MECP2 Mutation Analysis in Patients With Mental Retardation

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Mutations in the methyl-CpG-binding protein 2 (MECP2) gene are known to underlie Rett’s syndrome, the most common cause of mental retardation (MR) in girls. Since the original report, phenotypes resulting from MECP2 mutations have been shown to extend, for example, to several Rett variants, autism, atypical Angelman syndrome, and non-specific MR. It was earlier proposed that MECP2 mutations might account for ~2% of the male cases with non-specific MR. Thereby, the frequency of MECP2 mutations in the mentally retarded population would be comparable to that of Fragile-X syndrome. The aim of this study was to analyze well-characterized cases with MR and to clarify the role of the MECP2 gene in the etiology of MR and atypical Angelman syndrome. The coding sequence of the MECP2 gene was analyzed in a sample of 118 patients (103 males, 15 females) by direct sequencing. Two coding sequence variants, 602C>T (A201V) and 1189G>A (E397K), were identified. In addition, we identified four variants in the intronic or 3’ UTR regions. None of these variants is likely to be causal. We conclude that the evidence across all the mutation screening studies implies that MECP2 mutations do not represent a major cause of non-specific MR.

KEY WORDS: mental retardation; XLMR; Rett’s syndrome; Angelman syndrome; mutation screening; X-chromosome

INTRODUCTION
Methyl-CpG-binding protein 2 (MECP2) gene acts by binding to DNA methylated at CpG sites through a methyl-CpG-binding domain (MBD) and silences the transcription of downstream genes by recruiting corepressor complexes through a transcriptional repression domain (TRD) [Lewis et al., 1992; Jones et al., 1998; Nan et al., 1998; Shahbazian and Zoghbi, 2002]. Currently, mutations in the coding region of the MECP2 gene are known to account for 75%–90% of sporadic Rett’s syndrome cases (RTT; MIM 312750; F84.2) [Amir et al., 1999; Shahbazian and Zoghbi, 2002].

Besides RTT, a wide spectrum of phenotypes has currently been reported to be associated with MECP2 mutations in females. These have included Rett variants [Shahbazian and Zoghbi, 2002], non-specific mental retardation (MR) [Kleefstra et al., 2004], as well as some cases with autistic [Carney et al., 2003] and Angelman-like phenotypes [Watson et al., 2001; Kleefstra et al., 2004]. The MECP2 mutations found in female RTT patients are thought to be lethal in males. However, several mutations similar to those observed in female RTT cases have been reported in males with either Klinefelter syndrome (47,XXY) or somatic mosaicism [Moog et al., 2003]. In addition, some late truncating mutations and missense mutations not found in RTT cases have also been reported in males. These mutations would likely have produced a mild phenotype in females but led to severe MR in males with a normal karyotype [Shahbazian and Zoghbi, 2002]. The clinical features associated with MECP2 mutations in males vary from neonates with severe encephalopathy to adults with MR, as reviewed in detail elsewhere [Moog et al., 2003].

It was recently suggested that the frequency of MECP2 mutations might be comparable to the frequency of mutations in the FMR1 gene among the mentally retarded population, and that systematic screening for MECP2 mutations in male patients MR might be feasible [Couvert et al., 2001]. The aim of this study was to elucidate the frequency of MECP2 mutations in the study population of 118 carefully phenotyped patients with MR and to obtain data about the rationality of screening for MECP2 mutations in patients with non-specific MR.

SUBJECTS AND METHODS

Patients

We recruited a total of 118 unrelated mentally retarded patients (103 males, 15 females) mainly via Finnish central hospitals and homes for people with MR (Table I). A total of 109 patients were Finnish and 9 Estonian. The cases were selected after thorough clinical and medical examination by an experienced child neurologist or pediatrician. Detailed questionnaire concerning the clinical features of the patients was obtained from the referring clinicians. We excluded all patients with Rett’s syndrome, Rett variants, infantile autism, cerebral

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palsy, fetal alcohol syndrome, or other known etiology. Fragile-X syndrome was excluded in all patients. All patients were verified to have normal karyotypes. A total of 20 individuals (15 males, 5 females) were considered to have atypical Angelman syndrome. Angelman syndrome was excluded by methylation test and FISH analysis. In addition, UBE3A mutations were excluded in 9 of 20 patients with Angelman-like phenotype. The study was approved by the Ethical Committee of the Helsinki University Hospital.

**Mutation Screening**

DNA was extracted from EDTA blood according to standard procedures. We analyzed the entire 1,461 bp protein coding sequence and the flanking splice sites of the MECP2 gene by direct sequencing. Polymerase chain reactions were performed, as described elsewhere [Auranen et al., 2001], and the specificity of the PCR products was assessed by 1.5% agarose gel electrophoresis. The sequencing reactions were performed from the PCR products using the Big Dye Terminator v. 2.0 kit (Applera Corporation, Norwalk, CT) according to the manufacturer's instructions. Electrophoresis was performed on an ABI377 DNA sequencer (Applera Corporation). The sequence analyses were performed with Sequencher 4.0.5 software (Gene Codes Corporation, Ann Arbor, MI). For splice site predictions we used the GeneSplicer Web Interface (http://www.tigr.org/db/GeneSplicer/gene_spl.html), the Berkley Drosophila Genome Project web page (http://www.fruitfly.org/seq_tools/splice.html), the NetGene2 Server (http://www.cbs.dtu.dk/services/NetGene2/), and the splice view of the Institute of Advanced Biomedical Technologies (ITBA) web page (http://l25.itba.mi.cnr.it/~webgene/wwwspliceview.html).

**Table I. Description of the Study Sample**

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>103 (87)</td>
</tr>
<tr>
<td>Females</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Degree of MR</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>13 (11)</td>
</tr>
<tr>
<td>Moderate</td>
<td>53 (45)</td>
</tr>
<tr>
<td>Severe</td>
<td>43 (36)</td>
</tr>
<tr>
<td>Profound</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Not specified</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Angelman-like phenotype</td>
<td>20 (17)</td>
</tr>
</tbody>
</table>

**RESULTS**

A total of 118 patients with MR were screened for the MECP2 mutations in this study (Table I). The entire protein coding sequence and the flanking splice sites of the MECP2 gene were analyzed. We observed only two coding sequence variants in two unrelated male patients with severe MR. These were a 602C>T transition leading to A201V amino acid substitution and a 1189G>A transition leading to the E397K amino acid change. Both of these variants have been reported several times earlier and are considered to represent rare polymorphisms [Wan et al., 1999; Amano et al., 2000; Buyse et al., 2000; Hampson et al., 2000; Lam et al., 2000; Nicolao et al., 2001; Moncla et al., 2002]. Intrinsic or 3’UTR sequence variants were detected in five patients. One patient had a 377+22C>G substitution and two patients carried a 378-17delT variant, which both had earlier been reported to be noncausative polymorphisms [Lam et al., 2000; Laccone et al., 2001; Beyer et al., 2002]. These variants were predicted not to affect splicing. The 3’UTR region of MECP2 gene extends around 8.5 kbs and is well conserved across species, but its function is currently unknown. No pathogenic 3’UTR variations are currently known. Two patients in the present study sample had 1461*93G>A, which is listed in the IRSA MECP2 Variations Database as polymorphism [http://mecp2.chw.edu.au/; Christodoulou et al., 2003]. Finally, a patient carrying a 1461*93G>A change had also an intronic variant 378-61C>G [Orrico et al., 2000]. This variation was predicted not to affect splicing and therefore it is unlikely that it could be associated with the etiology of MR. The sequence variants detected in this study are listed in Table II.

**DISCUSSION**

In their initial report, Couvert et al. [2001] identified four MECP2 mutations among 185 male patients with MR, which equals a frequency of ~2%. Based on these results, they suggested that systematic screening of MECP2 gene should be considered in all unexplained cases of MR. Later on one of these variants, P399L, has been found to be present in a healthy male, indicating that this variant most probably is not causative [Laccone et al., 2002]. Furthermore, Couvert et al. [2001] reported one R453Q mutation and a recurrent A140V mutation in two patients. To our knowledge, the R453Q mutation has not been reported elsewhere. Since data of segregation or functional analyses concerning this mutation do not exist, it should probably be considered as unclassified at this stage. The A140V mutation has been detected in two X-linked mental retardation (XLMR) families [Orrico et al., 2000; Winnepenninckx et al., 2002], in a patient with schizophrenia associated with language disorder [Cohen et al., 2002] and in the PPM-X syndrome

**Table II. MECP2 Sequence Variants Detected in 118 Patients With Mental Retardation**

<table>
<thead>
<tr>
<th>Variant number</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Frequency in this study</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>602C&gt;T</td>
<td>A201V</td>
<td>1</td>
<td>Amano et al. [2000]; Lam et al. [2000]; Nicolao et al. [2001]</td>
</tr>
<tr>
<td>2</td>
<td>1189G&gt;A</td>
<td>E397K</td>
<td>1</td>
<td>Wan et al. [1999]; Buyse et al. [2000]; Hampson et al. [2000]; Lam et al. [2000]; Nicolao et al. [2001]; Moncla et al. [2002]</td>
</tr>
<tr>
<td>3</td>
<td>377+22C&gt;G</td>
<td>Intrinsic</td>
<td>1</td>
<td>Couvert et al. [2001]; Beyer et al. [2002]</td>
</tr>
<tr>
<td>4</td>
<td>378-61C&gt;G</td>
<td>Intrinsic</td>
<td>1</td>
<td>Orrico et al. [2000]; Laccone et al. [2001]; Beyer et al. [2002]; Bourdon et al. [2003]</td>
</tr>
<tr>
<td>5</td>
<td>378-17delT</td>
<td>Intrinsic</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1461*93G&gt;A</td>
<td>3’UTR</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
represent relatively rare causes of MR. Recent findings [Yntema et al., 2002; Bourdon et al., 2003; Couvert et al., 2001; Klauck et al., 2002]. Our results are in line with the data across all published datasets containing a total of 1,117 mentally retarded patients, the frequency of causative mutations in the material by Couvert et al. [2001] 185 MR, negative for CGG expansion in FRAXA 0–3 a No. of mutations

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of cases</th>
<th>Description of the study sample</th>
<th>No. of mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Couvert et al. [2001]</td>
<td>185</td>
<td>MR, negative for CGG expansion in FRAXA 0–3*</td>
<td>1</td>
</tr>
<tr>
<td>Yntema et al. [2002]</td>
<td>475</td>
<td>MR, negative for CGG expansion in FRAXA 0</td>
<td></td>
</tr>
<tr>
<td>Bourdon et al. [2003]</td>
<td>354</td>
<td>MR, negative for CGG expansion in FRAXA 0</td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>103</td>
<td>Carefully selected set of patients with MR of unknown etiology</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,117</strong></td>
<td></td>
<td><strong>1–4 (0.1–0.4%)</strong></td>
</tr>
</tbody>
</table>

*Two out of three mutations listed here are A140V variants, which have been suggested to be designated as unknown. The P399L mutation reported by Couvert et al. [2001] is likely to be a noncausative polymorphism and is not included in the number of mutations in the table [Laccone et al., 2002].

A197M variant was reported, but its role is currently unknown.

A wide spectrum of MECP2 mutations is currently known to cause a variety of phenotypes. In addition, a large number of noncausative variants are known to exist [http://mecp2.chw.edu.au/; Christodoulou et al., 2003]. Therefore, it is important to critically evaluate the role of identified variants prior to the genetic counseling of families [Laccone et al., 2002; Moncla et al., 2002; Bourdon et al., 2003]. Our results are in line with recent findings [Yntema et al., 2002; Bourdon et al., 2003; Kleefstra et al., 2004] and suggest that MECP2 mutations represent relatively rare causes of MR.

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