Molecular Genetic Analysis of FOXL2 Gene in Two Iranian Families with Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome

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Abstract

Background: Blepharophimosis-ptosis-epicanthus syndrome (BPES) is a rare genetic disorder with autosomal dominant inheritance. There are two distinct phenotypes: BPES type I, which is associated with eyelid abnormalities as well as female infertility or premature menopause due to ovarian resistance to gonadotropins, whereas in type II only eyelid abnormalities are present. Mutations in the forkhead transcription factor 2 (FOXL2) gene are responsible for both types of BPES.

Objectives: The purpose of this study was to identify mutations in FOXL2 in two Iranian families (from Tehran) with BPES who were referred to Tehran Medical Genetics laboratory.

Methods: The peripheral blood was collected from the affected members of two BPES families and genomic DNA was extracted using salting out method. Then, direct sequencing of whole exon of FOXL2 gene was performed.

Results: Two frameshift mutations were identified in FOXL2 gene in two familial cases including NM_023067:c.102_103insA (p.G35Rfs*61) as a novel mutation and NM_023067:c.855_871dup (p.H291Rfs*71) (17-bp insertion). Both mutations cause the protein to be truncated and are responsible for a severe phenotype (BPES type I) which was in harmony with our finding.

Conclusions: Our results increased the spectrum of FOXL2 mutations and confirm the mutations associated with BPES type I.

Keywords: BPES, FOXL2, Premature Ovarian Failure, Eyelid Abnormalities

1. Background

Blepharophimosis ptosis epicanthus inversus syndrome (BPES; MIM 110100) is a rare genetic disorder with autosomal dominant inheritance (1). Its prevalence is estimated to be 1 in 50,000 (2). This disease has two types; type I is associated with eyelid abnormalities and premature ovarian failure (POF) and type 2 manifests only eyelid abnormalities. The penetrance of BPES type I and type II are 100% and 96.5% respectively (3). The major clinical symptoms in BPES are Blepharophimosis (narrow horizontal aperture of the eyelids), inverted inner canthal, ptosis (2). The other clinical features include low nasal bridge, hypoplasia of eyelid, strabismus, amblyopia, arched eyebrow and cupping ear (2, 4). Some of the patients with deletion in FOXL2 gene have mental retardation (5, 6). The mutations of FOXL2 gene which is located at 3q23 cause BPES disease (7). This gene has only one exon (FOXL2; MIM 605597) and encodes a forkhead transcription factor with two domain regions comprising of a hundred amino acids DNA binding forkhead domain and a polyalanine domain of fourteen alanines (8). The FOXL2 gene is expressed in the developing stage of eyelid formation and fetal and adult ovaries (9-11). It has an important role in follicular development (8, 9, 12, 13). Recent investigations suggest a wider spectrum of tissues in which FOXL2 is expressed such as pituitary, macrophages, blood reticulocytes, hepatocytes, colon and heart (14). Infertility has a different range in affected females of type I (15, 16). These females have the normal ovaries and gonads but have the disorder of the hypothalamic pituitary-ovarian axis that causes their ovarian failure (8, 12). Defect of FOXL2 may result in large cyst and corpus luteum cyst in patients (17). High levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH),are associated with these defects (8). Genotype phenotype correlation studies revealed that haploinsufficiency of FOXL2 causing by deletion of one allele results in type I and expression of extended proteins causes type II (8). In this study, the FOXL2 gene mutations were investigated in two Iranian BPES families.
2. Methods

Patients: Two families with BPES syndrome were included in this study. In the first family, there were six male patients with eyelid abnormalities in three generations (Figure 1). The second family had eight patients (three males and five females) in three generations with eyelid abnormalities observed in all, and infertility in the female patients. Examination by the ophthalmologist was performed for all the patients following a written consent for taking part in this research. This study was approved by the local ethics committee.

In the first familial case all of the affected members had the disorder’s major traits including microphthalmia, blepharophimosis, ptosis, epicanthus inversus, telecanthus, strabismus and high arch eyebrow. In the indivial III-2, mental retardation was also seen.

In the second familial case, all of the patients had microphthalmia, blepharophimosis, ptosis, epicanthus inversus, telecanthus and low set ears. The female patients in second familial case had either primary amenorrhea or developed premature ovarian failure (POF) later in life. The proband and her sister had high levels of FSH, LH and decreased serum concentrations of estradiol and progesterone.

Molecular study: Sampling was performed in Tehran Medical Genetics Laboratory. Five mL peripheral blood was taken in EDTA tubes from each patient, and DNA was extracted from leukocytes of peripheral blood by standard salting out method (18). Because the FOXL2 gene has only one long exon, the primers were designed to amplify the six overlapping fragments of this exons (Table 1). Primer designing was performed by using Gene Runner software and primer blast web site. PCR reactions carried out in 30 µL containing 100 ng DNA, 3 µL PCR Buffer, 0.25 mmol/L of each dNTP, 5 µL GC-rich solution from Roche, 0.2 unit Taq DNA polymerase and 50 µmol/L of each primer. Cycling conditions included 95°C for 5 minutes, 30 cycles of 95°C for 30 seconds and 50 µmol/L of each primer. Cycling conditions included 95°C for 5 minutes, 30 cycles of 95°C for 50 seconds, annealing temperature (Table 1) for 50 seconds, and 72°C for 1 minute, followed by 72°C for 10 minutes. Sequencing was performed using BigDye Terminator v1.1 Cycle Sequencing Kit on ABI 3130 genetic Analyzer and the data was compared with FOXL2 reference sequence (NG_029796.1).

3. Results

Cycle sequencing revealed MN_023067:c.102_103insA (p.G35Rfs*61) novel mutation in FOXL2 gene in the patients of the first family (Figure 2). This frameshift mutation results in stop codon at amino acid 95 and causes truncation of FOXL2 protien and BPES type 1 phenotype.

In the second family, a 17 bp duplication NM_023067:c.855_871dup (p.H291Rfs*71) was detected in the patients (Figure 3). This frameshift mutation results in stop codon at amino acid 361 and causes truncation of FOXL2 protien and BPES type 1 phenotype.

4. Discussion

FOXL2 gene encodes a human protein composed of 376 amino acids with two domains: DNA binding forkhead domain (52 - 152 residues) and polyalanine domain (221 - 234 residues) (19, 20). So far, many different mutations have been reported for this gene, which were classified into seven specific groups by De Bae and his colleagues (21). Group A lacks entire forkhead domain; Group B lacks part of the forkhead domain; In group C, the forkhead domain is intact whereas the polyalanine domain is lacking; Group D has both forkhead and polyalanine domains but is truncated and has lost part of its carboxyl end; Group E is similar to Group D in having intact forkhead and polyalanine domains but the proximal end of protein is elongated as a result of frameshift mutation; Group F contains in-frame alterations in the polyalanine domain with no length variation. The length of the protein is not altered in group F and G: the former contains in-frame changes, and the latter contains missense mutations. Another group includes variants with cytogenetic abnormalities (21).

Different molecular genetic testing are used to detect the mutations in FOXL2 gene. Seventy two percent of probands with a pathogenic variant are detectable by sequence analysis. Also Deletion/duplication analysis of FOXL2 gene and its upstream region contains the disease causing mutations in 10%-15% and 5% of cases respectively (22).

In this study, we used cycle sequencing which is an accurate method and given that the FOXL2 gene has only one exon, it is cost effective. Similar study was performed on an Iranian family with BPES type II by sequencing of FOXL2 coding region in which a heterozygous missense mutation was revealed (23).

Other methods such as array CGH are reported to have been used for detecting microdeletions covering the FOXL2 gene (24).

In this study we found two frameshift mutations in FOXL2 gene in two familial cases including NM_023067:c.102_103insA (p.G35Rfs*61) as a novel mutation and NM_023067:c.855_871dup (p.H291Rfs*71).

The novel mutation in the first family (c.102_103insA) causes a loss of forkhead and polyalanine domains (Figure 4B). This mutation results in changing amino acid composition from amino acid 35 and premature termination by a
The probands are shown by arrows. There are six male patients in the first family (A) and eight patients (three males and five females) in the second family (B) all with eyelid abnormalities. Also infertility was observed in all female patients in the second family (B). The provided patients’ figures are related to 3 males and 2 females in the first and second families respectively.

Table 1. PCR Primers for Six Overlapping Regions of FOXL2 Coding Exon and Their Annealing Temperature

| Primer Sequence (5′ → 3′) | Reverse Sequence (5′ → 3′) | Annealing Temperature, °C |
|---------------------------|---------------------------|--------------------------|
| GTGGAGCCCATACGAAACAG      | GTAGGAGTACGGGGCTTCT       | 58                       |
| CAGGCGGCTGAGGAGGAGG       | CGTGGCGGGCTGAAGTGC        | 65                       |
| GACCCGGGCGGGAAGGAGA       | GGCGCCGTCGCGATGGTGT       | 64                       |
| CCGGGGGGTGCTGCTACAGG      | CTCGGGGGGGCTGCTGTC        | 68                       |
| CCTCCTTIGCTCCCTAGTGA      | CGGTGTAACCGAGTACAGG       | 52                       |
| AGAAAGGGACGGCAAATAC       | ATTTATCGGAAATCCAGAAG      | 54                       |

The identified mutation in second family which was 17 bp duplication (NM_023067:c.855_871dup) although does not affect forkhead and polyalanine domains but results in changing amino acid composition from amino acid 291 onwards and premature termination by a stop codon at amino acid 361 leading to a truncated protein with short-
Figure 3. The nm_023067:c.855_871dup (p.H291Rfs*71) Mutation in the foxl2 Gene in the Patients of the Second Family.

Figure 4. Schematic Representation of the Mutant FOXL2 Protein with c.102_103insA (B) and c.855_871dup (C) Mutations in Comparison with the Wild Type Protein (A).

ened carboxyl end (Figure 4C) (25). Therefore, it belongs to group D. This mutation results in a proline-arginine rich domain at the carboxyl end of the protein affecting its three-dimensional structure, which disturbs its function (25) and causes a truncated FOXL2 protein which is associated with BPES type I (8, 25, 26).

All females in the second family were infertile. The exact mechanism leading to infertility in females with BPES type I is unclear, but it is believed that this is caused by abnormalities in hypothalamic pituitary ovarian axis whose outstanding symptom is increased plasma level of FSH and LH in females of BPES type I (16), which was also observed in our patients.

In conclusion, in the present study, we identified mutations in two extended Iranian families spanning three generations with BPES type I. The NM_023067:c.102_103insA (p.G35Rfs*61) mutation is a novel mutation which is submitted to ClinVar (Submission accession: SCV000328205.1) and NM_023067:c.855_871dup (p.H291Rfs*71) mutation is the first report of BPES type I in Iran. The mutations found in this study demonstrate a genotype-phenotype correlation. Our results may help patients and genetic counselors for disease management and prenatal diagnosis. In cases the cycle sequencing of FOXL2 gene does not reveal any disease causing mutation, deletion/duplication analysis of FOXL2 gene and its upstream region is suggested.

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