

## SHORT REPORT

# Low frequency of *MECP2* mutations in mentally retarded males

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A high frequency of mutations in the methyl CpG-binding protein 2 (*MECP2*) gene has recently been reported in males with nonspecific X-linked mental retardation. The results of this previous study suggested that the frequency of *MECP2* mutations in the mentally retarded population was comparable to that of CGG expansions in *FMR1*. In view of these data, we performed *MECP2* mutation analysis in a cohort of 475 mentally retarded males who were negative for *FMR1* CGG repeat expansion. Five novel changes, detected in seven patients, were predicted to change the *MECP2* coding sequence. Except for one, these changes were not found in a control population. While this result appeared to suggest a high mutation rate, this conclusion was not supported by segregation studies. Indeed, three of the five changes could be traced in unaffected male family members. For another change, segregation analysis in the family was not possible. Only one mutation, a frameshift created by a deletion of two bases, was found to be *de novo*. This study clearly shows the importance of segregation analysis for low frequency mutations, in order to distinguish them from rare polymorphisms. The true frequency of *MECP2* mutations in the mentally retarded has probably been overestimated. Based on our data, the frequency of *MECP2* mutations in mentally retarded males is 0.2% (1/475).

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Mutations in the methyl-CpG binding protein 2 (*MECP2*; MIM 300005) gene have first been described in Rett syndrome (RTT; MIM 312750), a progressive neurologic developmental disorder.<sup>1</sup> As RTT occurs almost exclusively in females, it has been proposed that RTT is caused by an X-linked dominant mutation with lethality in hemizygous males. However, *MECP2* mutations have now been reported in severely retarded males with progressive neurological symptoms,<sup>2–6</sup> in males with non-progressive encephalopathy,<sup>7</sup> and in males with an Angelman-like phenotype.<sup>8</sup> Recently, recessive mutations have also been described in

males from families with nonspecific X-linked mental retardation (MRX).<sup>9–11</sup>

Of all reported MRX genes, *MECP2* may have the highest mutation frequency. *MECP2* mutations that do not cause RTT in females have been found in families with X-linked mental retardation that were previously linked to Xq28.<sup>5,10,11</sup> In addition, based on an analysis of the *MECP2* gene in 185 sporadic MR males, Couvert *et al*<sup>10</sup> suggested that mutations in *MECP2* are responsible for ~2% of mental retardation in males. This indicates that the frequency of *MECP2* mutations equals that of Fragile-X syndrome.<sup>12</sup> This hypothesis prompted us to perform *MECP2* mutation analysis on the DNA of 475 mentally retarded males, who were negative for the CGG repeat expansion in the *FMR1* gene, and had a normal 46(X,Y) karyotype. Informed consent was obtained in all instances.

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No pre-selection based on degree of mental retardation and presence of additional dysmorphic or neurologic abnormalities was made. We performed mutation analysis of the coding region and splice sites of the *MECP2* gene by direct sequencing of PCR-amplified genomic DNA (five fragments, primer sequences are available upon request). Fourteen different sequence changes were detected. Of these changes, four were reported previously as non-pathogenic variants, and five were novel silent changes (Table 1). Five changes were possible mutations not described before (Table 2). The latter group included four amino acid changes and a deletion of two bases resulting in a frameshift and premature stopcodon. The T196S change was found in three patients of different families.

Following the detection of four novel amino acid changes, we investigated the DNA of 150 control males for these changes by allele-specific oligonucleotide hybridization. The T196S mutation was found in one of the controls. The other amino acid changes were not detected in the control population. Sequence analysis revealed that all mothers of the patients were heterozygous (carriers) for the missense mutation. For three amino acid changes segregation studies were performed. All changes were found in male family members with normal IQ, including grandfathers, maternal uncles and healthy brothers of the index patient. These results indicate that the changes T196S,

E394K, and P480S are rare polymorphisms instead of disease causing mutations.

The T228S amino acid change was inherited from the mother, but no other family members were available for further segregation analysis. In this case, it is impossible to distinguish between a rare non-pathogenic variant and a causative mutation. The threonine at position 228 however is not conserved in mouse (alanine), rat (alanine) and chicken (glycine), indicating that it is unlikely that the change has a dramatic effect on the *MECP2* protein.

The 1415–1416del mutation was found hemizygotously in the patient and was absent in the DNA of the mother. Thus, this is the first reported *de novo* *MECP2* mutation in a male. Somatic mosaicism for a *MECP2* mutation in males,<sup>2,3</sup> as well as inherited *MECP2* mutations in males have been previously described.<sup>4–11</sup> The 1415–1416del mutation in the 3' part of the gene leads to a frameshift and premature stop codon. It is predicted that C-terminal 15 amino acids are replaced by 13 other amino acids (Figure 1). Previous clinical studies showed that the patient has moderate mental retardation (IQ 45–55), emotional disturbances, gynaecomastia and generalized obesity. Results of clinical re-examination will be published elsewhere.<sup>13</sup> *MECP2* mutations in males frequently comprise amino acid substitutions or truncating mutations at the C-terminus, outside the conserved MBD and TRD domain, like in the patient reported here.<sup>5–7,10,11</sup> Apparently, these mutations are milder than those seen in RTT, very likely because they will affect only subset of all *MECP2* target genes.

Our study supports the finding of Glatt *et al*<sup>14</sup> who suggested that rare missense variants might be more common than expected. Therefore, it is of crucial importance to perform segregation analysis in the families, once a possible mutation has been identified. Generally, a control population of males and females is screened for the change. In this study, three amino acid changes were found in 1/475 patients (0.2%). Therefore, at least 1500 chromosomes would need to be examined to determine if the frequency in normal males is significantly different from that in patients.<sup>15</sup> Furthermore, only male chromosomes should be used as a control because MRX acts as a

**Table 1** Silent changes and known non-pathogenic variants in 475 MR males

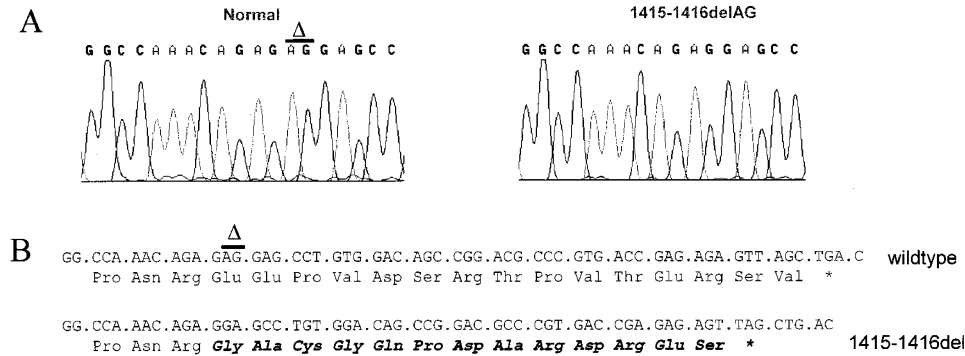
Nucleotide change	Amino acid effect	Frequency in this study	Reference
225 G→A	P75P	1/475	This study
528 C→G	P176P	1*/475	This study
573 C→G	P191P	1/475	This study
582 C→T	S194S	2/475	17
686 C→T	S229L	1/475	17
834 C→T	A287A	1/475	6
897 C→T	T299T	1*/475	17
942 C→T	I314I	1/475	This study
1189 G→A	E397K	1/475	18

\*One of the patients has two silent changes: T299T and P176P.

**Table 2** Novel amino acid changes, detected in 475 MR males

Patient ID	Mental impairment	nt change	aa change	Frequency in MR males	Frequency in controls	Mother	Segregation	Conclusion
17513	Moderate	587C→G	T196S	3/475	1/150	carrier	healthy grandfather positive	Non-pathogenic variant
17307	Mild	587C→G	T196S	3/475	1/150	carrier	healthy maternal uncle positive	Non-pathogenic variant
17309	Borderline	587C→G	T196S	3/475	1/150	ND	ND	Non-pathogenic variant
17296	Borderline	683C→G	T228S	1/475	0/150	carrier	NA	Non-pathogenic variant or mutation?
17529	Moderate	1180G→A	E394K	1/475	0/150	carrier	healthy grandfather positive	Non pathogenic variant
17293	Moderate	1415-1416del	Frameshift	1/475	ND	no carrier	<i>de novo</i>	<i>De novo</i> mutation
17012	Moderate-severe	1438C→T	P480S	1/475	0/150	carrier	healthy brother positive	Non-pathogenic variant

Mental impairment: borderline, IQ 70–85; mild, IQ 55–69; moderate, IQ 40–54; severe, IQ 25–39. ND: not determined. NA: not available.



**Figure 1** Mutation analysis of *MECP2* in a patient with moderate mental retardation. (A) PCR products of the 3' part of the last exon were sequenced and a deletion of two bases at position 1415–1416 (indicated by  $\Delta$ ) was detected in the patient. (B) Wildtype DNA sequence and predicted protein are shown at the top. Below the DNA sequence and the predicted protein sequence from the patient are depicted. The 13 novel C-terminal amino acids are shown in italics.

recessive trait and a change in a control female could actually cause a phenotype when transmitted to a son. Furthermore, it is important that all sequence changes identified worldwide are deposited in the *MECP2* mutation database (<http://homepages.ed.ac.uk/skirmis/>). For low frequency changes, the most efficient way to distinguish between a polymorphism and a disease causing mutation may be segregation analysis in the family.<sup>16</sup>

The frequency of *MECP2* in the Dutch Fragile-X negative mentally retarded males is 0.2 to 0.4% (the latter figure applies if the T228S change is regarded a causative mutation). In our institute, expanded CGG repeats in the *FMR1* gene are detected in 2.5% of an equal population of mentally retarded males, ie those mentally retarded males without a family history of Fragile-X syndrome (E Sistermans, unpublished observations). The difference between the *MECP2* mutation frequency in our study (1/475; 0.2–0.4%) and the 2% (4/185) previously obtained by Couvert *et al*<sup>10</sup> is statistically significant ( $P=0.002$ ; Fisher exact test). Our study revealed the importance of segregation analysis, which showed that several possible mutations were polymorphisms. In contrast to the present study, the design of the previous study did not allow contacting additional family members and segregation analysis could not be performed. The changes in the previous study were not found in 500 control chromosomes, but this amount is too low to exclude that some of the putative mutations in that study (eg P399L, R453Q) may in fact be rare polymorphisms. In conclusion, our results indicate that the incidence of *MECP2* mutations in sporadic male MR is 5–10-fold lower than that of Fragile-X syndrome.

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