Case Report:
A Novel Frameshift Mutation in the EYA1 Gene in a Korean Family with Branchio-Oto-Renal Syndrome

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Abstract. Branchio-oto-renal (BOR) syndrome is an autosomal dominant disorder characterized by branchial cleft fistulae or cysts, preauricular pits, ear malformations, hearing loss, and renal anomalies. Mutations in the human homologue of the Drosophila eyes absent gene (EYA1) are the most common cause of BOR syndrome. In this study, we found a Korean family showing clinical features of the disease. Mutation analysis of the EYA1 gene revealed a novel one-base-pair deletion resulting in truncated protein (c.321delT; p.Ala107fs). This is the first report of BOR syndrome caused by deletion mutation of the EYA1 gene in Korea.

Keywords: branchio-oto-renal syndrome, EYA1 gene mutation

Introduction

Branchio-oto-renal (BOR) syndrome (MIM# 113650) is an autosomal dominant disorder, associated with branchial cleft fistulae or cysts, preauricular pits, ear malformations, hearing impairment, and renal anomalies [1]. The incidence of BOR syndrome is approximately 1:40,000 and may account for 2% of children with profound hearing loss [2].

Mutations in the EYA1 gene, the human homologue of the Drosophila eyes absent gene, have been shown to cause BOR syndrome [3]. The EYA1 gene is composed of 16 exons spanning 156 kb on chromosome 8q13.3 [4]. Four different spliced transcripts have been identified [5]. The EYA1 gene is a member of the EYA family that is characterized by a divergent N-terminal activation domain and a conserved C-terminal Eya domain [3] and functions as transcriptional co-activator in the Eya-Six regulatory network for early development of different organs, including the ear and kidney [6]. Over 80 different disease-causing mutations of the EYA1 gene have been reported in various populations [7]. However, only a few mutations have been identified in Korean BOR families and all of them were private [8-10]. Here we report a novel frameshift mutation of the EYA1 gene for the first time in a Korean family with BOR syndrome and we describe the clinical features of the family members.

Case Report

The patient was a 20-yr-old man showing the 4 major common phenotypic features of BOR syndrome: preauricular pit, branchial fistula, hearing loss, and renal anomaly. His serum urea and creatinine levels were normal, but abdominal ultrasonography revealed ureteropyelojunction obstruction (Fig. 1A) and a left pyeloplasty was performed. Physical examination showed a preauricular tag and pit on the left side (Fig. 1B). The right ear had a narrow external auditory canal; the tympanic membranes appeared normal on both sides. Pure tone audiometry (PTA) showed mixed type hearing loss (Fig. 1C) and the patient wore a hearing-aid on the left side. Temporal bone computed tomography (CT) showed a small middle ear cavity and absent ossicles on the right side, laterally attached ossicles on the left side, and
hypoplastic cochlea on both sides. The patient underwent surgery for branchial fistula on the right side (Fig. 1D). Family history revealed that the patient’s parents both had severe hearing loss. His father had a preauricular pit and cervical branchial fistula with morphology similar to the proband, but he was not tested for renal abnormality. His mother had lost her hearing due to a febrile illness in childhood.

Methods

Blood samples were collected from the proband and his parents after obtaining informed consent and approval by the Institutional Review Board of Soonchunhyang University Hospital. Genomic DNA was extracted from peripheral blood leukocytes using a Wizard Genomic DNA Purification Kit following the manufacturer’s instructions (Promega, Madison, WI, USA). To see whether a mutation in the EYA1 gene caused the BOR syndrome in this family, the 16 exons of the gene were analyzed by direct sequencing. Each exon was amplified by PCR using the appropriate intronic primers (available upon request), designed by the Primer 3 program. The PCR products were purified and cycle sequencing was performed with an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) using an ABI3130XL Genetic Analyzer (Applied Biosystems). Mutation nomenclature was based on the EYA1 cDNA sequence of NM_000503.3 (http://www.ncbi.nlm.nih.gov).

Results

A novel one-base-pair deletion of thymidine was found at nucleotide position 321 in exon 6 (c.321delT) and the proband was heterozygous for this change (Fig. 2). This deletion causes a frameshift of amino acids and results in a truncated protein (p.Ala107fs). Further analysis of his parents’ samples revealed that the father had the same mutation, but the mother had wild type EYA1.

Discussion

BOR syndrome is characterized by a wide spectrum of clinical manifestations that represents a combination of branchial, otic, and renal anomalies [11]. Individuals with the branchio-otic (BO) syndrome (OMIM 602588) are affected by the same branchial and otic anomalies as in BOR without the associated renal findings [12]. Due to the wide spectrum of phenotypic findings and the phenotypic variability between and within families, phenotypic criteria for clinical diagnosis of BOR syndrome have been proposed [13]; the major criteria include branchial anomalies, deafness, preauricular pits, and renal anomalies, while minor criteria include external-, middle-, and inner-ear anomalies and preauricular tags. Positive diagnosis requires affected individuals to have ≥3 major criteria, or 2 major criteria and ≥2 minor criteria, or 1 major criterion and an affected first-degree relative with BOR syndrome. The proband described here has 4 major criteria and his father has ≥3 major criteria. There have been 2 other reports similar to the phenotypic characteristics of BOR syndrome in our case and 1 report of BO syndrome in Korea [8-10]. Compared to the other 2 cases with renal agenesis or hypoplasia, our case showed hydronephrosis.

Mutations in the EYA1 gene are the underlying genetic defects in approximately 40% of patients with BOR syndrome [13] and most have been identified in subjects of European ancestry. To date, 12 different mutations of EYA1 including 6 nonsense, 3 missense, 2 splicing site, and 1 frameshift have been reported in Japanese BOR/BO families [14-20]. In contrast, 3 mutations have been identified in Korean BOR/BO syndrome [8-10], and no mutations have been associated with BOR syndrome in Chinese populations. All these mutations were scattered through the EYA1 coding region; complex mutations involving large deletions or chromosomal rearrangements have not been found in Asians.

To determine whether there are ethnic differences in mutations of the EYA1 gene, more comprehensive studies of the EYA1 gene with large numbers of samples are needed. The results will have important implications for genetic testing in which assessing the ethnic origin may direct mutation analysis to specific regions of the gene. In addition, efforts should be made to develop more efficient screening methods for EYA1 and identification of other genes to increase the sensitivity of genetic testing for BOR syndrome. Recently, an additional locus associated with BOR syndrome was mapped on chromosome 14q23.1-q24.3, known as the SIX1 gene. This locus plays a role in EYA-SIX-PAX interaction in the development of the ear, kidney, and other organs [21]. Evaluation of SIX1 and its related target genes may provide
Fig. 1. The proband showed several clinical manifestations. (A) Kidney with markedly dilated pelvicalyceal system on the left side. (B) Preauricular pits and tag on the left side. (C) Mixed hearing loss on both sides. (D) Finding at operation of branchial cleft fistula on the right side.

Fig. 2. (a) The pedigree of the family with BOR syndrome. The arrow indicates the proband. The mother’s hearing loss is designated as normal since she lost her hearing due to a febrile disease in childhood. (b) A novel deletion mutation (c.321delT) in exon 6 of the EYA1 gene was identified in the proband and his father.

In summary, we have described a Korean family with BOR syndrome and identified a novel frameshift mutation caused by a one-base-pair deletion in the EYA1 gene. This report adds useful information regarding the genetic heterogeneity underlying this syndrome.
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References