Original article

Mutation analysis of methyl-CpG binding protein family genes in autistic patients

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Abstract

Methyl-CpG binding protein 2 gene (MECP2), the gene implicated in Rett syndrome, was also reported to be involved in mental retardation and autism. MECP2, MBD1, MBD2, MBD3, and MBD4 comprise a nuclear protein family sharing the methyl-CpG binding domain (MBD) and are related to transcriptional repression. In 65 Japanese autistic patients, all the exons of each gene were screened for mutations by DHPLC, and the results were confirmed by direct sequencing. An R269C mutation that resulted in the addition of cysteine near a cysteine rich region was found in the MBD1 gene in one patient. This mutation was also detected in the patient’s father with some phenotypes of autism and his normal sister, but not in 151 controls. Two repeat length polymorphisms, (GGGGCC)2 to 3 and (GGC)4 to 5, were detected in MBD2, and several polymorphisms were detected in each gene. Although our findings could not confirm that the genes of this family are responsible for the etiology in the majority of autistic patients, the R269C mutation in the MBD1 gene may relate to autism. The potential association of the high-polymorphic gene variants with autism needs to be studied further. Furthermore, these polymorphisms are useful for linkage analysis.

Keywords: Autism; Methyl-CpG binding protein genes; MECP2; MBD1

1. Introduction

Autism (MIM 209850) is a neurodevelopmental disorder that affects at least five in 10,000 individuals. It is characterized by impairments in reciprocal social interaction and communication, and restricted and stereotyped patterns of interests and activities. Thus far, only the mutations of the neureligin 3 and 4 genes have been detected in a few patients with autism [1].

Methyl-CpG binding protein 2 (MECP2) gene mutation was reported as the cause of Rett syndrome, a neurodevelopmental disorder associated with some autistic phenotypes [2], and in X-linked mental retardation. Additionally, a mutation in MECP2, IVS2+2delTAAG, was reported in one case of infantile autism [3]. MeCP2 selectively binds to methylated DNA and represses transcription, leading to gene inactivation, and is reported to correlate with neuronal maturation.

The methyl-CpG binding protein family consists of five members, MECP2 and MBD1-4, sharing a common methyl-CpG binding domain (MBD), and they were mapped to Xq28, 18q21.1, 18q21.3, 19p13, and 3q21, respectively [4]. All MBDs except MBD4, relating to DNA mismatch-repair, form complexes with histone deacetylase and are involved in recruiting histone deacetylases to methyl-CpG enriched regions in the genome to repress transcription [5]. A study on the expression of MBD genes showed that MBD1-3, all have a broad expression in multiple murine tissues including the brain [6]. Additionally, some patients with an 18q deletion, including the 18q21 deletion, where MBD1 and 2 were localized, had autism or atypical Rett syndrome [7]. From the above data, it is suggested that the MBD family genes can be candidate genes for autistic disorders. Thus, we screened these genes for disease-causative mutations in autistic patients.
2. Materials and methods

Patients with autism or pervasive developmental disorder (PDD) not otherwise specified who visited Jichi Medical School or Kori Hospital, Kansai Medical School were enrolled in this study after obtaining their parent’s written informed consent. Autism and PDD were diagnosed according to the DSM-IV criteria. Forty-eight patients had autism, and 17 had PDD. Of these, 49 patients were males and 16 were females. The age of the patients ranged from 3 to 23 years. Control samples were obtained from Japanese adult volunteers after obtaining their written informed consent.

Genomic DNA was extracted from the peripheral blood leukocytes. All exons of \textit{MECP2} and \textit{MBD1-3} were amplified separately by PCR. The primer sequences and annealing temperature are listed in Table 1. Primers for \textit{MECP2} were the same as those described by Amir [2].

Heteroduplex formation was induced by heat denaturation of PCR products at 94°C for 5 min, followed by gradual reannealing from 94 to 25°C for over 45 min. For the analysis of \textit{MECP2}, the PCR products of two patients were mixed before heteroduplex formation.

DHPLC analysis was performed with the WAVE DNA-fragment analysis system (Transgenomic, Omaha). Heterozygous profiles were detected as distinct elution peaks, in comparison to homozygous wild-type peaks. The PCR products that showed the heteroduplex by DHPLC were sequenced using Thermo Sequenase II dye terminator cycle sequencing premix kit (Amersham Pharmacia Biotech, Buckinghamshire) and an ABI 373 DNA sequencer (Applied Biosystems).

This study was approved by the Ethics Committee for research at Jichi Medical School.

3. Results and discussion

The screening results of each gene are summarized in Table 2.

3.1. MECP2

Three base changes, A201V, G232A, and E397K, which were missense mutations located in exon 4, were detected only in one patient each, but they have already been reported as polymorphisms. It was also reported that no mutations in the coding region of \textit{MECP2} were detected in autistic patients [8]. \textit{MECP2} may contribute to the occurrence of autism in some patients, but it is unlikely that this gene plays a major role in the etiology of autism.

3.2. MBD1

One patient (Aut34-1) had a novel missense mutation, C805T, which introduced an R269C amino acid change in exon 9. This base change was not detected in the 151 control individuals. This patient was a 4-year-old boy with autism and mild MR (his DQ was almost 60).
His father with some autistic phenotypes, and normal sister (6-year-old) had this base change. This boy had poor eye contact and communication difficulties, and spoke only a few words. His visual perception was better than his verbal perception. He was attached to the number. His father had normal intelligence and did not fulfil the criteria for autism; however, he had difficulties in social interaction, such as poor eye contact. He did not play with friends during his childhood. This changed base is not a conserved residue but is located in close proximity (10 bp) to the second CXXC region (Fig. 1), which also has a co-effect on MBD1 binding to methylated DNA [9]. This resulted in the addition of cysteine near a cysteine rich region. Therefore, it is possible that MBD1 with the R269C base change is related to autistic spectrum disorders. The fact that this patient’s asymptomatic sister was also heterozygous with R269C may be due to the involvement of other genes, or differential gene expression between males and females. Mbd1 knockout mice showed neurological abnormalities such as decreased neurogenesis, impaired spatial learning, and a significant reduction in long-term potentiation in the dentate gyrus of the hippocampus [10]. The relation between MBD1 and autism needs to be further analyzed.

3.3. MBD2

We found two kinds of repeat-length polymorphisms in exon 1. In one patient a (GGGGCC)2 to 3 change occurred that induced the insertion of glycine and arginine in the GR-repeat region. The other is the (GGC)4 to 5 change that caused the insertion of one glycine in four patients. The prevalence of both the expanded alleles was not different between autistic patients and the control population. However, this region of the gene may have some unstable factors because there are two repeat variations within a 100 bp interval in exon 1. An Mbd2 knockout mice study showed a remarkable deficit in maternal behavior [11], which is disturbed in autism. However, we could not detect the relationship between the MBD2 and autism.

3.4. MBD3

We detected no disease relating mutation. In each gene, we detected some polymorphisms in the exons and introns. Most base changes in introns are located near the exon/intron boundaries (from 8 to 74 bp), which are highly conserved sequences between human and murine homologs. The highly conserved sequence within noncoding human
and mouse DNA may serve important functional roles, such as regulating gene expression. Whether the high-polymorphic changes in these regions are involved in the MBD gene regulation is not yet clear, but the fact that MBDs are highly polymorphic suggested the possible involvement of SNPs of these genes in the pathology of autism. Furthermore, these data are useful for linkage analysis.

From our results, it is unlikely that the MBD genes are responsible for the etiology in the majority of autism patients. However, it is possible that in some autistic patients, the alteration of the MBD genes may contribute, at least partly, in expressing the autistic phenotype, as shown by the R269C mutation in MBD1 and the highly polymorphic nature of the MBD genes.

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### Fig. 1. Protein alignment of mouse and human MBD1.

The arrow indicates the mutated amino acid (R269C) in one patient. The residues underlined with CXXC show cysteine-rich domains.
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References


