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Novel *EYA1* Variants Causing Branchio-Oto-Renal Syndrome

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Abstract

Introduction—Branchio-oto-renal (BOR) syndrome is an autosomal dominant genetic disorder characterized by second branchial arch anomalies, hearing impairment, and renal malformations. Pathogenic mutations have been discovered in several genes such as *EYA1, SIX5*, and *SIX1*. However, nearly half of those affected reveal no pathogenic variant by traditional genetic testing.

Methods and materials—Whole Exome sequencing and/or Sanger sequencing performed in 10 unrelated families from Turkey, Iran, Ecuador, and USA with BOR syndrome in this study.

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Results—We identified causative DNA variants in six families including novel c.525delT, c. 979T>C, and c.1768delG and a previously reported c.1779A>T variants in *EYA1*. Two large heterozygous deletions involving *EYA1* were detected in additional two families. Whole exome sequencing did not reveal a causative variant in the remaining four families.

Conclusions—A variety of DNA changes including large deletions underlie BOR syndrome in different populations, which can be detected with comprehensive genetic testing.

Keywords

Branchiootorenal syndrome; *EYA1*; Branchial arch anomalies; Hearing Loss; Whole exome sequencing

1. Introduction

Branchio-oto-renal spectrum disorders include Branchio-oto-renal (BOR) syndrome and Branchio-oto syndrome (BOS). BOR syndrome is a rare genetic disorder characterized by a distinct phenotype including branchial arch anomalies, hearing impairment and renal malformations. In contrast, the absence of structural renal anomalies defines BOS. The prevalence of this spectrum disorder is estimated to occur in 1 out of 40,000 and accounts for 2% of childhood deafness [1]. Clinical manifestations include branchial clefts, cysts and fistulas. Otologic findings include preauricular pits and tags, auricular malformations, external auditory canal stenosis and atresia, and multiple middle and inner ear anomalies. Hearing loss is present in 90% of cases [2]. Renal anomalies include hypoplasia, dysplasia and agenesis. However, only 6% of those with renal involvement have severe clinical effects [3]. The clinical heterogeneity is due to variable expressivity amongst and within families.

A clinical diagnosis of BOR syndrome is based on family history and clinical features. In the absence of family history, three major criteria or two major and two minor criteria must be met. The major diagnostic criteria include deafness, preauricular pits, auricular malformations, renal anomalies, and second branchial arch anomalies. The minor diagnostic criteria include external auditory canal anomalies, middle ear anomalies, inner ear anomalies, preauricular tags, facial asymmetry and palatal abnormalities [4].

BOR syndrome is transmitted in an autosomal dominant manner. Mutations within the *EYA1* gene have been detected in 40% of those affected [4, 5]. *EYA1* functions as a protein phosphatase and as a transcriptional co-activator whose role is important during embryogenesis. Other pathogenic variants within the *SIX5* and *SIX1* genes make up 5% and 4% of cases, respectively [6]. The products of these genes interact with *EYA1* gene product directly, forming transcription factor complexes. Unfortunately, in the rest of the patients with a clinical diagnosis of BOR syndrome, no pathogenic mutation is detectable by traditional genetic testing.

This article describes the identification of three novel pathogenic variants in *EYA1*, and two copy number variant (CNV) deletions through the use of whole exome and Sanger sequencing.

2. Materials and Methods

2.1 Statement of Ethics

Participants enrolled in this study were approved by the IRB at the University of Miami, Growth and Development Research Ethics Committee of Iran, the Ethics Committee of Ankara University, and Bioethics Committee COBI-IRB of Ecuador. Blood samples were obtained from affected and unaffected individuals after informed consents were obtained.

2.2 Subjects

This study includes 10 unrelated families in which probands were clinically diagnosed with BOR syndrome. Families were from Turkey (7), Iran (1), Ecuador (1), and USA (1). In four of the 10 families, the proband was accompanied by at least one affected first-degree relative. Clinical evaluations were completed both by an otorhinolaryngologist and a clinical geneticist. Evaluations included a thorough family history, physical examination, renal ultrasound and hearing test. Blood samples were taken from probands, affected siblings and unaffected family members. DNA was extracted from peripheral leukocytes.

2.3 Genetic Analysis

Capture and sequencing was completed by Agilent SureSelect Human All Exon 50 Mb and a HiSeq 2000 instrument (Illumina) based on our previously published protocol [7]. Variants were called and filtered using online software as previously described [8]. CoNIFER (Copy Number Inference From Exome Reads) was used to identify CNVs from whole exome sequencing (WES) data [9]. TaqMan® Copy Number Assay (Probe: Hs02221533_cn, overlaps Intron 4-Exon 4) was performed to confirm the CNV deletions in *EYA1* (NM_000503.5) by using previous protocol [10]. Sanger sequencing was used for the confirmation and co-segregation of the *EYA1* in the families. In two families exome sequencing was not performed since causative *EYA1* variants were detected via Sanger sequencing.

3. Results

Eighteen individuals from 10 unrelated families were included in this study. Identified mutations and their segregation in each family are shown in figures 1 and 2 and the summary of clinical features are shown in Table 1.

Family 1374 included four affected family members. WES revealed a novel frameshift variant within *EYA1* c.525delT (p.G176Dfs*65) which was then confirmed by Sanger sequencing (Fig. 1A). Family 2103 included a 26 year-old male with two other affected family members. Sanger sequencing was used to reveal a heterozygous variant that changes the stop codon to tyrosine and extend the protein six amino acids, c.1779A>T (p.*593Yext*6) (Fig. 1B). This variant has been previously described to cause BOR syndrome [11]. Family 2353 had a 4 year-old male proband and an affected mother. Sanger sequencing showed a novel *EYA1* variant c.979T>C (p.W327R) (Fig. 1C). Both the proband and his mother were heterozygous for the variant. Family 2397 included a 2 year-old female proband and an affected mother. Sanger sequencing revealed a novel deletion variant c. 1768delG (p.E590fs*49) (Fig. 1D).

Family 528 included a 5 year-old male proband and an affected father. A ~1.97 Mb CNV deletion was revealed by WES which has been previously described [6] (Fig. 2A and C). The deletion was confirmed by TaqMan ® copy number assay qPCR (Fig. 2D). Family 954 included a 62 year-old male proband. A separate, smaller CNV deletion was revealed by WES and confirmed by TaqMan ® copy number assay qPCR (Fig. 2B, D, and E). This CNV has also been previously described [12]. The summary of mutation analysis and clinical phenotype is shown in (table 1) [13–16].

4. Discussion

BOR syndrome is a rare autosomal dominant disorder characterized by branchial fistulas or cysts, hearing loss and renal malformations. Clinical evaluation is complicated due to the presence of reduced penetrance and variable expressivity [17]. The responsible locus was first mapped to chromosome 8q13 [18–23]. Eventually mutations and deletions within the *EYA1* gene were identified as causal variants in the pathogenesis of BOR syndrome [24]. Over 100 causative variants including point mutations, small indels and CNVs have since been discovered [4, 11, 25–27].

In this study, a clinical evaluation followed by genetic screening was performed in patients affected by BOR syndrome. The clinical observations were consistent with characteristics previously reported and all patients included in this study fit clinical criteria for the diagnosis of BOR syndrome [4]. Genomic analysis revealed four molecular variations in the *EYA1* gene including two novel frameshift deletions, a novel missense variant, as well as one previously identified variant (Fig. 1). Additionally, two CNV deletions including *EYA1* were identified in two separate families via WES and later confirmed with TaqMan ® copy number assay qPCR. The molecular variations identified were distributed in the coding regions of *EYA1* and several flanking genes on chromosome 8.

In comparison to other reported mutations, evaluation of the novel mutations identified in this study appear to be consistent with the clinical findings of previously reported *EYA1* mutations associated with BOR syndrome. As the two novel mutations described here represent frameshift variants, there is a greater biological plausibility that these variants represent the genetic etiology for BOR syndrome in the affected patients as frameshift mutations result in radical alterations of the final protein structure. Additionally, the novel mutations identified in this study appear to be transmitted in an autosomal dominant inheritance pattern, consistent with BOR variants reported in the literature.

In four families no causative variants in *EYA1*, *SIX1* and *SIX5* were identified via WES. It is likely that variants that are located in uncovered regions of these genes or in novel genes are present in those families.

5. Conclusion

This study utilized genomic analyses to discover three novel variants within the *EYA1* gene of unrelated probands who were clinically diagnosed with BOR syndrome. In conclusion, the application of WES appears to be effective in the discovery of pathogenic variants for BOR syndrome.

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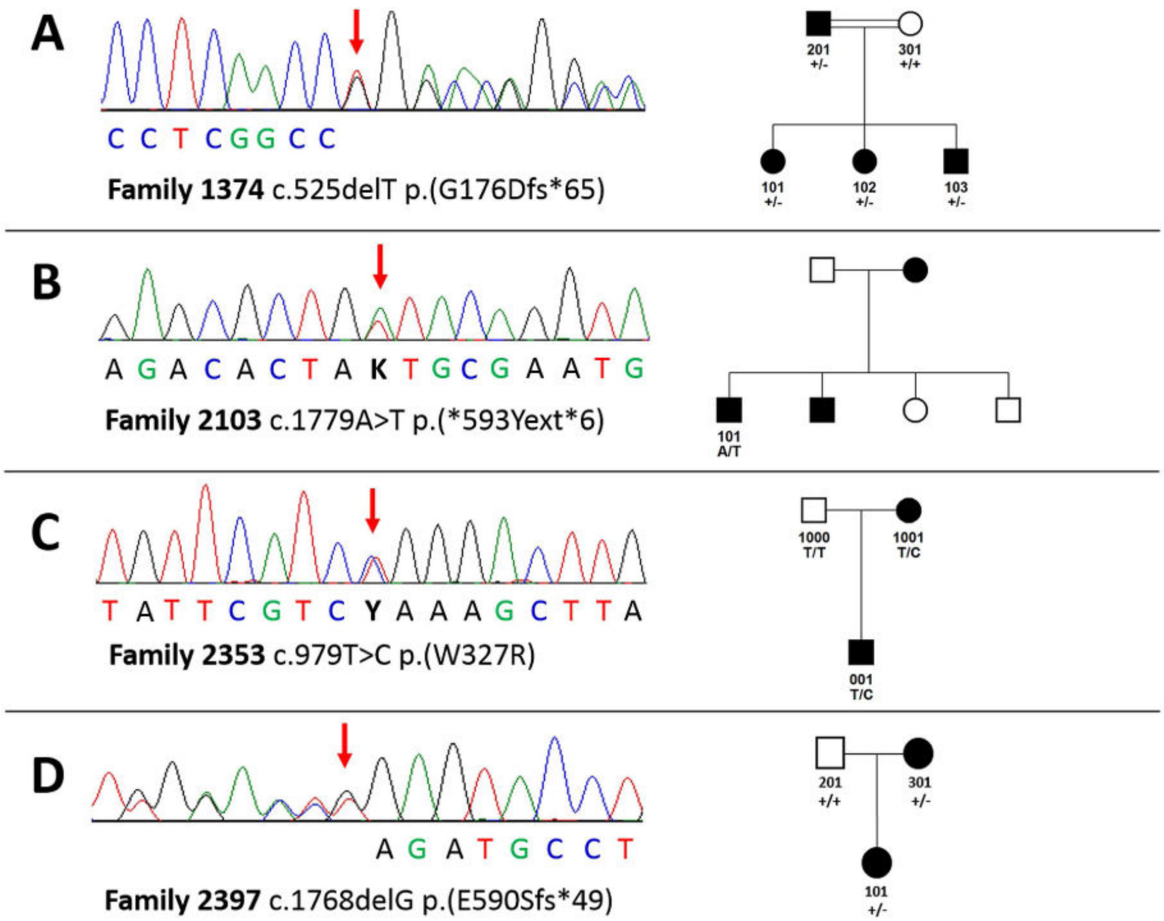

Figure 1.

Sanger sequencing results for four affected families confirming variants in *EYA1*. (A) depicts a novel variant representing a single-base deletion in Family 1374 at position 525 resulting in a frameshift mutation. (B) reveals a previously reported stop-loss variant in family 2103. (C) depicts a novel missense variant in family 2353. (D) an additional novel frameshift variant at position 1768 in Family 2397.

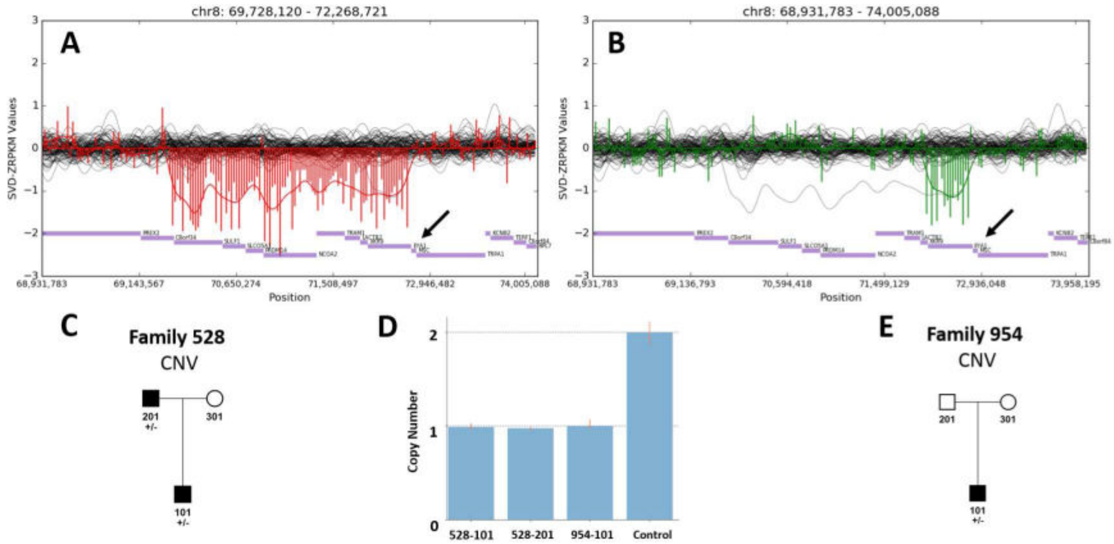

Figure 2.

Whole exome sequencing analysis reveals copy number variants (CNVs) encompassing the *EYA1* gene. (A,C) shows a large ~1.9Mb deletion affecting a contiguous segment of chromosome 8 that includes the coding region for *EYA1*. (B,E) shows a smaller deletion, ~336Kb affecting only *EYA1* and surrounding region. (D) CNV confirmation via TaqMan® copy number assay showing heterozygous loss of one copy of *EYA1* gene in families 954 and 528.

Table 1

Summary of clinical features of the probands.

Family	**528-101**	**954-101**	**2353-001**	**1374-101**	**2103-101**	**2397-101**	**Literature**
Proband Age/Gender	5/Male	62/male	4/Male	20/male	26/male	2/female	
Country of Origin	Turkey	USA	Ecuador	Turkey	Iran	Turkey	
# of affected in family	2	1	2	4	3	2	
EYA1 Variant	CNV het deletion (chr8:70476 154-72448242) (Hg19)	CNV het deletion (chr8:72111472-72448242) (Hg19)	c.979T>C p.(W327R)	c.525delT p.(G176Dfs*65)	c.1779A>T p.(*593Ye xt*6)	c.1768delG p.(E590Sfs*49)	
Method	WES/TaqMan	WES/TaqM an	Sanger Sequencing	WES/Sanger	WES/Sanger	Sanger sequencing	
Reference	Sanchez-Valle A (2010) [6]	Rickard S (2001) [12]	This Study	This Study	Rickard S (2000) [11]	This Study	
Major Criteria							
Second branchial arch anomalies	Right fistule	Bilateral	Left fistule		Bilateral	Left fistule	50–68.5%[4][12]
Deafness	Congenital, Bilateral, moderate	Congenital, mixed bilateral	Congenital, bilateral, Severe	Congenital	Congenital	Hearing loss	93–98.5%[4][12]
Preauricular pits	Bilateral pits	none	Left pit	Bilateral present	None	Bilateral pits	82–83.6%[4][12]
Auricular malformation	Bilateral prominent	Anteverted ears	Microtia Type 1		None	Malformed right ear	36–50 % [12][13]
Renal anomalies	Unilateral renal agenesis	Small cystic lesion on upper left lobe		Hypoplasic kidney	None	None	38.2–67%[4][12]
Minor Criteria							
External auditory canal anomalies		none			None	None	31.5–60%[4][13]
Middle ear anomalies		Bilateral Otosclerosis			None		25–50%[12]
Inner ear anomalies		Bilateral Mondini Malformation		Bilateral cochlear aplasia	None		25–66%[12][14]
Preauricular tags		none			None	Right ear	13% [12]
Other: facial asymmetry, palate abnormalities		Underdeveloped malar area, bilateral extra nipples, bracydactyly of digits				facial asymmetry, high arched palate	2–7% [12][15]