ELECTRONIC LETTER

Submicroscopic duplication in Xq28 causes increased expression of the *MECP2* gene in a boy with severe mental retardation and features of Rett syndrome

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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.[1] Mutations in the gene encoding the methyl-CpG binding protein 2 (MECP2) were demonstrated as the cause of Rett syndrome,[2] and confirmed by a number of studies. The vast majority (95%) of *MECP2* mutations occurs 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.[3] [4]

It has recently been shown that missense mutations in *MECP2* can cause severe neonatal encephalopathy in boys.[5] Classic Rett phenotypes in boys have so far only been reported in rare cases of somatic mosaicism or XXY karyotypes.[6–11] In girls, larger intragenic deletions are responsible for about 11–16% of typical Rett syndrome without point mutations in the coding exons.[12] [13] 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 cytogenetic 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.

Key points

- Rett syndrome has been recognised as one of the major causes of mental retardation in girls. Both point mutations and deletions affecting the *MECP2* gene have been identified in girls with this neurodevelopmental disorder. Only a few boys with *MECP2* mutations have been described, most of whom have a severe neonatal encephalopathy.

- We describe a complete duplication of *MECP2* due to a submicroscopic duplication of approximately 430 kb within Xq28 in a boy with severe mental retardation and features of Rett syndrome. *MECP2* is the only gene involved in the duplication that is known to be relevant in mental retardation or essential for neural development, and was found to be completely duplicated.

- The size of the duplication was analysed by quantitative PCR. The proximal border of the duplicated segment was located to a region at least 8.4 kb 5′ from the first exon of *L1CAM*. The distal border lies within the *FLNA* gene, but leaving the functional gene copy intact. We provide evidence that the duplication leads to elevated *MECP2* mRNA levels in lymphoblastoid cells.

- The mother of the patient carries the same duplication, but is asymptomatic, which may be attributed to extremely skewed X inactivation.

- These findings complement the understanding of pathogenesis of Rett syndrome and may have important implications for development of potential gene therapy strategies.

- This is the first report showing that, in a manner equal to gene deletion with loss of *MECP2* function, duplication and overexpression of *MECP2* can cause mental retardation with features of Rett syndrome.

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

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Abbreviations: FISH, fluorescence in situ hybridisation; MRI, magnetic resonance imaging; SSCP, single strand conformational polymorphism

hyperopia, 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 6¾ 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 4.5 years. At 6 years, the parents observed phases of absence in the child. Diagnostic evaluation confirmed generalised epilepsy with absences, and myotonic–astatic and tonic seizures. EEG was markedly abnormal, with hypersynchronic activity (spike and sharp slow 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 sulthiame 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 6¾ 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 mild peripheral vasomotor disturbance. The patient is now 8 years old and has increasing problems with swallowing, resulting in salivation and decrease in weight gain. 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 I 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 ex4F1/ex4R1, and Rett ex4F2/ex4R2) and one for the third (primers Rett ex3F/ ex3R), 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℃ for 2 minutes, 95℃ for 15 minutes, and 40 cycles of 95℃ for 30 seconds, then 62℃ for 1 minute (data collection step), followed by a melting curve analysis (95℃ for 1 minute, 55℃ for 10 seconds, temperature increase by 0.5℃ every 10 seconds (data collection) up to 95℃) 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 Primer pairs for amplicons used for quantitative PCR. Position of amplicons in Mb refers to UCSC

Name	Nucleotide sequence	Position
Test amplicons		
L1CAM int1 F	TCGGGAGGTATTTGACCACG	151 608
L1CAM int1 R	AACACCATCACGGTAACACCC	
LCA10 int1 F	CCTTGAAGCGCTGATTGTCC	151 618
LCA10 int1 R	TCCCTGGTTTGCTACACCG	
AVPR2 int1 F	GTGGGCAGAGTGGGTTTGAC	151 639
AVPR2 int1 R	CTCATTGGCAGCCTGGAGAG	
HCFC ex17 F	TCTGATCACGGCACCTAGTGG	151 689
HCFC ex17 R	TCGGCGATGGTAACTGTGG	
IRAK1 int9 F	CAGCCAGTAATTGCTTCACTGC	151 749
IRAK1 int9 R	CTGAATCCTGACGGAGACCC	
Rett ex4 F1	AGACGGTCAGCATCGAGGTC	151 764
Rett ex4 R1	CCTTTCCCGCTCTTCTCACC	
Rett ex4 F2	TTCACGGTAACTGGGAGAGGG	151 765
Rett ex4 R2	TGCCAGTTCCTGGAGCTTTG	
Rett ex3 F	ATGTATGATGACCCCACCCTG	151 766
Rett ex3 R	AGCGGCCAGATTTCCTTTG	
XQ3273 F	TTGGCCACTGAGAAACCCTC	151 848
XQ3273 R	CGGAGAGCAGGACCAGGTAG	
TKTL ex12 F	GAATTCCCTTCGCTGTTTGC	152 027
TKTL ex12 R	GGAGGCTCTCATCACAAGCAG	
FLNA 3'F	TCAGCTTTCTCGCCACATCC	152 044
FLNA 3'R	TCAGAAGGTCTGGGTTTGACG	
FLNA int29 F	ACAGGCATGGAGGGTCACC	152 053
FLNA int 29 R	TGCCGCAAAACTCTAGCCAC	
FLNA int25 F2	ATGGGACTGGTCACTGAGCAG	152 055
FLNA int25 R2	TCTGGCACGAAAGACACCC	
FLNA ex21 F	GCTCGAGTGCTTGGACAATG	152 057
FLNA ex21 R	GGGTGTCAGCGAAGAGGATG	
FLNA exInt18 F	CTGTCCAGCAGGTAGCCACAC	152 058
FLNA exInt18 R	AGAAAGTCCCTCGGAGCTGTC	
FLNA int14 F	TCAAGTGGTTGTGGCACACC	152 060
FLNA int14 R	GCAAAGCCACAGCAGAAAGC	
FLNA int8 F2	CTCACGCTTTCACCTTCGG	152 062
FLNA int8 R2	AGTACCGTTGACCCTGTGGG	
FLNA int1 F	CACGCAGGAGTTGGTTGGTAG	152 066
FLNA int1 R	GAGAACGAATTGGGCTGCTG	
EMD 3'UTR F	CATGCACGCTACCAGCAGTC	152 078
EMD 3'UTR R	AGAATGATGTGCCAGAGACGC	
Reference amplicons		
PMP22 ex3 F	GCCACCATGATCCTGTCGAT	17p11.2
PMP22 ex3 R	CCCTTGGTGAGGGTGAAGAGT	
ALB ex12 F	AATGCTGCACAGAATCCTTGGT	4q11–q13
ALB ex12 R	TCATCGACTTCCAGAGCTGAAA	

amplicons in *L1CAM*, *LCA10*, and *AVPR2*, plus *FLNA* amplicons int25, ex21, exInt18, 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 *Taq*Man 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 LLNLc110C1837Q (GenBank accession no. AF031077), containing a total of 12 kb of genomic sequence from the large second intron of *MECP2*. The cosmid clone was obtained from the Resource Center of the German Human Genome Project (RZPD, Berlin, Germany). After culturing and DNA preparation, cosmid DNA was briefly sonicated and labelled directly with dGreen using the ULS universal linkage system (Q-Biogene) according to the instructions of the manufacturer. Dual colour FISH was performed with the cosmid probe, and with a probe mix generated from PCR amplified fragments of the *FLNA* gene (four PCR fragments containing a total of 5.5 kb between *FLNA* introns 18 and 29) labelled directly with rhodamine. Hybridisations were carried out for 12–18 h at 37℃ following a standard protocol.[18] Slides were evaluated using an Axioscope 2 Plus microscope (Zeiss, Germany) and the Isis software (Metasystems, Germany).

X chromosome inactivation

X chromosome inactivation was analysed by PCR amplification in the androgen receptor gene (*AR*) as described.[19] Genomic DNA of the probands was digested with either *Msp*I or methylation sensitive *Hpa*II, or incubated with restriction buffer only. A 280 bp fragment of *AR* exon 1 was then amplified incorporating ^{32}P labelled dCTP, separated on denaturing polyacrylamide gels, and evaluated using a Storm860 phosphoimager (Molecular Dynamics, CA, USA). The ratio of inactivation of the alleles in informative females was calculated from the band intensities measured by the ImageQuant 5.1 software (Molecular Dynamics).

Test of integrity of *FLNA*

The restriction map of *FLNA*[20] shows a central 21.2 kb *Eco*RI fragment (*Eco*RI sites in intron 1 and exon 41 of *FLNA*). To test for integrity of one functional gene copy of *FLNA*, 10 μg of genomic DNA of the index patient were digested with *Eco*RI, and a Southern blot performed. The blot was hybridised with a ^{32}P labelled probe generated from a PCR fragment made with *FLNA* primers int8F and int14R (outside the duplicated region), and evaluated by autoradiography.

MECP2 expression analysis

Total RNA of the patient, his mother, and male and female controls was extracted from lymphoblastoid cells generated by EBV transformation. Expression of *MECP2* was analysed by one step RT real time PCR with SYBR Green detection and the comparative threshold cycle method. Primers Rett 2F and Rett 3-2R were used to amplify a 277 bp *MECP2* mRNA fragment. Quantification was performed against a control amplicon of the *GAPDH* mRNA, using primers GAPDH-F (AGGTCGGAGTCAACGGATTTG) and GAPDH-R (AAGCAG CCCTGGTGACCAG). Reactions for *MECP2* and *GAPDH* were carried out three times in duplicate using the Quantitect SYBR Green RT-PCR kit (Qiagen, Germany). Reaction tubes contained 0.4 μmol/l each primer of a pair, 10 μl QuantiTect SYBR Green, 0.2 μl QuantiTect RT mix, and 20 ng RNA (total volume 20 μl). Fluorescein (0.2 μl diluted 1:1000) was added for internal calibration of the iCycler system. Cycling conditions were 50℃ for 30 minutes, 95℃ for 15 minutes, and 40 cycles of 94℃ for 15 seconds, then 60℃ for 30 seconds, and 72℃ for 30 seconds (data collection step), followed by a melting curve analysis. Quantification of relative *MECP2* expression was performed as described for relative gene dosage calculation (see above).

RESULTS

The analysis of *MECP2* gene dosage was performed repeatedly by independent comparison of three amplicons in *MECP2* to autosomal reference amplicons, and suggested existence of two gene copies in the index patient, identical to the gene dosage found in girls. Chromosome analysis by conventional cytogenetics had earlier revealed a normal karyotype 46,XY in the boy (not shown). To verify a *MECP2* duplication, additional amplicons 5′ and 3′ of the gene, situated in *IRAK1* intron 9 and in STS XQ3273 (GenBank accession no. G66748) were analysed. Double dosage was found for both amplicons, indicating a complete duplication of *MECP2* (fig 1). No such findings were found in a cohort of 75 female and 17 male patients suspected with Rett syndrome who had tested negative for smaller intragenic mutations.

EDTA blood samples had been received after informed consent for dosage analysis in the family of the index patient (fig 2A). An increased dosage for all *MECP2* amplicons was found for the patient himself (III.2) and his mother (II.2), indicating two gene copies in the index patient and three copies in his healthy mother. Normal *MECP2* dosage was detected in the healthy brother (III.1), father (II.1), and maternal uncle (II.3) (fig 2B).

The extent of the duplication was determined by application of further amplicons in Xq27–q28, situated in *HCFC1*, *AVPR2*, *L1CAM*, and *FMR1* (centromeric to *MECP2*) and *TKTL1*, *FLNA*, *EMD*, *G6PD*, and *F8* (telomeric to *MECP2*). The evaluation showed that the duplication involves a region including *AVPR2* to *TKTL1*, but not the amplicons situated in *L1CAM* and in *FLNA* intron1 (fig 1). Dosage of the more distant genes *FMR1*, *EMD*, *G6PD*, and *F8* was also normal (not shown).

An amplicon in the first intron of *LCA10* (GenBank accession no. AF514420), a predicted gene encoding for lung carcinoma associated protein, was analysed to determine possible involvement of *L1CAM*. Single dosage for the *LCA10* amplicon was found repeatedly in the assay. This suggests localisation of the proximal breakpoint to a 21 kb region between *LCA10* intron 1 and *AVPR2* intron 1, at least 8.4 kb 5′ of the first exon of *L1CAM*. No regulatory sequences of *L1CAM* are known further upstream, thus practically excluding *L1CAM* contribution to the phenotype of this patient (fig. 1).

Further amplicons in the *FLNA* gene were applied for fine mapping of the distal breakpoint between *TKTL1* exon 12 and *FLNA* intron 1. The distal breakpoint was finally located to an approximately 2 kb segment between an amplicon in *FLNA* intron 14 and an amplicon at the border of exon 18 to intron 18 of *FLNA* (fig 1). The aberration thus includes complete duplication of 12 genes and predicted transcripts (*AVPR2*, *ARHGAP4*, *ARD1*, *RENBP*, *HCFC1*, *IRAK1*, *MECP2*, *OPN1LW*, *OPN1MW*, *TKTL1*, *Cxorf12*, and *Cxorf2*), plus partial duplication of *FLNA* (and possibly of predicted transcript *LCA10*).

The mother of the index patient was informative for the trinucleotide repeat polymorphism at the *AR* site. The analysis demonstrates severely skewed X inactivation in the mother. Quantification of the X inactivation of the two alleles
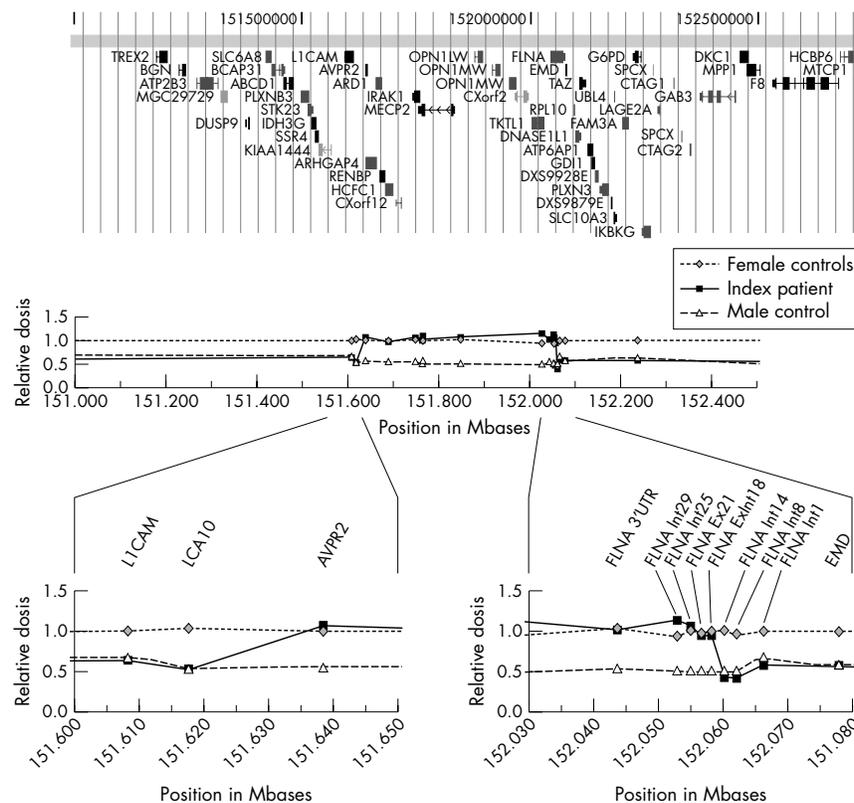
Figure 1 Characterisation of the duplication by dosage analysis for amplicons in Xq28 for the index patient, and female and male controls. Data points refer to amplicons amplified with primer pairs in table 1. Gene dosage was calculated relative to autosomal reference amplicons. Graphical representation of genes on Xq28 is adapted from the UCSC genome browser.

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 at Xq11 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 *Eco*RI 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*, *L1CAM*, *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,[6–10] while in most other cases, *MECP2* mutations in boys have been associated with a more severe clinical course culminating in neonatal encephalopathy.[5 11] 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 6¾ years led to suspicion of Rett syndrome. Quantitative PCR revealed a duplication involving *MECP2*. As there are at least 12 genes
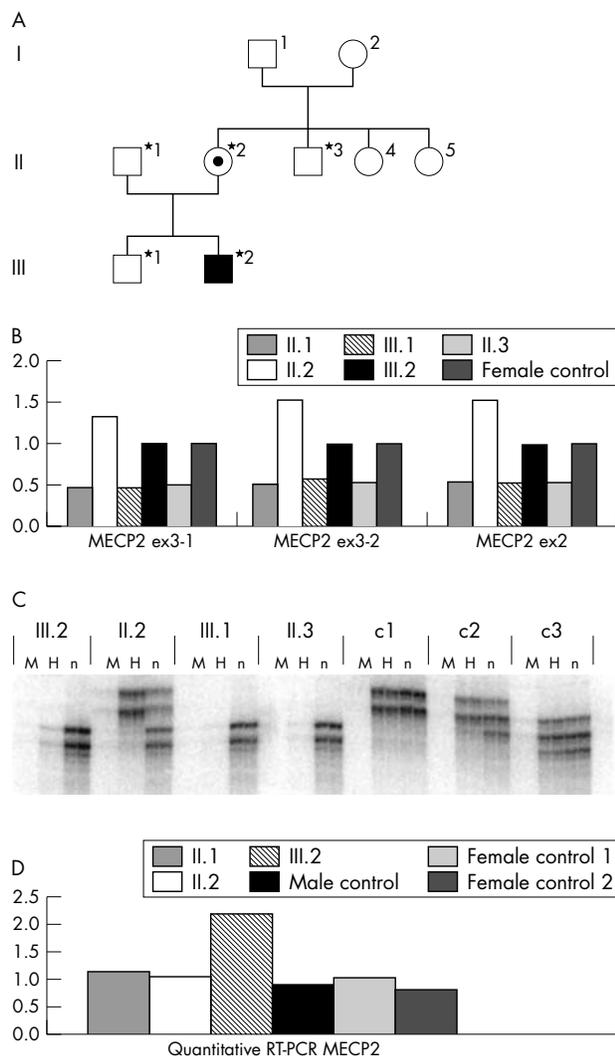
Figure 2 (A) Family tree of the index patient (III.2). *Subjects available for molecular genetic analysis. (B) Dosage analysis of three amplicons in MECP2 for the index patient (III.2), his parents (II.1 and II.2), his maternal uncle (II.3), and female controls. (C) X inactivation analysis at the AR locus. Methylation status at the polymorphic site in AR exon 1 was studied by PCR either after digest with MspI (M), HpaII (H), or no digest (n). (D) Results of quantitative RT PCR in lymphoblasts of the patient, his mother, and male and female controls.

Figure 3 FISH analysis in the index patient with an MECP2 probe (green) generated from cosmid LLNLc110C1837Q. The signal in the telomeric region of Xq could not be further resolved, suggesting an intrachromosomal duplication with integration next to the originating segment.

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.[28]

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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REFERENCES

1 **Zoghbi H**. Genetic aspects of Rett syndrome. *J Child Neurol* 1988;**3**(suppl):S76–8.
2 **Amir RE**, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999;**23**:185–8.
3 **Hagberg B**. Clinical manifestations and stages of Rett syndrome. *Ment Retard Dev Disabil Res Rev* 2002;**8**:61–5.
4 **Huppke P**, Held M, Laccone F, Hanefeld F. The spectrum of phenotypes in females with Rett Syndrome. *Brain Dev* 2003;**25**:346–51.
5 **Schanen NC**, Kurczynski TW, Brunelle D, Woodcock MM, Dure LS IV, Percy AK. Neonatal encephalopathy in two boys in families with recurrent Rett syndrome. *J Child Neurol* 1998;**13**:229–31.
6 **Schwartzman JS**, De Souza AM, Faiwichow G, Hercowitz LH. (Rett phenotype in patient with XXY karyotype: case report). *Arq Neuropsiquiatr* 1998;**56**:824–8.
7 **Salomao Schwartzman J**, Zatz M, dos Reis Vasquez L, Ribeiro Gomes R, Koiffmann CP, Fridman C, Guimaraes Otto P. Rett syndrome in a boy with a 47,XXY karyotype. *Am J Hum Genet* 1999;**64**:1781–5.
8 **Clayton-Smith J**, Watson P, Ramsden S, Black GC. Somatic mutation in MECP2 as a non-fatal neurodevelopmental disorder in males. *Lancet* 2000;**356**:830–2.
9 **Armstrong J**, Pineda M, Aibar E, Gean E, Monros E. Classic Rett syndrome in a boy as a result of somatic mosaicism for a MECP2 mutation. *Ann Neurol* 2001;**50**:692.
10 **Schwartzman JS**, Bernardino A, Nishimura A, Gomes RR, Zatz M. Rett syndrome in a boy with a 47,XXY karyotype confirmed by a rare mutation in the MECP2 gene. *Neuropediatrics* 2001;**32**:162–4.
11 **Moog U**, Smeets EE, van Roozendaal KE, Schoenmakers S, Herbergs J, Schoonbrood-Lenssen AM, Schrander-Stumpel CT. Neurodevelopmental disorders in males related to the gene causing Rett syndrome in females (MECP2). *Eur J Paediatr Neurol* 2003;**7**:5–12.
12 **Van den Veyver IB**, Zoghbi HY. Genetic basis of Rett syndrome. *Ment Retard Dev Disabil Res Rev* 2002;**8**:82–6.
13 **Laccone F**, Junemann I, Whatley S, Morgan R, Butler R, Huppke P, Ravine D. Large deletions of the MECP2 gene detected by gene dosage analysis in patients with Rett syndrome. *Hum Mutat* 2004;**23**:234–44.
14 **Mnatzakanian GN**, Lohi H, Munteanu I, Alfred SE, Yamada T, MacLeod PJ, Jones JR, Scherer SW, Schanen NC, Friez MJ, Vincent JB, Minassian BA. A previously unidentified MECP2 open reading frame defines a new protein isoform relevant to Rett syndrome. *Nat Genet* 2004;**36**:339–41.
15 **Wilke K**, Duman B, Horst J. Diagnosis of haploidy and triploidy based on measurement of gene copy number by real-time PCR. *Hum Mutat* 2000;**16**:431–6.
16 **Ponchel F**, Toomes C, Bransfield K, Leong FT, Douglas SH, Field SL, Bell SM, Combaret V, Puisieux A, Mighell AJ, Robinson PA, Inglehearn CF, Isaacs JD, Markham AF. Real-time PCR based on SYBR-Green I fluorescence: an alternative to the TaqMan assay for a relative quantification of gene rearrangements, gene amplifications and micro gene deletions. *BMC Biotechnol* 2003;**3**:18.
17 **Karolchik D**, Baertsch R, Diekhans M, Furey TS, Hinrichs A, Lu YT, Roskin KM, Schwartz M, Sugnet CW, Thomas DJ, Weber RJ, Haussler D, Kent WJ, University of California Santa Cruz. The UCSC Genome Browser Database. *Nucleic Acids Res* 2003;**31**:51–4.
18 **Rooney DE**. *Human cytogenetics: a practical approach*, 3rd ed. Oxford: Oxford University Press, 2001.
19 **Allen RC**, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 1992;**51**:1229–39.
20 **Small K**, Iber J, Warren ST. Emerin deletion reveals a common X-chromosome inversion mediated by inverted repeats. *Nat Genet* 1997;**16**:96–9.
21 **Laporte J**, Hu LJ, Kretz C, Mandel JL, Kioschis P, Coy JF, Klauck SM, Poustka A, Dahl N. A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat Genet* 1996;**13**:175–82.
22 **Mosser J**, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, Poustka AM, Mandel JL, Aubourg P. Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature* 1993;**361**:726–30.
23 **Rosenthal A**, Jouet M, Kenwrick S. Aberrant splicing of neural cell adhesion molecule L1 mRNA in a family with X-linked hydrocephalus. *Nat Genet* 1992;**2**:107–12.
24 **Fox JW**, Lamperti ED, Eksioglu YZ, Hong SE, Feng Y, Graham DA, Scheffer IE, Dobyns WB, Hirsch BA, Radtke RA, Berkovic SF, Huttenlocher PR, Walsh CA. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* 1998;**21**:1315–25.
25 **Bione S**, Maestrini E, Rivella S, Mancini M, Regis S, Romeo G, Toniolo D. Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. *Nat Genet* 1994;**8**:323–7.
26 **Trappe R**, Laccone F, Cobilanschi J, Meins M, Huppke P, Hanefeld F, Engel W. MECP2 mutations in sporadic cases of Rett syndrome are almost exclusively of paternal origin. *Am J Hum Genet* 2001;**68**:1093–101.
27 **Kriaucionis S**, Bird A. DNA methylation and Rett syndrome. *Hum Mol Genet* 2003;**12**(suppl 2):R221–7.
28 **Luikenhuis S**, Giacometti E, Beard CF, Jaenisch R. Expression of MeCP2 in postmitotic neurons rescues Rett syndrome in mice. *Proc Natl Acad Sci USA* 2004;**101**:6033–8.