Case Report: A Novel Mutation in the MECP2 Gene in a Korean Patient with Rett Syndrome

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Abstract. Rett syndrome (RTT) is a severe X-linked dominant neurodevelopmental disorder characterized by apparently normal psychomotor development during the first 6 months of life, followed by gradual loss of acquired skills, deceleration of head growth, initiation of stereotypic hand movements, and autistic behavior by the age of 3-4 years [1]. RTT affects about 1 in 10,000 to 15,000 new-born females [2], and there is wide variability in the rate of progression and severity of the disease. Clinically, the disease is present in either classical or variant forms [3].

Mutations in the methyl-CpG-binding protein 2 (MECP2) gene, which is located on chromosome X (Xq28), have been found in the majority of patients with RTT [4]. MECP2 is thought to selectively bind methyl-CpG islands in the mammalian genome, functioning as a regulator (mostly a repressor) of gene expression [4]. MECP2 seems to function in neuronal maturation and synaptic transmission [5-8], but it is unknown how the loss of MECP2 functions causes the RTT phenotype [9].

Sporadic cases of RTT are the rule; 99.5% of cases are single occurrences within the family [10]. To date, more than 400 different mutations in the MECP2 gene have been reported to the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/all.php) and most are gross deletions or missense/nonsense mutations.

We report a Korean RTT patient with a novel MECP2 mutation. According to molecular genetic testing of the MECP2 gene, the patient had a deletion of 41 base pairs in exon 4, causing a premature stop codon.

Introduction

Rett syndrome (RTT) is a severe X-linked dominant neurodevelopmental disorder characterized by apparently normal psychomotor development during the first 6 months of life, followed by gradual loss of acquired skills, deceleration of head growth, initiation of stereotypic hand movements, and autistic behavior by the age of 3-4 years [1]. RTT affects about 1 in 10,000 to 15,000 new-born females [2], and there is wide variability in the rate of progression and severity of the disease. Clinically, the disease is present in either classical or variant forms [3].

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The institutional review board approved this study.

**Materials and Methods**

Genomic DNA was isolated from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit according to the manufacturer’s protocol (Promega, Madison, WI, USA). The *MECP2* gene was amplified via PCR using the appropriate primers as designed by the authors and a thermal cycler (Applied Biosystems, Foster City, CA, USA). Bi-directional sequencing of all three coding exons along with the flanking intron regions of the *MECP2* gene was performed twice with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in conjunction with an ABI Prism 3100 automated genetic analyzer (Applied Biosystems).

**Results**

Direct sequencing analysis of the proband revealed a heterozygous 41-bp deletion (c.1152_1192del41) in exon 4 of the *MECP2* gene, which resulted in a frameshift leading to premature termination of the 487 amino acid protein at the 390th codon (Fig. 2). This mutation was absent in the proband’s parents and in 100 control chromosomes. We also identified a missense mutation (c.201C>T) previously described as a polymorphism (rs61748381) in exon 4 of the *MECP2* gene.

**Discussion**

Mutations in the *MECP2* gene on chromosome Xq28 have been shown to be the cause of RTT [4]. According to the literature, mutations are found in more than 80% of classical RTT cases and in more than 40% of variant cases [11]. Human *MECP2* consists of 4 exons, and the majority of causative alterations (including missense, nonsense, and frameshift mutations) occur in exons 3 and 4 [12]. Exons 3 and 4 encode the *MECP2* functional domains: MBD (methyl-CpG binding domain), capable of recognizing and physically associating with methylated CpG islands, which are known to be abundant in gene promoter regions; and TRD (transcription repression domain), responsible for the interaction with co-repressor mSin3A and histone deacetylases. The C-terminal region located downstream of the TRD is prone to deletions of various sizes resulting in the production of truncated proteins [13].

The patient described in this study appeared to have suspicious phenotypes of RTT according to international criteria [3] so we sequenced her *MECP2* gene. We identified a novel heterozygous 41-bp deletion (c.1152_1192del41) and a missense mutation (c.201C>T) previously described as a polymorphism (rs61748381) in exon 4 of the *MECP2* gene. The novel mutation resulted in a frameshift leading to premature termination of the 487 amino acid protein at the 390th codon. The c.1152_1192del41 mutation is located in the C-terminal region of the *MECP2* gene, which is in the C-terminal segment downstream of the TRD [14]. The C-terminal segment shares...
homology with neuronal-specific transcription factors, suggesting that the protein may have additional, more complex, and possibly neuron-specific functions [15]. This region also facilitates binding to the nucleosome core [16]. The C-terminal region is prone to deletions of various sizes, and deletions in this region represent about 10% of RTT genotypes [17,18].

The association between the severity of RTT phenotype and X-chromosome inactivation (XCI) has been studied. XCI is the process by which one of the two X chromosomes carried by females is inactivated to achieve gene expression patterns similar to those found in males, who carry only one copy of the X chromosome [19]. The RTT phenotypes in females are different according to the degree of inactivation between the mutant and wild-type alleles [19-22]. However, recent studies show that skewed XCI is insufficient to explain the phenotypic manifestations of RTT [19,23-24].

Another factor affecting the severity of phenotype is the mutation site. Hoffbuhr et al [25] reported that patients with missense mutation within the MBD and mutations truncating the entire TRD had more severe clinical presentations compared with patients with missense and nonsense mutations within the TRD and frameshift deletions within the C-terminus. A recent study showed that overall severity of C-terminal cases appears to be in the middle of the range, significantly more severe than those with the mildest mutation, p. R133C, and yet significantly milder than the most severe mutations, p. R270X [26]. Smeets et al [17] studied 10 RTT female patients with deletions in the C-terminal region and found that despite an atypical or “milder” course, these patients eventually develop symptoms clinically recognizable as RTT.

The main symptom is gradual decline of gross motor ability and rapidly progressive spine deformation despite preservation of simple communicative and cognitive abilities. Recently, Bebbington et al [26] studied 832 RTT patients and determined that the cases with C-terminal deletions were more likely to have normal head circumference, weight, and ambulation.

The mutation types are also associated with the severity of RTT phenotype. Patients with truncating mutations have higher incidence of awake respiratory dysfunction [27] and severe disease [28] compared to patients with missense mutations. However, extremely late truncating mutations (1152 del44bp and 1157 del41bp) had been excluded prior to this analysis [28]. Bebbington et al [26] reported that the frame shift C-terminal deletions were less severe than the in-frame C-terminal deletion. So correlation of phenotype and extremely late truncating mutation due to frame shift C-terminal deletion, as in this case, remains uncertain.

In order to confirm the presence of germline mosaicism, DNA analysis must be done on the parents’ sperm or oocyte. We do not know whether the mutation in our patient is de novo or not, because we could not test the sperm or oocyte due to lack of specimens. Further studies are needed to evaluate the possibility of germline mosaicism in order to determine whether this mutation is de novo or inherited.

References

13. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone


