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Report

A Mutation in the Rett Syndrome Gene, *MECP2,* Causes X-Linked Mental Retardation and Progressive Spasticity in Males

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Heterozygous mutations in the X-linked *MECP2* gene cause Rett syndrome, a severe neurodevelopmental disorder of young females. Only one male presenting an *MECP2* mutation has been reported; he survived only to age 1 year, suggesting that mutations in *MECP2* are male lethal. Here we report a three-generation family in which two affected males showed severe mental retardation and progressive spasticity, previously mapped in Xq27.2-qter. Two obligate carrier females showed either normal or borderline intelligence, simulating an X-linked recessive trait. The two males and the two obligate carrier females presented a mutation in the *MECP2* gene, demonstrating that, in males, *MECP2* can be responsible for severe mental retardation associated with neurological disorders.

Several reports have shown that heterozygous de novo mutations in the *MECP2* gene (MIM 300005) cause Rett syndrome (MIM 312750), a sporadic severe neurodevelopmental disorder of young females (Bienvenu et al. 2000; Cheadle et al. 2000; De Bona et al. 2000; Huppke et al. 2000; Kim and Cook 2000; Xiang et al. 2000). The few known familial cases are due to the normal fitness of nonpenetrant carriers who either bear the mutation in a mosaic state or have a nonrandom X inactivation (Amir et al. 1999; Wan et al. 1999). There have been no reports of males with *MECP2* mutations surviving beyond age 1 year, which suggests the hypothesis of lethality in males. However, since mutations in the same gene often cause different phenotypes, we tested whether *MECP2* mutations may be found in males in whom mental retardation segregated as an apparently X-linked recessive trait.

In one of the families, segregating a very severe mental retardation mapped in Xq27.2-qter, an *MECP2* mutation was found. In this family, mutations in other candidate genes (*L1CAM, GDI, 16A,* and *SEX*) were excluded (Claes et al. 1997). Screening of the *MECP2* gene was performed by SSCP followed by direct sequencing of PCR products showing abnormal migration, as reported elsewhere (De Bona et al. 2000). A C→T transition in position 1216 was found in exon 3. The mutation causes a substitution of glutamine (CAG) 406 with a stop codon (TAG). Since the mutation introduces an *Avr*II restriction site, we tested all family members by restriction analysis (fig. 1). The mutation was present in the two affected males, as well as in the carrier females, and was absent in all unaffected males in the family. The mutation was never described in Rett cases and was not found among 100 chromosomes, analyzed by *Avr*II digestion, from unaffected females.

Nonsense and frameshift mutations are supposed to act in two different ways. In some cases, they have been shown to cause production of a truncated protein. Alternatively, they may cause a null phenotype because of the so-called nonsense-mediated mRNA decay, a mechanism that recognizes and degrades mRNAs containing nonsense mutations. To define the exact consequence of the mutation C1216T, we performed semiquantitative RT-PCR on RNA from lymphoblastoid cell lines from one of the affected males and two unaffected controls. After 35 PCR cycles, in the exponential phase of amplification, the amount of transcript was identical in the patient and in the controls (fig. 2), indicating that the

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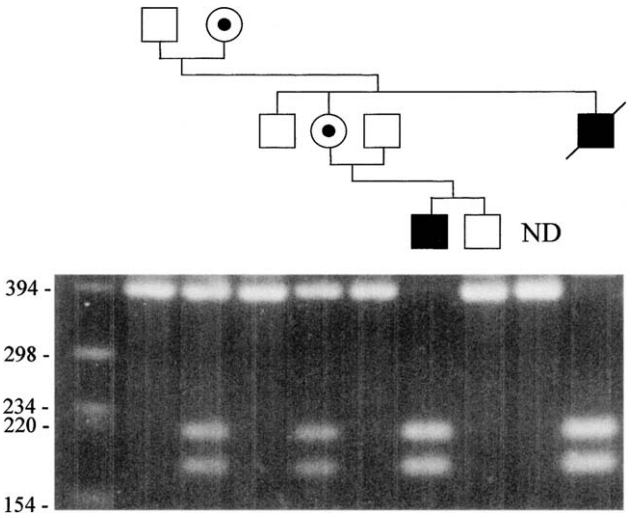

Figure 1 Segregation of the mutation in the family; 3% agarose gel of *Avr*II-digested PCR products of segment D of exon 3 (396 bp) obtained as reported elsewhere (De Bona et al. 2000). The C→T transition in position 1216 creates the "ccTagg" *Avr*II restriction site from the normal "cccagg" sequence, generating two fragments of 213 and 183 bp. Line 1, molecular weight marker VI (Roche), following lines as reported in pedigree above; ND = nondigested control.

transcript is not degraded and that a truncated protein may be produced.

In the few familial cases of Rett syndrome, nonpenetrant females have nonrandom X inactivation. We studied X inactivation in DNA from blood cells of both carrier females (Allen et al. 1992). The assay showed balanced X inactivation in both females (fig. 3). Band intensities were measured: the ratio between the two alleles was 56:44 in one female and 60:40 in the second.

A detailed phenotypic analysis of the family has been reported elsewhere (Claes et al. 1997). In brief, males showed delayed development (first steps at 2–5.5 years) and were never able to speak. They showed facial hypotonia and sialorrhea and a habitus suggesting complicated spastic paraplegia; their head circumferences were at the 75th–90th percentile. One of them had choreoathetotic movements in the right arm, a global bradyarrhythmia as indicated by electroencephalogram, and bilateral juvenile cataract; he was confined to a wheelchair and died from pneumonia at age 39 years. A thorough phenotypic analysis of the family and comparison with signs of Rett syndrome reveals some similarities, including absence of language, ataxic gait, seizures, grinding of teeth, and sialorrhea (table 1). Moreover, spastic paraparesis is a frequent end-stage finding of most cases of Rett syndrome. Among salient differences are the absence (1) of growth retardation, (2) of loss of acquired purposeful hand skills, and (3) of acquired microcephaly. The latter is one of the major diagnostic criteria and contrasts with the macrocephaly of this family. Moreover, stereotypic hand movements versus choreoathetotic movements (present in only one male) and constipation versus diarrhea are notable differences.

The only documented case of a male with an *MECP2* mutation is an infant bearing an early truncating mutation (806delG) who died, at age 1 year, from congenital encephalopathy (Wan et al. 1999). Two other reported cases were presumed to carry the same mutation segregating in the family (Wan et al. 1999; I. Meloni, M. Zappella, and A. Renieri, unpublished data). However, direct evidence was hampered by the absence of biological samples from both boys. We now demonstrate that hemizygosity of an *MECP2* mutation may be compatible with life.

To our knowledge, the mutation described in the present report has never been described in Rett patients. The presence of a normal amount of *MECP2* mRNA in the patient analyzed suggests that a shorter protein, lacking 80 amino acids at the C terminus, is synthesized. The abnormal protein maintains the methyl-binding domain, the transcription repression domain, and only part of the highly conserved histidine and proline-rich C-terminal domain (Huppke et al. 2000). This C-terminal region of the protein shows an overall homology of 35% identity and 46% positivity in a 100–amino acid region with two brain-specific factors, BF-1 and FKH4, which are members of the fork head gene family (Murphy et al. 1994). Moreover, the portion between amino acids 404 and 467 has been shown to facilitate MeCP2 binding to DNA (Chandler et al. 1999).

Altogether, our results suggest that a late truncation of the MeCP2 protein (at amino acid 406) is compatible

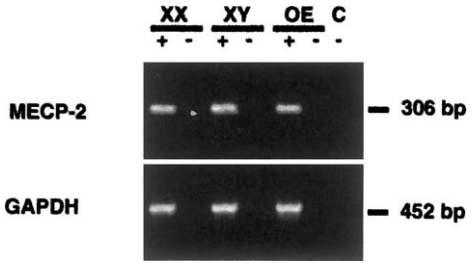

Figure 2 Quantitative RT-PCR of total RNA from lymphoblastoid cells of patient (OE) and male (XY) and female (XX) controls. Primers were MECP-2 RT-S: 5′-CGC TCC ATC ATC CGT GAC-3′; MECP-2 RS-A: 5′-TCT GCC AGT TCC TGG AGC-3′; GAPDH-F: 5′-AAC ACA GTC CAT GCC ATC AC-3′; GAPDH-R: 5′-TCC ACC ACC CTG TTG CTG TA-3′. The RT was done with (+) and without (−) retrotranscriptase, as described elsewhere (Bione et al. 1996), using 1 μg of total RNA. We amplified 2.5 μl of the RT reaction in a 50-μl PCR reaction. C = PCR control without template DNA. PCR conditions were the following: GAPDH: 5 min at 94°C; 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C for 25 cycles; MECP-2: 5 min at 94°C; 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C for 35 cycles.

with some of the functions of MeCP2 and allows survival of the patients while nevertheless causing very severe neurological defects. How the mutation described in this paper is similar to the group of late frameshift *MECP2* mutations clustered between amino acids 384 and 455 cannot be determined at this point; unfortunately, no studies could be performed on RNA stability or the presence of a truncated protein in those patients.

The absence of a skewed X inactivation in carrier females was perhaps the most striking result of this study. Since a notable variation between tissues is reported, the presence of a completely skewed X inactivation in relevant tissues cannot be excluded. The milder effect of the mutation described here could, however, explain the lack of skewed pattern of X inactivation in females. Alternatively, Rett syndrome may be considered a digenic disease that develops when a de novo mutation in the *MECP2* gene occurs in the presence of a mutation in another gene.

Following this digenic model, the *MECP2* mutation alone produces a recessive phenotype (as in this family), whereas a mutation in the second gene alone may produce no phenotypic effect at all. The model fits with the evidence that, in familial and sporadic Rett cases, the X-inactivation pattern plays a central role in modulating the disease (Amir et al. 2000; Van den Veyver and Zoghbi 2000).

In conclusion, we suggest that mutations in the *MECP2* gene account for a subset of cases of apparently recessive, very severe X-linked mental retardation associated with neurological disorders and that mutation analysis of the *MECP2* gene should also be performed in mentally retarded males with progressive encephalopathy. Extensive analysis of the *MECP2* gene in such cases will give an indication of the percentage of X-linked mental retardation due to an *MECP2* mutation.

Table 1

Comparison of Signs Present in Rett Syndrome and in Males of This Family

Symptom	Rett Syndrome	Males of This Family
Absence of language	+	+
Seizures	+	+
Spasticity	+	+
Ataxic gait	+	+
Sialorrhea	+	+
Grinding of teeth	+	+ (Only one)
"Acquired" microcephaly	+	−
Stereotypic hand movements	+	−
Growth retardation	+	−
Loss of acquired purposeful hand skills	+	−
Constipation	+	− (Diarrhea)
Choreoathetotic movements	−	+ (Only one)
Facial hypotonia	−	+

NOTE. A plus sign (+) indicates presence, and a minus sign (−) indicates absence.

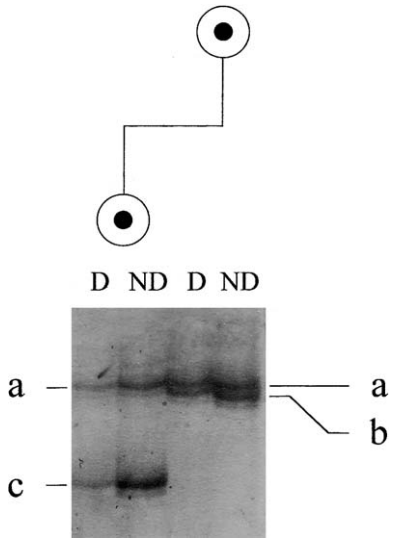

Figure 3 Results of X-inactivation assay: 6% polyacrylamide silver-stained gel of PCR product of CAG repeat of the androgen-receptor gene (Allen et al. 1992) from *Hpa*II digested (D) and non-digested (ND) DNA of the carrier females. Primers used for PCR amplification were RS-6: 5′-GTC CAA GAC CTA CCG AGG AG-3′ and RS-7: 5′-CCA GGA CCA GGT AGC CTG TG-3′. Band intensities were measured by Diversity Database program (Biorad), and the values were corrected for preferential allele amplification (Pegoraro et al. 1994). Results showed a balanced X-inactivation: ~56% of X-bearing allele b active (b) and 44% of X-bearing allele a active (a) in the mother (*right panel*); ~60% of normal paternal X active (c) and 40% of mutated X active (a) in the daughter (*left panel*).

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Electronic-Database Information

Accession numbers and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for the *MECP2* gene [MIM 300005] and Rett syndrome [MIM 312750])

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