, and fine mapping of the duplication breakpoints were performed using a quantitative PCR approach with SYBR-Green 1 detection, and the comparative threshold cycle method.15 16 Analysis was carried out on the iCycler iQ real time PCR detection system (Bio-Rad, CA, USA) and performed repeatedly for three independent MECP2 amplicons (each three times, in duplicate). Two primer pairs for the fourth exon (primers Rett exhF1/exhR1, and Rett exhF2/exhR2) and one for the third (primers Rett exhF3/exhR), and reference amplicons in the ALB and PMP22 genes were used (table 1). Size of the duplication was analysed using a primer set for amplicons covering parts of Xq27–q28 (table 1). Genomic sequences were obtained from the UCSC site (http://genome.ucsc.edu/).17 Primers were designed with lengths of 18–24 nucleotides, G+C contents 50–65%, PCR product length 75–150 bp. Specificity and exact position (physical distance) of primers were verified using the BLAT interface at the UCSC site. Standard curves and PCR efficiency were run to confirm applicability of the comparative threshold cycle method.

Quantitative PCR reactions contained 0.6 μmol/l each primer and 12.5 μl SYBR Green PCR Master Mix (ABGene, UK) in a total of 25 μl. Assays included DNA standards, no template control, or 2.5 ng/μl of the patient DNA in two replicates. Cycling conditions were 50°C for 2 minutes, 95°C for 15 minutes, and 40 cycles of 95°C for 1 minute, 55°C for 10 seconds, then 62°C for 1 minute (data collection step), followed by a melting curve analysis (95°C for 1 minute, 55°C for 10 seconds, temperature increase by 0.5°C every 10 seconds (data collection) up to 95°C) to confirm specificity of the PCR reaction.

Quantification of target amplicons was performed relative to reference amplicons with the comparative threshold cycle method. Quantitative data were normalised against the average of two or four female controls (MECP2 amplicons, table 1).
amplicons in L1CAM, LCA10, and AVPR2, plus FLNA amplicons int25, ex21, ex1118, and int14 were run with four female and two male controls. All other amplicons were run with two female and one male control. Calculation of standard deviations for normalised values (as for assays using the TaqMan technology) is not possible for SYBR green detection because of the independent amplifications of target and control. Normalised values of 1.0 indicate a diploid (female) situation, values of 0.5 or 1.5 indicate partial haploidy or partial triploidy respectively.

**Cytogenetic analysis**

Cytogenetic analysis on cultured blood lymphocytes had been performed earlier. GTG and QFQ banded metaphases at a resolution of 550–600 bands revealed no numerical or structural aberration (not shown).

Fluorescence in situ hybridisation (FISH) analyses were carried out on metaphase spreads using a probe generated from cosmid LNLIC110C1837Q (GenBank accession no. AF031077), containing a total of 12 kb of genomic sequence from cosmid LLNLc110C1837Q (GenBank accession no. AF031077), containing a total of 12 kb of genomic sequence from cosmid LLNLc110C1837Q (GenBank accession no. AF031077), containing a total of 12 kb of genomic sequence from cosmid LLNLc110C1837Q (GenBank accession no. AF031077), containing a total of 12 kb of genomic sequence from cosmid LLNLc110C1837Q (GenBank accession no. AF031077), containing a total of 12 kb of genomic sequence from cosmid LLNLc110C1837Q (GenBank accession no. AF031077), containing a total of 12 kb of genomic sequence from cosmid LLNLc110C1837Q (GenBank accession no. AF031077), containing a total of 12 kb of genomic sequence from cosmid LLNLc110C1837Q (GenBank accession no. 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indicated a ratio of more than 10:1 for the patient’s mother, whereas a ratio of 7:3 and 6:4 was found for the two informative female controls c2 and c3 (fig 2C). The index patient, his brother, and his maternal uncle all carry the same, active allele at the AR locus. This is also the allele preferentially unmethylated in the mother, indicating recombination between the AR gene and MECP2 at Xq28 in the index patient (fig 2C).

FISH analysis with a probe for MECP2, generated from cosmid LLNLc110C1837Q, showed only one signal in the telomeric region of Xq in all analysed metaphases (fig 3). This suggested an intrachromosomal duplication with integration next to the originating segment. Dual colour FISH was applied with the same probe and a probe mix from the duplicated region of FLNA. In one metaphase, a pattern of fluorescent signals compatible with an orientation cen–FLNA(partial)–MECP2–MECP2–FLNA–tel was observed. This result, however, which suggests an inverted tandem duplication with integration into the distal breakpoint, could not be verified in other metaphases, probably due to a limited resolution of this ~430 kb duplication in the FISH analysis. Southern blot analysis with a probe outside the duplicated region revealed the expected 21 kb central EcoRI fragment of FLNA in the index patient, his mother, and controls, confirming integrity of FLNA (data not shown).

Expression of MECP2 was analysed at the mRNA level in lymphoblastoid cells. No significant difference was found between male and female samples, as expected for a gene underlying X inactivation in females. In contrast, quantification of transcript levels in the index patient suggests a double dose of MECP2 transcription (fig 2D). The patient’s healthy mother had normal values.

**DISCUSSION**

Xq28 is a chromosomal region frequently affected by rearrangements, and contains several genes mutated in mental retardation and neurological disease, such as MTM1, ABCD1, LICAM, MECP2, FLNA, and EMD.21–25 The gene product of MECP2, the gene mutated in Rett syndrome, is involved in regulation of transcription by interaction with methylated DNA. In vitro, MECP2 has been found to act as a transcriptional repressor, although the target genes of MECP2 have yet to be identified. The precise effect of MECP2 mutations is only partly understood. It was previously speculated that MECP2 mutations would be lethal in boys, although it is now thought that the much higher incidence of Rett syndrome in girls is mainly caused by an overwhelming ratio of mutations on paternally derived X chromosomes.26 Nevertheless, relatively few reports of Rett syndrome in males exist. The classic Rett phenotype has been described in males with Klinefelter syndrome or with somatic mosaicism,27,28 while in most other cases, MECP2 mutations in boys have been associated with a more severe clinical course culminating in neonatal encephalopathy.29–31 In contrast, there have been numerous reports on point mutations, small intragenic deletions, and larger deletions in MECP2 in girls, and a possible genotype–phenotype correlation has been studied in detail. Some authors have suggested that partial deletions and truncating mutations may be associated with a more severe phenotype. Other investigators have not observed this correlation, leading to the suggestion that Rett syndrome may be caused by loss of MECP2 function regardless of the exact mutation involved.27

We report here a familial duplication of a submicroscopic Xq28 segment in a boy with severe mental retardation and features of Rett syndrome. Psychomotor development was primarily retarded. Regression and loss of purposeful hand use and other acquired abilities, stereotypic movements, autistic features, epileptic seizures, and feeding problems occurring between the ages of 4½ and 6½ years led to suspicion of Rett syndrome. Quantitative PCR revealed a duplication involving MECP2. As there are at least 12 genes...
and predicted transcripts included in the duplication, we cannot rule out that other genes may contribute to the clinical phenotype, although none of these genes other than MECP2 (and the colour opsin genes) is known to play a fundamental role in neural development and maintenance. There is convincing experimental evidence that complete, functional gene copies exist for both L1CAM and FLNA, the two nearest genes responsible for well known neurological phenotypes. Although L1CAM is not included in the duplication, fine mapping of the distal breakpoint indicated partial duplication of FLNA. Protein synthesis from the partially duplicated gene copy lacking the promoter and at least exons 1–13 is most unlikely, especially as periventricular heterotopia was excluded by MRI. In combination with the clinical phenotype, the results indicate that duplication of MECP2 may be the major if not the only cause for the mental retardation syndrome in our patient. Even though primary psychomotor retardation and normal head growth are atypical findings, the patient shows many diagnostic signs of Rett syndrome.

Owing to the uninformative family history, it remains unclear whether submicroscopic duplications involving MECP2 could cause Rett syndrome or other deleterious conditions in female patients. The finding of extremely skewed X inactivation in the index patient’s healthy mother, who also carries the duplication, suggests this hypothesis. This is further supported by the finding of elevated MECP2 mRNA levels in lymphoblastoid cells of the index patient, but not in his mother, compared with male and female controls. Importantly, in Mecp2 transgenic mice, the overexpression of murine Mecp2 causes a neurological phenotype similar to that in Mecp2 deficient mice.

In conclusion, our results indicate that duplications of the MECP2 gene caused by intrachromosomal duplications of Xq28 are rare, but are sufficient to cause a clinical phenotype similar to Rett syndrome in boys and possibly girls. These findings may also have implications for development of potential gene therapy strategies for Rett syndrome. The case demonstrates that alteration of methylation patterns due to pure loss of MECP2 function is not the only mechanism involved in pathogenesis of Rett syndrome, but that elevated dosage of MECP2 may be more critical than previously thought.

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