MECP2 Structural and 3′-UTR Variants in Schizophrenia, Autism and Other Psychiatric Diseases: A Possible Association With Autism

Akane Shibayama,1 Edwin H. Cook Jr.,2 Jinong Feng,1 Cecile Glanzmann,1 Jin Yan,1 Nick Craddock,3 Ian R. Jones,3 David Goldman,4 Leonard L. Heston,5 and Steve S. Sommer1*

1Department of Molecular Genetics, City of Hope National Medical Center, California
2Department of Psychiatry, University of Chicago, Chicago, Illinois
3Department of Psychiatry, NIAAA, NIH, Bethesda, Maryland
4Department of Psychiatry, City of Hope National Medical Center, California
5Division of Neuroscience, University of Birmingham, Queen Elizabeth Psychiatric Hospital, Birmingham, UK

INTRODUCTION

Protein truncating and certain missense mutations in the methyl-CpG-binding protein 2 gene (MECP2) cause Rett syndrome (RTT) and have also been reported in a number of X-linked mental retardation syndromes. Furthermore, putative mutations have been described in autistic patients and a boy with language disorder and schizophrenia. In this study, DNA samples from individuals with schizophrenia and other psychiatric diseases were scanned in order to explore whether the phenotypic spectrum of mutations in the MECP2 gene can extend beyond the traditional diagnoses of RTT in females and severe neonatal encephalopathy in males. The coding regions, adjacent splicing junctions, and highly conserved segments of the 3′-untranslated region (3′-UTR) were examined in 214 patients, including 106 with schizophrenia, 24 with autism, and 84 patients with other psychiatric diseases by detection of virtually all mutations-single strand conformation polymorphism (SSCP) (DOVAM-S). To our knowledge, this is the first analysis of variants in conserved regions of the 3′-UTR of this gene. A total of 5.2 kb per haploid gene was analyzed (1.5 Mb for 214 patients). A higher frequency of missense and 3′-UTR variants was found in autism. One missense and two 3′-UTR variants were found in 24 patients with autism versus one patient with a missense change in 144 ethnically similar individuals without autism (P = 0.009). These mutations suggest that a possible association between MECP2 mutations and autism may warrant further study. © 2004 Wiley-Liss, Inc.

KEY WORDS: MECP2; 3′-UTR; schizophrenia; autism; psychiatric diseases; mutation detection

Mutations in the gene coding for methyl-CpG-binding protein 2 (MECP2) cause Rett syndrome (RTT) and have also been reported in a number of X-linked mental retardation syndromes. Furthermore, putative mutations have been described in autistic patients and a boy with language disorder and schizophrenia. In this study, DNA samples from individuals with schizophrenia and other psychiatric diseases were scanned in order to explore whether the phenotypic spectrum of mutations in the MECP2 gene can extend beyond the traditional diagnoses of RTT in females and severe neonatal encephalopathy in males. The coding regions, adjacent splicing junctions, and highly conserved segments of the 3′-untranslated region (3′-UTR) were examined in 214 patients, including 106 with schizophrenia, 24 with autism, and 84 patients with other psychiatric diseases by detection of virtually all mutations-single strand conformation polymorphism (SSCP) (DOVAM-S). To our knowledge, this is the first analysis of variants in conserved regions of the 3′-UTR of this gene. A total of 5.2 kb per haploid gene was analyzed (1.5 Mb for 214 patients). A higher frequency of missense and 3′-UTR variants was found in autism. One missense and two 3′-UTR variants were found in 24 patients with autism versus one patient with a missense change in 144 ethnically similar individuals without autism (P = 0.009). These mutations suggest that a possible association between MECP2 mutations and autism may warrant further study. © 2004 Wiley-Liss, Inc.

SUBJECTS AND METHODS

Subjects

The study was approved by the Institutional Review Board. The details on patient ascertainment were described previously [Feng et al., 1998, 2001].
Mutation Detection

The coding regions, associated splice junctions, and conserved regions of the 3'-UTR were amplified in 20 segments and scanned for mutations with DOVAM-S, a robotically enhanced, highly redundant form of single strand conformation polymorphism (SSCP), which detects virtually all mutations by blinded analysis [Liu et al., 1999; Buzin et al., 2000]. In brief, the above regions were amplified robotically in 20 segments ranging in size from 149 to 360 bp, pooled, denatured, and electrophoresed under five nondenaturing conditions in which gel matrix, buffer, temperature, and additive were varied. PCR primers for the MECP2 gene are available upon request. PCR products with mobility shifts were sequenced with the ABI model 377 (Perkin-Elmer Model 377, Norwalk, CT).

RESULTS AND DISCUSSION

The coding region, splice junction, and nine highly conserved segments of 3'-UTR were scanned (1.5 megabases total) in 214 patients, including schizophrenia (106), autism (24), attention-deficit hyperactivity disorder (ADHD) (25), bipolar illness (BPI) (24), alcoholism (17), puerperal psychosis (15), and phobia (3). Eleven different MECP2 variants within coding exon 4, flanking intron 3 and 3'-UTR were identified; six variants are novel (Fig. 1 and Table I). Two novel missense variants were identified: (i) P376R in the C-terminal portion of MECP2 in a female patient with autism, (ii) T196S between the MBD and the TRD in a female patient with schizophrenia.

Three novel 3'-UTR variants were identified in one male and one female Caucasian patient with autism and in a male African–American patient with ADHD. G > C at c.1638 and insertion A at c.1558 occur in the middle of a region in which 306 of 308 nucleotides (99%) are identical in mouse and human. T > C at c.6809 occurs within the less conserved region in which 243 of 286 nucleotides are identical in mouse and human (85%).

In contrast, in the factor IX gene and most other genes, it is not possible to find any segments that are 50% identical when comparing 3'-UTRs from different classes of mammals. The putative mutations were present in the heterozygous mother in the two autism cases.

One missense and two 3'-UTR variants were found in 24 autistic patients of Western European Caucasian descent, while only one patient with a missense change in 144 ethnically similar individuals without autism \( (P = 0.009, \text{ Fisher's exact test}) \). The results remain significant if only 3'-UTR variants are analyzed \( (2/24 \text{ vs. } 0/144; P = 0.02) \).

The data herein raise the question of whether milder MECP2 mutations can be found in male and female patients with autistic disorder. Two of 69 analyzed females with autistic disorder were found to have de novo mutations in the MECP2 gene [Carney et al., 2003]. These two females had a frameshift mutation and a nonsense mutation previously described in patients with RTT, respectively. Their Kerr scores may be interpreted in the lower range of the RTT spectrum [Carney et al., 2003]. However, four studies utilizing a variety of mutation scanning methods have not found MECP2 structural variants in the coding region in a combined group of 202 patients with autism [Vourc'h et al., 2001; van Karnebeek et al., 2002; Lobo-Menendez et al., 2003; Zappella et al., 2003]. In another study, analysis of 21 autistic females led to the identification of a putative 5' splice site mutation in intron 2 [Lam et al., 2000]. Beyer et al. [2002] identified six structural variants in 184 unrelated patients with autism, although three of these either did not cosegregate in pedigrees or represented polymorphisms previously described.

![Genomic organization of the MECP2 gene and MECP2 variants.](image)

The MECP2 gene spans 113 kb in Xq28. It is comprised of four exons, and nucleotide positions relative to the ATG codon are indicated above the gene. Untranslated regions are shaded gray. The 5'-UTR is relatively short (167 nucleotides). The coding sequence for the methyl-CpG-binding domain (MBD) is split between exon 3 and 4. The region encoding the transcription repression domain lies in the fourth exon. T196S in a patient with schizophrenia as indicated by the diamond while the squares indicate the missense and 3' UTR changes in autism. The arrow indicates the 3' UTR variant found in an Afro–American patient with ADHD. Silent and intronic variants are denoted by circles below the gene. Recurrent variants are denoted by symbols adjoined by lines at identical positions on the gene.
<table>
<thead>
<tr>
<th>No.</th>
<th>NT change</th>
<th>AA change</th>
<th>Conservation</th>
<th>Exon/</th>
<th>Region</th>
<th>ID</th>
<th>Ethnicity</th>
<th>Gender</th>
<th>Disease</th>
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<td>V1</td>
<td>c.587 C &gt; G</td>
<td>T196S</td>
<td>Mouse, rat</td>
<td>E4</td>
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<td>Schizophrenia</td>
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<td>P376R</td>
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<td>E4</td>
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<td>M</td>
<td>ADHD</td>
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*The sequence reference number was from Amir et al. [1999] (GenBank accessions number AF030876 for the gene locus and AF158180 for the mRNA).

*ID, patients' ID.

*The articles reporting the sequence change in the MECP2 gene.

*Parent was not available to investigate in the ADHD case.
To our knowledge, no study examining highly conserved regions of the 3'-UTR region in autism has been reported previously. We scanned nine highly conserved segments of the 3'-UTR. A higher frequency of missense and 3'-UTR variants was found in patients with autism compared to that of controls (P = 0.02). Missense and 3'-UTR variants are not common in psychiatric diseases with the exception of autism. At present, a role of MECP2 as a modifier gene in the context of a potential polygenic and multifactorial disorder like autism cannot be ruled out.

In conclusion, mutation scanning in 214 well-characterized patient samples does not suggest that mutations in the coding sequence of the MECP2 gene play a major role in the etiology of schizophrenia, ADHD, alcoholism, BP1, and puerperal psychosis. The presence of a missense variant and two single nucleotide substitutions in conserved regions of the 3'-UTR in 24 patients with autism suggests that a case control analysis including 3'-UTR conserved regions may be warranted with a larger sample of patients with autism.

ACKNOWLEDGMENTS

We thank Wenyan Li and Christina Ticsay for DNA sequencing.

REFERENCES


