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SHORT REPORT

No mutations in the coding region of the Rett syndrome gene MECP2 in 59 autistic patients

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Autistic disorder is a pervasive developmental disorder considered to have a multigenic origin. Mental retardation is present in 75% of autistic patients. Autistic features are found in Rett syndrome, a neurological disorder affecting girls and associated with severe mental retardation. Recently, the gene responsible for the Rett syndrome, methyl CpG-binding protein (MECP2) gene, was identified on the X chromosome by a candidate gene strategy. Mutations in this gene were also observed in some mentally retarded males. In this study we tested MECP2 as a candidate gene in autistic disorder by a DGGE analysis of its coding region and intron-exon boundaries. Among 59 autistic patients, 42 males and 17 females, mentally retarded or not, no mutations or polymorphisms were present in the MECP2 gene. Taking into account the size of our sample, we conclude that MECP2 coding sequence mutations are not an important factor (less than 5% of cases) in the aetiology of autistic disorder. *European Journal of Human Genetics* (2001) **9**, 556–558.

Keywords: autism; Rett syndrome; MECP2 gene; DGGE analysis

Introduction

Autistic disorder is a pervasive developmental disorder of unknown aetiology which affects males more often than females. Epidemiological studies suggest a multigenic origin.[1] However, at this time, no genes have been identified which are clearly associated to autism.

Rett syndrome is another pervasive developmental disorder which affects mainly females with a much lower prevalence than autism.[2] Some Rett patients present autistic features.[3] Recently mutations in the MECP2 gene have been identified as being responsible for more than 70% of Rett syndrome cases.[4–6] The MECP2 gene encodes a methyl CpG-binding protein.[7] Moreover, a MECP2 gene mutation has been discovered in one family with recessive X-linked mental retardation with progressive spasticity.[8] Systematic screening of males with sporadic mental retardation negative for FRAXA CGG expansion showed rare MECP2 mutations (T Bienvenu, C Beldjord, J Chelly, personal communication). These observations raise the possibility that MECP2 mutations might be involved in other developmental disorders with mental retardation, like autism. To test this hypothesis, we have screened the coding sequence of the MECP2 gene in 59 sporadic autistic patients.

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Materials and methods

Subjects

The autistic patients were 17 females and 42 unrelated males, aged from 4 to 20 years at the time of examination. The DSM IV criteria were used by a team of practicioners to ascertain the diagnosis of autistic disorder.[9] Recognisable genetic diseases have been excluded by clinics and cytogenetics. The FRAXA mutation at the CGG repeat was excluded by classical PCR technique and Southern analysis.[10,11] The mean of the developmental quotient (DQ) was 36 (range 12–109), with 44 severe mental retardation (DQ < 50), 12 mild mental

retardation ($50 \leqslant DQ < 70$) and three normal DQ. We have used the denaturing gradient gel electrophoresis (DGGE) assay to screen the MECP2 gene.

Mutation analysis

Genomic DNA was extracted from peripheral blood using standard methods. The three exons and the flanking intronic sequences of the MECP2 gene were separately amplified by nine different polymerase chain reactions (PCR) using primers with psoralen clamps. PCR were performed in a total volume of 25 μl containing 100 ng of genomic DNA, $1 \times$ GibcoBRL PCR Buffer, $MgCl_2$ 1.5 mM, 250 μM of each dNTP, 20 pmol of each primer (Table 1) and 0.5 U of Taq DNA polymerase Platinium (GibcoBRL). Amplifications were carried out for 30 s at 95°C, 30 s at Tm (see Table 1) and 30 s at 72°C for 40 cycles. Heteroduplex formation consisted of incubation for 5 min at 94°C and 30 min at 56°C. For DGGE, gels were run at 60°C and the denaturing solution contained 40% formamide and 7 M urea. The conditions of electrophoresis are summarised in Table 1.

Results

No abnormal pattern of migration could be detected. So we did not observe mutations in the MECP2 coding sequence in 59 autistic patients. The polymorphisms described in some Rett syndrome patients were absent in the autistic population tested.

Discussion

MECP2 gene could be involved in several disorders with mental retardation and not only in Rett syndrome. This hypothesis was confirmed by the observation of mutations in MECP2 gene in males with mental retardation. Here we tested for the participation of the MECP2 gene in autistic disorder. We used a DGGE analysis by overlapping primer sets encompassing the complete coding region. DGGE is one of the most sensitive methods for screening mutation.[12] However, no current analytical methods including sequence analysis and DHPLC, have 100% sensitivity.[13] The MECP2 gene contains two introns. Mutations in the intron-exon boundaries could produce alternative splicing events. In order to screen these regions, we localised the primer sequences in the introns at 9 to 111 bp of the exon-intron junctions.

No abnormal migration pattern of the PCR products was detected in any of our autistic patients, mentally retarded or not. This observation rules out the possibility that the MECP2 gene is mutated in a significant fraction of autistic patients. Taking into account the size of our sample (59), the percentage of mutations in the autistic population can be estimated to be lower than 5% (binomial distribution, n=59 with $P < 0.05$). However, as our sample contained only 17 females, we cannot exclude that the MECP2 gene is more frequently mutated in a subgroup of autistic females. Moreover, our method did not test for mutations in introns, promotor region, 5′UTR and 3′UTR.

The high prevalence of autism in males has focused the attention of researchers on X chromosome for a long time. Indeed, two genetic studies have highlighted a possible susceptibility region in Xq23.[9,14] On the other hand, a number of full genome screen studies did not find significant increased allele sharing in the Xq28 region.[15–17] These data, together with our results, make it very unlikely that MECP2 coding region mutations play a significant role in autistic disorder.

Table 1 Conditions for PCR amplification of the MECP2 gene fragments and for DGGE

Primer	*Sequence of primer*	*Length (bp)*	*Tm (°C)*	*Gradient (%ds)*	*Voltage (V)**
1F	5′-P-ta-tttctttgttttaggctcca-3′	190	55	40–70	300
1R	5′-ggccaaaccaggacatatac-3′				
2.1F	5′-P-ta-gagcccgtgcagccatcagc-3′	170	55	40–90	250
2.1R	5′-cgtgtccagccttcaggcag-3′				
2.2F	5′-atgtatgatgaccccaccct-3′	150	48	25–65	180
2.2R	5′-P-ta-ctgtagagataggagttgct-3′				
2.3F	5′-gtgatacttacatacttgtt-3′	200	55	20–70	180
2.3R	5′-P-ta-ggctcagcagagtggtgggc-3′				
3.1F	5′-P-ta-tgtgtctttctgtttgtccc-3′	182	58	30–80	200
3.1R	5′-gatttgggcttcttaggtgg-3′				
3.2F	5′-P-ta-cctcccggcgagagcagaaa-3′	240	58	45–80	240
3.2R	5′-tgacctgggtggatgtggtg-3′				
3.3F	5′-tgccttttcaaacttcgcca-3′	344	55	40–85	380
3.3R	5′-P-ta-tgaggaggcgctgctgctgc-3′				
3.4F	5′-gcagcagcagcgcctcctca-3′	244	56	40–90	300
3.4R	5′-P-ta-tggcaaccgcgggctgagtca-3′				
3.5F	5′-P-ta-tgccccaaggagccagctaa-3′	200	55	40–80	240
3.5R	5′-gctttgcaatccgctccgtg-3′				

P: Psoralen; ds: denaturing solution; *: running conditions during 5 h.

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