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Spectrum of *MECP2* mutations in Iranian Azeri Turkish Rett syndrome patients

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Abstract

Rett syndrome is an X-linked dominant neurodevelopmental disorder that occurs mostly in females. De novo mutations in the *MECP2* gene have an important role in the appearance of the features of this syndrome. We planned to study spectrum of *MECP2* mutations in Rett syndrome patients and their clinical symptoms. A cohort of 29 patients referred by neurologists from Iranian Azeri Turks was screened. Then direct sequencing was utilized to characterize the spectrum of mutations in the *MECP2* gene in Rett syndrome patients. A total of 10 different mutations on *MECP2* gene were detected in 22 patients. We identified 2 (9%) frameshift, 10 (45.64%) nonsense, 8 (36.4%) missense mutations, and 2(9%) large deletions. In this cohort, one of the detected deletions was novel, namely 1023-1096del74nt. Random X chromosome inactivation in females' cells and different *MECP2* mutations can cause a phenotypic variability between patients. This is the first report regarding the spectrum of *MECP2* mutation and phenotypic spectrum in Iranian Azeri Turks with Rett syndrome. Our finding confirms a high mutation frequency (75.8%) of *MECP2* gene in Iranian Rett syndrome patients.

Keywords: Iranian Azeri Turks; *MECP2* gene; Novel Mutation; Rett syndrome; X-linked disorder

INTRODUCTION

Rett syndrome (RTT) is a severe neuro-developmental X-linked dominant pervasive developmental disorder that occurs mostly in females. After Down syndrome, RTT is thought the most common genetic disorder causing mental retardation in girls.[1] The prevalence of RTT has been estimated nearly 1 in 10,000 to 1 in 15,000 girls worldwide in all ethnic groups. So far, there is no reported treatment for RTT. Affected patients seem to have initial normal development for the first 6-18 months, but then they experience loss of speech and purposeful hand use, stereotypic hand movements, deceleration of head growth, seizures, autistic features, and breathing problems.[2] Because of random X chromosome inactivation (XCI) and a mixture of cellular mosaicism, which causes expression of either the normal or mutant version of an X-linked gene in females, there are different severity and manifestation among RTT patients.[3] The most common reason for RTT is due to de novo mutations of *MECP2* (Methyl-CpG binding protein 2) located on Xq28. *MECP2* is a four-exon gene, expressed almost in all tissues; however, a most essential gene for nerve cells functions. *MECP2* has multiple isoforms, and the one expressed in the brain lacks exon 2.[4] The encoded protein, *MECP2*, has two important domains including, methyl-binding domain (MBD) and transcription repression domain (TRD); function of MBD is to binding methylated CpG's, and TRD is responsible for recruiting other repressors proteins. Since the recognition of *MECP2* mutations as the leading cause of RTT, more than 200 different mutations have been identified.[5] Notably, *MECP2* duplication syndrome has been reported in males in which duplication of Xq28 (involving the *MECP2* gene) is accompanied with severe developmental delay. In spite of RTT, *MECP2* duplication syndrome is gain-of-function mutations condition.[6] Therefore, both deficiency and excess dosage of the *MECP2* gene lead to neurological disorders.

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The objective of this study was to investigation of the spectrum of *MECP2* mutations in Iranian Azeri Turkish patients. Given that some mutations may have a different modulatory effects on the severity and manifestation of RTT[7], characteristics and clinical features of patients along with their mutations are also investigated.

METHODS

Subjects

All patients screened in this study were sporadic cases. A total of 29 patients (female) with RTT originated from the Iranian Azeri Turk ethnic group were analyzed in this study. All the children were diagnosed with classical RTT according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) (DSM IV) by a neurologist specialist.

MECP2 gene mutations

Participant parents were informed about the study, and consent was obtained from them. Peripheral blood samples were collected from the patient, and genomic DNA was extracted from peripheral blood leukocytes using standard protocols. PCR amplification of the four coding exons was carried out using gene-specific primers. The exon 4 was amplified in a total of five overlapping fragments. PCR products were sequenced using gene-specific primers on ABI3730XL DNA sequencer (Macrogen, Korea). Sequence alignment was carried out using DNAMAN program several times. To evaluate the homology of the deleted region with other species, several sequence alignments re-claim from NCBI database, and those include amino acids sequences of Macaca mulatta, simum simum, Jaculus jaculus, Mus musculus, Saimiri boliviensis, and Papio Anubis were carried out. All the patients were followed up for several years.

RESULTS

Genetic test

Mutations in the coding sequence of the *MECP2* gene in 22 of 29 patients were identified by sequencing. A total of 10 different mutations was detected. We classified the patients into 4 mutations groups: 10 (45.6%) nonsense, 8 (36.4%) missense mutations, 2 (9%) frameshift, and 2 (9%) large deletions. Altogether, missense and nonsense mutations have higher frequencies in this cohort (Figure 1). R106W and T158M are located in MBD, R306C, R270X and R255X are located in TRD, and R168X is located in the inter-domain region. Among the frameshift mutations, 806delG and 695delG caused one nucleotide deletion, and the large deletion (1023del74nt and 1156del 44nt) showed a defective sequence. 1023del74nt was a novel mutation identified in a girl who was deceased at 9 years old. It was observed that the deleted region in the novel mutation is well conserved (96%) among mammalian species. The novel mutation, 1023-1096del74nt (cctgggcggaaaagcaaggagagcagccccaaggggcgcag cagcagcgcctcctcacccccaagaaggagca), causes deletion of 25 amino acid residues at C-terminal domain of the protein. The clinical features of this girl were as follow: abnormal head circumference and seizure (under control), unable to walk and speak; however, her growth and nutrition were normal. Deletion of 44nt at 1157-1200, located at C-terminal domain, was found in a 19-year-old girl. This girl is not able to walk and hold things by hands. She has lost her verbal expressive language and also suffers from seizure. Surprisingly she has a brother with more severe symptoms; though, no mutation was identified in his *MECP2* gene. The distribution and frequencies of identified mutations in the *MECP2* gene along the coding sequence are depicted in Figure 1.

Characteristics and clinical features of patients

Our dataset consists of 29 patients followed up for the last 10 years. The average age of cases was about 12 ± 6.12 years, and the average age at diagnosis was about 16.3±8.88 months. There was no significant difference in clinical features between various mutations; this maybe because of our small group. Notably, all RTT patients, irrespective to their mutation types, have no speech ability. Summary of the clinical features are shown for 22 patients in Table 1.

DISCUSSION

In this study, we searched for mutations by sequencing the *MECP2* coding region in 29 sporadic Iranian Azeri Turkish RTT patients. Iran consists of the different ethnic groups, and 15–20 million Azeri Turks living in northwestern Iran. They are ethnically identical to Azeri and closely related to Turks, and consisting about 25% of the Iran population. Disease-causing mutations in this study were detected in 22 (75.8%) patients, which is a rather same percentage of mutation in comparison to other studies were reported by

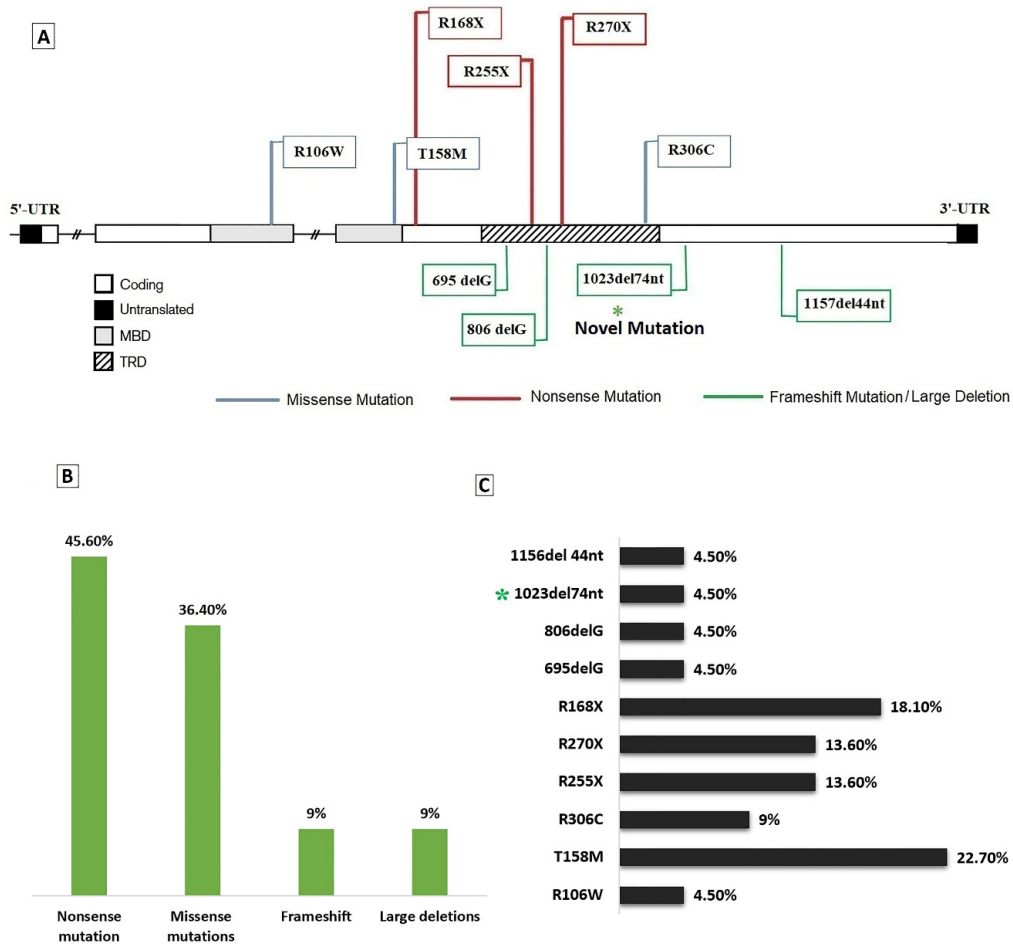

Figure 1. Distribution and frequencies of the identified mutations in the MECP2 gene containing exon 2, 3, and 4 (the entire coding region of the frequent MECP2 isoform) in Azeri Turk's RTT patients. A) Mutation bearing regions of the MECP2 gene in Azeri Turk's RTT patients, enriched in exon 4 and especially in the encoding region of TRD. Among the 10 identified mutations, 1023del74nt mutation has not been previously described. (R106, T158, R168, R255, R270, and R306 are because of C>T transition in CpG island of exon 3 and 4). B) Frequencies of the mutation groups. C) Frequencies of the different mutations.

Djarmati *et al*. in Siberian population (79%) and Chae *et al*. in Korean population (about 70%), and Dragich *et al*. in the USA (>65%).[8-10] However, the study of Matijevic *et al*. in Croatia population reported a lower percentage of mutation (about 47%).[11]

According to previous studies, point mutations include missense and nonsense mutations account for the major proportion of the detected mutations in RTT cases.[7,8,12] According to our knowledge, T158M, R168X, R255X, R270X, and R306C were the most frequent in all populations.[7-9,13] In the present study, the rate of nonsense and missense mutations was significantly high (0.45% for nonsense and 0.36% for missense). Six of these recurrent mutations (R106W, T158M, R306C, R168X, R255X, and R270X) involve C>T transitions at CpG dinucleotides. These C>T transitions mutations probably arose due to spontaneous deamination of methylated cytosine in transcriptionally inactive *MECP2* during spermatogenesis. A number of factors have been found to accelerate deamination- e.g., cytosine protonation is responsible for aberrant base-pair formation or base modification.[14]

In this cohort, one unknown coding-region mutation 1023-1096del74nt also identified. Sequence homology for 1023-1096 region

Table 1: Clinical features of Iranian Azeri Turk RTT patients along with their *MECP2* mutation

Mutation	Ability to walk	Hand skills	Speech	Seizures	Age	Unusual feature
Missense						
R106W	no	no	no	yes	14yr	
R306C	NA*	NA	NA	NA	NA	
R306C	NA	NA	NA	NA	NA	
T158M	On toes	no	no	yes	16yr	No perception and scoliosis
T158M	no	no	no	yes	16yr	Sleep disturbances and scoliosis
T158M	yes	no	no	no		No perception
T158M	On toes	no	no	yes	7yr	Heel bended, finger bent, and stereotypic hand movement
T158M	yes	yes	no	no	1/5yr	
Nonsense						
R255X	NA	NA	NA	NA	26yr	
R255X	yes	no	no	no	14yr	Sleep disturbances and abnormal respiratory
R255X	yes	no	no	no	2yr	Sleep disturbances and no perception
R270X	NA	NA	NA	NA	9yr	
R270X	yes	no	no	yes	Death at 9yr	
R270X	NA	NA	NA	NA	NA	
R168X	NA	NA	NA	NA	11yr	
R168X	yes	no	no	yes	16yr	Abnormal respiratory and scoliosis
R168X	yes	no	no	no	2yr	Sleep disturbances and abnormal respiratory
R168X	yes	no	no	no	2yr	Sleep disturbances and abnormal respiratory
Frameshift						
806 delG	yes	no	no	no	12yr	
695 delG	yes	no	no	no	24yr	Disrupt brain maturating
Deletion						
1157-1200 44nt	yes	no	no	yes	19yr	Sleep disturbances
1023-1096 74nt	yes	no	no	yes	Death at 9yr	Abnormal head circumference

NA: not available

revealed that their encoded amino acids (PGPKSKESSPKGRSSSASSPPKKEH) are well-conserved (96%) across different mammalian species. As Guy et al. reported, large deletions in *MECP2* occur in 37.7% of classical RTT and 7.5% of atypical RTT.[15] In our study, 9% of mutations were categorized in large deletion mutations group, and both of them are located in the C-terminal of the gene. In the previous study,[15] it has been presumed that mutations in

the C-terminal region of the protein could lead to mild symptoms; however, in this novel mutation the symptoms were so severe that resulted in death of the affected child.

In the present small cohort, the mutations of T158M with a frequency of 22.7% and R168X with a frequency of 18.1% were found to be the most common compared to other mutations. Reports from USA population[8] and Chines population[13] have also shown similar frequencies as ours. Whereas, in a study performed by Fong *et al*. on the Malaysian population, the absence of these mutations in their cohort was reported.[16]

The phenotypic variations have been seen among patients with the same mutation. This could be due to random X inactivation or unknown reasons, which could modulate clinical symptoms.[1] However, Caffarelli *et al*. showed that R168X, R255X, and R270X mutations are more severe mutations and considered to have more effect on the deterioration of bone status, e.g., inability to walk and loss of hand skills.[7] In accordance with the aforementioned study, patients of our study who have each of the nonsense mutation also lost their hand movements. In the seven RTT patients, no mutation in the *MECP2* gene was detected; therefore, the phenotypes could be due to mutation in other RTT-related genes, which need to be identified in the future.

Taken together, our study represents the first spectrum of *MECP2* mutations in Iranian Azeri Turkish patients with RTT. Based on our results, a high frequency of mutations occurred in the *MECP2* gene, especially in its 4th exon. Here also, we report a novel mutation located in 4th exon, accompanied by detailed clinical features of patients adding to our knowledge of genotype-phenotype correlations in RTT.

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DISCLOSURE

Conflict of interest: None

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