

MECP2 mutation in non-fatal, non-progressive encephalopathy in a male

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Abstract

To study the clinical overlap between Rett (RTT) and Angelman syndromes (AS), we screened the MECP2 gene in a cohort of 78 patients diagnosed as possible AS but who showed a normal methylation pattern at the UBE3A locus. MECP2 missense (R106W, G428S), nonsense (R255X, R270X), and frameshift mutations (803 delG) were identified in 6/78 patients including 4/6 female cases consistent with RTT, one female case with progressive encephalopathy of neonatal onset, and one isolated male case with non-fatal, non-progressive encephalopathy of neonatal onset. This study shows that MECP2 mutations can account for a broad spectrum of clinical presentations and raises the difficult issue of the screening of the MECP2 gene in severe encephalopathy in both males and females.

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Rett syndrome (RTT) is an X linked condition characterised by the cluster of acquired microcephaly, ataxia, psychomotor regression with loss of purposeful hand skills in females, and lethality in males (MIM 312750). The vast majority of cases are sporadic, but rare familial forms have allowed the disease gene to be mapped to chromosome Xq28.¹ Recently, RTT has been ascribed to mutations of the MECP2 gene, which encodes a protein involved in transcription repression and gene silencing, via a methyl-CpG binding domain (MBD) and a transcription repression domain (TRD).^{2,3} Screening RTT patients for MECP2

mutations has shown random X inactivation in over 90% of RTT females,⁴ the paternal origin of de novo MECP2 mutations,⁵ and the maternal inheritance of mutations in familial forms, with a skewed X inactivation pattern in circulating white blood cells of healthy female carriers.^{6,7}

Angelman syndrome (AS) is an epileptic encephalopathy with microcephaly, absent speech, ataxia, and inappropriate laughter mapped to chromosome 15q11 and ascribed to deletion, uniparental disomy, and mutations of either the imprinting centre or the UBE3A gene.⁸ Considering that RTT shares common clinical features with AS, we hypothesised that some patients regarded as possible AS cases might have been misdiagnosed and could in fact suffer from RTT. For this reason, we studied the MECP2 gene in a series of male and female children whose encephalopathy was not accounted for by AS.

Methods

A total of 78 children (40 females and 38 males), referred for clinical features consistent with the diagnosis of Angelman syndrome but displaying a normal methylation pattern at the UBE3A locus, were further screened for MECP2 gene mutations.

The MECP2 coding sequence and flanking introns were amplified by PCR with a set of nine pairs of primers (table 1) and analysed by silver staining single strand conformation polymorphism (SSCP). For PCR amplification, the reaction mixture (20 µl) contained 100 ng of leucocyte DNA, 20 pmol of each primer, 0.1 µmol/l dNTP, and 1 IU Taq DNA polymerase (Roche). Thirty five cycles of amplification were performed, each cycle consisting of 30 seconds denaturation at 96°C, 30 seconds annealing, and 30 seconds elongation. PCR products were heated for 10 minutes at 96°C, loaded onto a GeneGel Excel 12.5/24 kit (Amersham Pharmacia Biotech), and electrophoresed for 2.5 hours at 15°C. When an abnormal SSCP pattern was identified, genomic DNA was sequenced on both strands by the fluorometric method (Big Dye Terminator Cycle Sequencing kit, Applied Biosystems).

Results

Nucleotide variations were identified in 6/78 patients including 4/6 female cases consistent with Rett syndrome, one female case of severe early onset encephalopathy, and one male case (tables 2 and 3). MECP2 mutations in females included missense (R106W) or nonsense mutations (R255X, R270X) or frameshift deletion (803delG), which involved either the

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Table 1 Primers and PCR conditions used for the MECP2 coding sequence screening

Exons	Primers	Annealing T (°C)	PCR product (bp)
1	F: ATTTTCTTTGTTTTAGGCTCCA R: GGCCAAACCAGGACATATAC	50	185
2	A F: GGCATGTTCTCTGTGATACT R: TGCCTCTGCGGGCTCAGCA	58	182
	B F: AAGAGAAAGAGGGCAAGCAT R: CTGTAGAGATAGGAGTTGCT	58	296
3	A F: TGTGTCTTTCTGTTTGTCCC R: GATTTGGGCTTCTTAGGTGG	58	181
	B F: CCTCCCGGCAGAGCAGAAA R: TGACCTGGGTGGATGTGGTG	64	243
C	F: TGCCTTTTCAAACCTTCGCCAG R: TTGACCTCGATGCTGACCGT	58	280
D	F: GATCTGTGCAGGAGACCGTA R: TTTGGGACTCTGAGTGGTG	62	250
E	F: GCAGCAGCAGCGCTCCTCA R: TGGCAACCGGGCTGAGTC	68	264
F	F: TGCCCAAGGAGCCAGCTAA R: GCTTTGCAATCCGCTCCGTG	64	202

Table 2 Clinical presentation in the 6/78 patients with a *MECP2* gene mutation

Patients	Sex	Clinical data	<i>MECP2</i> mutation	Protein domain
Case 1	F	RTT	316 C>T R106W	MBD
Case 2	F	RTT	763 C>T (R255X)	TRD
Case 3	F	RTT	763 C>T (R255X)	TRD
Case 4	F	RTT	803delG	TRD
Case 5	F	Progressive encephalopathy of neonatal onset	808 C>T (R270X)	TRD
Case 6	M	Non-progressive encephalopathy of neonatal onset	1282 G>A (G428S)	C-ter

C-ter: carboxyl-terminal domain.

methyl binding domain or the transcription repression domain of the protein (table 2). It is worth noting that all mutations occurred in CpG dinucleotides. Most interestingly, a male patient with non-fatal, non-progressive encephalopathy was found to carry a point mutation in a CpG doublet (1282 G>A in exon 3), changing a glycine into a serine at the carboxyl-terminal end of the protein.

This 3 year old boy was born to a healthy mother after a normal pregnancy and delivery. Family history was unremarkable. Hypotonia and hyperlaxity were noted early, but he thrived normally on breast feeding. However, his motor milestones were severely delayed (head

control 7 months, sitting unaided 13 months, voluntary grasp 9 months, fingertip grasp 18 months, standing with support 2 years). The mother had noticed frequent periods of fatigue during the day and a phobia of doors shutting. He was anxious and uncomfortable with frequent facial grimacing and poor eye contact. At 3 years of age, balance remained poor with restless, uncoordinated, and rough movements of the limbs and trunk and he had no speech. However, he did not lose skills and retained purposeful hand skills. Neurological examination showed no ataxia, spasticity, acquired microcephaly, or cranial nerve or fundus involvement. Mild distal lower limb hypotrophy and mild dysmorphic features were noted (fig 1). Electroencephalogram showed bradyarrhythmia and absence of paroxysmal anomalies. Brain MRI was unremarkable and RG banded karyotype at 800 band resolution showed normal chromosomes, 46,XY. The G428S mutation was inherited from the healthy carrier mother (II.3) who has not passed it to her healthy son (III.6, fig 2). Random X inactivation in her white blood cell DNA was found at the androgen receptor locus (not shown). The G428S mutation was also found in the two normal aunts (II.1 and II.2) but was absent from I.2, III.2, III.4, and a panel of 220 control chromosomes. Finally, haplotype analysis at the *MECP2* locus in the family makes germinal mosaicism in I.1 the most likely hypothesis.

Discussion

The systematic study of the *MECP2* gene in a series of 78 children previously tested for possible Angelman syndrome has resulted in the detection of 6/78 mutant genotypes. Three lines of evidence support the deleterious nature of these base changes namely, (1) the involvement of CpG dinucleotides which are mutation hot spots, (2) the occurrence of the mutations in domains of the protein which are highly conserved across species (not shown), and (3) the absence of the corresponding base changes in 220 control chromosomes.

While 4/6 cases were clinically consistent with the diagnosis of RTT, 2/6 cases failed to meet the classical diagnostic criteria (table 3). Indeed, one female had a progressive encephalopathy of neonatal onset, a feature which has long been regarded as an exclusion criterion in RTT. More importantly, while this condition is currently regarded as lethal in males, a *MECP2* mutation was found in a male patient with non-progressive encephalopathy, no seizures, and a normal head circumference. It is worth noting also that, at variance with classical RTT, the patient had no history of hand stereotypy, hyperventilation, or teeth grinding. Similarly, at variance with classical AS, he had no ataxia, epilepsy, typical EEG, or bursts of laughter. His abnormal movements were difficult to describe and raise the question of a combined neurological and behavioural phenotype. *MECP2* mutations have seldom been described in males.^{6,7,9-13} In fact, a total of seven male cases have been reported so far and 3/7 had maternal relatives with either typical or variant forms of



Figure 1 (A) Front view of the male patient and (B) profile. Note deep set eyes, arched eyebrows, prominent nasal bridge, and down turned corners of the mouth.

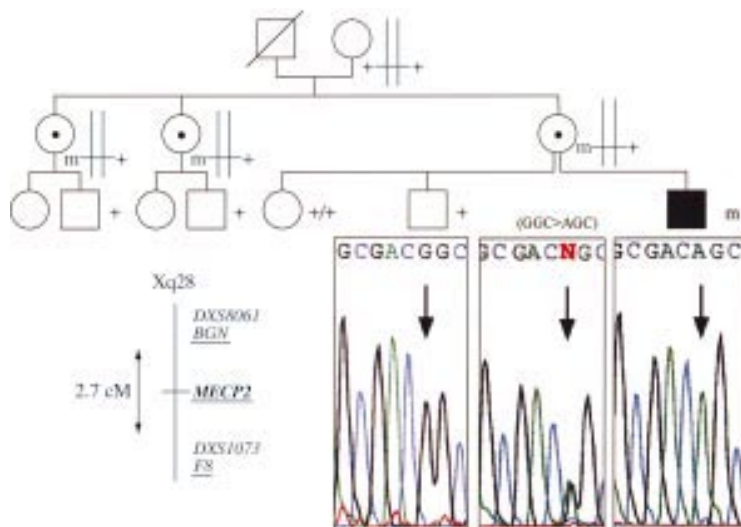


Figure 2 Segregation of the *MECP2* mutation in the family of case 6.

Table 3 Clinical data from female patients with a MECP2 gene mutation

	Case 1	Case 2	Case 3	Case 4	Case 5
OFC at birth	M	?	-2 SD	M	-1 SD
Acquired microcephaly	-2 SD at 2 y	-1.5 SD at 2 y	-2 SD at 18 mth	-2 SD at 2 y	-4 SD at 3,5 y
Psychomotor development until 6 months	Mild hypotonia	?	?	Mild hypotonia	Hypotonia, poor contact from birth
Loss of purposeful hand skills	—	?	+ at 18 mth	+ at 2 y	Never acquired
Hand stereotypies	+	++	++	++	++
Social contact	Poor/bursts of laughter	Poor/bursts of laughter	Inappropriate smile	Poor/inappropriate smile	Absent
Truncal ataxia and apraxia of gait	+	+	++	++	Poor spontaneous movements
Hyperprnoea	—	—	—	—	—
EEG anomalies/epilepsy	—	Bradyarrhythmia/ paroxysmal anomalies	Paroxysmal anomalies	—	Bradyarrhythmia/ paroxysmal anomalies
Spasticity	—	+	+	—	+
Kyphoscoliosis	—	+	—	—	++
Growth retardation	—	—	—	—	-2 SD

RTT, which helped in diagnosing their disease.^{6,7,9} To our knowledge, however, sporadic MECP2 mutation in a male has only been reported once (table 4).¹² Considering, on the one hand, the unexpectedly variable clinical expression of MECP2 mutations and, on the other hand, the possibility of non-lethal forms in males, the present observation raises the difficult issue of the putative involvement of MECP2 in sporadic forms of non-progressive encephalopathy in males. In order to decide which males should be screened for MECP2 mutations, identifying some specific clinical clues would be particularly helpful. At this point, however, owing to the absence of specific clinical features and the non- or slowly progressive nature of the disease in the male patient reported here, as well as in the previously reported cases, our study suggests that only a broad systematic screening could help diagnosis this condition among males with severely delayed motor development. This study also prompts one to extend screening for MECP2 mutations to females with delayed motor development, regardless of age at onset and even if they fail to meet the classical inclusion criteria of RTT.

Why most MECP2 mutations in males were lethal in the neonatal period while others were consistent with long survival is still unknown. In the isolated case reported by Clayton-Smith *et al*,¹² somatic mosaicism is likely. In the family reported by Orrico *et al*,¹³ the disease causing mutation was located in the MBD and was believed to alter the MECP2 function mildly.

Both in the two generation family reported by Meloni *et al*¹¹ (Q406X) and our male patient (G428S), the MECP2 mutation was located downstream from the MBD and TRD, in the C-terminal domain of the protein, known to facilitate MECP2 binding to DNA and chromatin in vitro.^{11,14} It is therefore tempting to speculate that hemizyosity for mild MECP2 mutations could be consistent with long survival in males, while heterozyosity for the same mutation would fail to trigger the disease in female carriers. Accordingly, the asymptomatic proband's mother showed a random X inactivation pattern at variance with known asymptomatic female carriers of MECP2 gene mutations responsible for classical RTT.^{6,7}

Conclusion

Continuing studies will hopefully help to refine the clinical and behavioural phenotype of RTT in males. Considering the different modes of inheritance, the variable recurrence risks, and long term prognosis in RTT and AS respectively, an accurate diagnosis is of particular importance in children with overlapping RTT/AS features.

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Table 4 Clinical findings in males with a MECP2 mutation presenting with severe, non-fatal encephalopathy

Data	Claes <i>et al</i> (1997), Meloni <i>et al</i> ¹¹	Clayton-Smith <i>et al</i> ¹²	Orrico <i>et al</i> ¹³	Our case
Familiarity	2 cases (uncle and nephew)	Sporadic	4 brothers	Isolated
Age at onset	Infancy	Neonatal	?	Neonatal
Growth parameters	?	?	?	50th (3 y)
OFC (centile)	75th-90th in adulthood	10th-25th (6 y)	Normal in adulthood	50th (3 y)
Severe mental retardation	+	+	+	+
Seizures	+	+	?	—
	(2 y in 1 case, adulthood in 1 case)	(3 y)		
Neurological findings	Ataxic gait	Ataxic gait	Resting tremors	Restless, uncoordinated movements, no spasticity, mild distal muscular atrophy
	Spasticity, distal muscular atrophy	Lower limb muscular atrophy	Slowness of movements	
	Unilateral choreoathetosis in 1 case			
Purposeful hand skills	?	+/-	?	+
Loss of skills	+/-	+	—	—
Electroencephalogram	Bradyarrhythmia	Excess of slow wave activity	?	Bradyarrhythmia
Brain imaging	Normal in 1 case*	Cortical atrophy	?	Normal
Dysmorphic facial features	—	—	?	Deep set eyes, arched eyebrows, prominent nasal bridge
MECP2 mutation	G406X (inherited)	241del TA (de novo)	A140V (inherited)	G428S (inherited)

*In one case, the necropsy of the brain showed diffuse cortical atrophy.

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