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 neurodevelopmental 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.
The objective of this study was to investigate
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, 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 gene-specific primers of 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 (1023del174nt and 1156del
44nt) showed a defective sequence. 1023del174nt
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, 1023-1096del174nt
(ctgggcggaaagcaaggacagccccaaaggggccag
cagcagcgcctctcctccaccccccagggagaca), 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%). However, the study of Matijevic et al. in Croatia population reported a lower percentage of mutation (about 47%).

According to previous studies, point mutations include missense and nonsense mutations account for the major proportion of the detected mutations in RTT cases. According to our knowledge, T158M, R168X, R255X, R270X, and R306C were the most frequent in all populations. 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.

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

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

NA: not available

revealed that their encoded amino acids (PGPKSKESSPKGRSSASSPCKKEH) 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. 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, 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 and Chinese population 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.

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. 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. 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 phenotype correlations in RTT.

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DISCLOSURE

Conflict of interest: None

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