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Short Report

A novel nonsense mutation in the *EYA1* gene associated with branchio-oto-renal/branchiootic syndrome in an Afrikaner kindred

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Branchio-oto-renal (BOR) syndrome is an autosomal dominant disorder characterized by the associations of hearing loss, branchial arch defects and renal anomalies. Branchiootic (BO) syndrome is a related disorder that presents without the highly variable characteristic renal anomalies of BOR syndrome. Dominant mutations in the human homologue of the Drosophila eyes absent gene (*EYA1*) are frequently the cause of both BOR and BO syndromes. We report a South African family of Afrikaner descent with affected individuals presenting with pre-auricular abnormalities and either hearing loss or bilateral absence of the kidneys. Genetic analysis of the pedigree detected a novel *EYA1* heterozygous nonsense mutation in affected family members but not in unaffected family members or a random DNA panel. Through mutational analysis, we conclude that this particular mutation is the cause of BOR/BO syndrome in this family as a result of a truncation of the EYA1 protein that ablates the critical EYA homologous region. To the best of our knowledge, this is the first case of BOR/BO syndrome reported in Africa or in those of the Afrikaner descent.

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Branchio-oto-renal (BOR) syndrome (OMIM 113650) is an autosomal dominant developmental disorder of the kidney and urinary tract, accompanied with hearing loss (1) and presenting with a wide intrafamilial variability and variable penetrance (2). Branchiootic (BO) syndrome (OMIM 602588) is a related disorder that presents without the highly variable renal anomalies of BOR syndrome. However, it has been demonstrated that allelic variants of *EYA1* mutations can be the cause of both BOR and BO syndromes (3–6). It is estimated that BOR/BO syndrome occurs at a frequency of 1:40,000 live births and affects approximately 2% of profoundly deaf children (7).

EYA1 (NM_172060) (8q13.3) functions as a transcription cofactor in the conserved EYA-SIX-PAX developmental network (8). In mammals,

EYA1-mediated gene regulation is critical to the proper formation of many organ systems and tissues (4, 9–12) and is one of the earliest known markers of the metanephric mesenchyme (13). The EYA1 protein does not contain a known DNA-binding domain but does contain a highly conserved EYA homologous region (EYAhr), which is encoded by exons 9–16 of the gene (Fig. 1a). The EYAhr is necessary for the protein to bind with its respective binding partners, such as SIX1, in order to form transcription complexes and regulate transcription properly (9, 12, 14, 15). In humans, more than 50 mutations in the *EYA1* gene have been associated with BOR/BO syndromes (16), all of which seem to either impair the function of the EYAhr or ablate the region altogether.

We report the identification of a novel nonsense mutation as well as a novel single nucleotide polymorphism (SNP) within the *EYA1* gene, as found in a South African Afrikaner kindred. To the best of our knowledge, this is the first reported case of BOR/BO syndrome in Africa or in individuals of Afrikaner descent.

Materials and methods

Informed consent was obtained from all participants before enrolling into the study (University of Michigan IRBMED # 2004-0322). Informed consent documents were downloaded from our research group's Web site at www.kidneygenes.com by the family's physician.

EYA1 exons were amplified by PCR using methods previously reported by Abdelhak et al. (3). Heterozygous mutations were screened against a random panel of healthy controls utilizing capillary heteroduplex analysis as described by Hoskins et al. (17).

Maximum entropy (MaxENT) scores were determined to assess the strengths of the 5′ and 3′ splice sites (ss) of introns 7 and 15 of *EYA1* utilizing the MaxENTScan software available at http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq_acc.html (18).

Results

Family JCA7 is of Afrikaner descent and was identified during pre-natal ultrasound screening where the proband (III.3) was diagnosed as having oligohydramnios and bilateral renal agenesis/Potter's sequence. The family's medical review indicated a history of bilateral renal agenesis/Potter sequence in a previous pregnancy (III.1, unavailable for analysis). In addition, it was found that the father of the proband (II.3) had a unilateral pre-auricular pit (Fig. 2a) with an

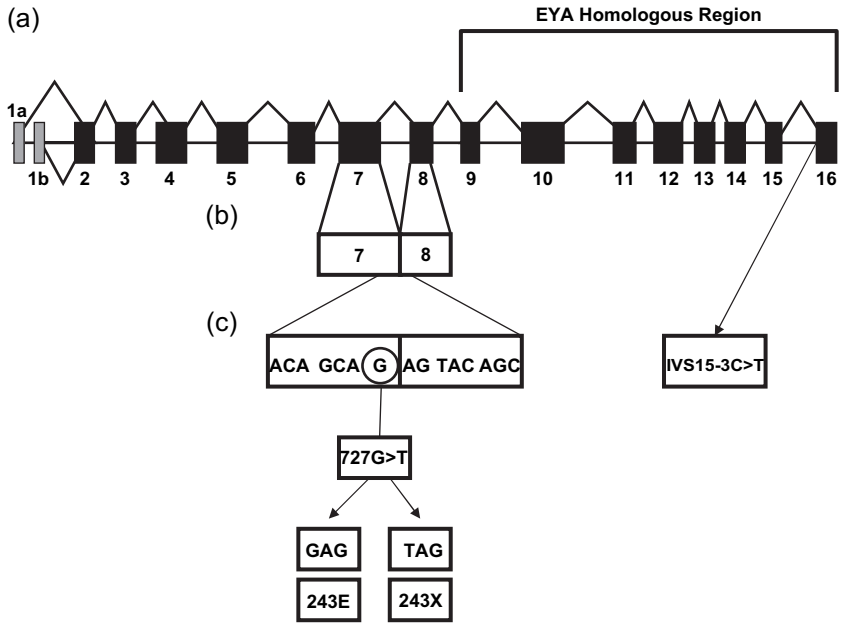

Fig. 1. (a) Schematic of the 156 kb EYA1 gene. Alternative first exons are denoted by the gray boxes and the black boxes denote exons 2–16. The EYA homologous region is encoded by exons 9–16. (b) Schematic of exons 7 and 8 with the introns removed. (c) In-frame partial sequences of exons 7 and 8 following splicing of intron 7 and position of the 727G>T transversion (circled) identified in family members II.3 and III.3. This transversion would result in a stop codon instead of glutamic acid at position 243. Analysis of the pedigree also revealed a novel single nucleotide polymorphism, IVS15-3 C>T, in intron 15 adjacent to the exon 16 acceptor site (arrow).

ipsilateral overfolded superior helix and hearing loss of an unknown etiology. Pre-auricular pits and hearing loss are present in 82% and 93% of individuals diagnosed with BOR/BO syndrome, respectively, and malformations of the external ear are present in approximately 36% (19). Ultrasound scans of II.3, as well as of the mother and unaffected sibling of the proband, II.7 and III.2 respectively, were negative for abnormalities of the urogenital system. The pregnancy was terminated at 28 weeks of gestation and post-mortem analysis indicated that the kidneys were absent but that the ureters were present and blind ending (Fig. 2b). The fetus also presented with a rudimentary bladder, bilateral pre-auricular skin tags and pulmonary hypoplasia. Based on the available information, we hypothesized that this was a case of familial BOR/BO syndrome and elected to directly sequence the *EYA1* gene for mutational analysis.

Analysis revealed that II.3 and III.3 carried a heterozygous mutation, EYA1 727G>T, which altered the last coding nucleotide (nt) of exon 7 (Fig. 1a,b,c). Translation of this mutation (Fig. 1 c) would result in an in-frame stop codon instead of a glutamic acid residue at amino acid position 243, and presumably truncate the protein and ablate the EYAhr (Fig. 1a,b,c). We also detected a novel SNP in intron 15 adjacent to the acceptor site of exon 16 in this family (Fig. 1c). The IVS15-3 C>T SNP as well as the causative 727G>T mutation were used to establish an *EYA1* haplotype map of this family (Fig. 3). Both the mutation and the SNP were screened against a panel of 155 and 165 random healthy human controls, respectively. The 727G>T mutation was not found in any individuals in this panel; however, the IVS15-3 C>T SNP was detected in seven individuals (4.2%). While patient II.3 carries both the 727G>T mutation and the SNP, it was determined that the IVS15-3 C>T SNP was not associated with a BOR/BO syndrome phenotype as noted in other family members (Fig. 3). We determined that not only were the mutation and the SNP on separate alleles but also that only the 727G>T mutation is associated with the BOR/BO syndrome phenotype and is *de novo* to patient II.3 as neither of his parents have the mutation (Fig. 3).

Due to the positions of the mutation and the IVS15-3 C>T SNP, MaxENT scores were assessed to determine if either alteration had an effect on the strengths of the 5′ and 3′ss (18). The C>T variation of the SNP had negligible effect on the recognition of the 3′ss of intron 15, with MaxENT scores of 7.99 (CTGCAG) and 7.34 (CTGTAG). However, the G>T variation at nt 727 changed the MaxENT score of the 5′ss of intron 7 from 8.55 (GCAGGT) to 1.05 (GCATGT), suggesting that the mutant transcript would have less likelihood for being properly spliced. In this case, it is then expected that the translation of exon 7 would likely continue in frame into intron 7. When intron 7

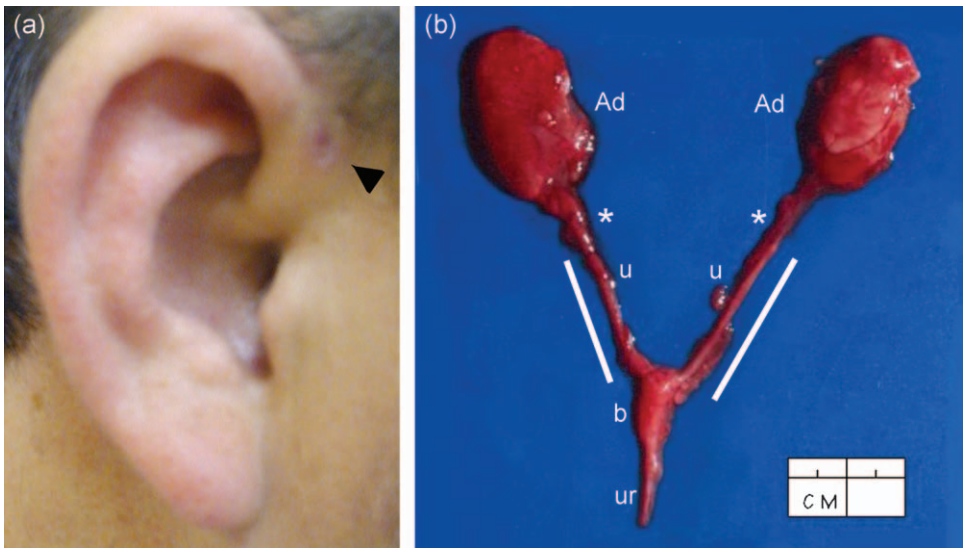

Fig. 2. Variable familial phenotypes. (a) The right ear of II.3. The black arrowhead indicates a unilateral pre-auricular pit. Additionally, this individual has hearing loss of an unknown etiology in this ear and an apparently overfolded superior helix. (b) The urinary tract of the proband III.3 at 28 weeks of gestation. The kidneys are absent; however, the presence of the ureters, the lengths of which are marked by the white bars, indicates that the metanephros was capable of inducing the ureteric bud from the nephric duct. The approximate position of where the kidneys should be is noted by the white asterisks. Ad, adrenal gland; b, bladder; u, ureter; ur, urethra.

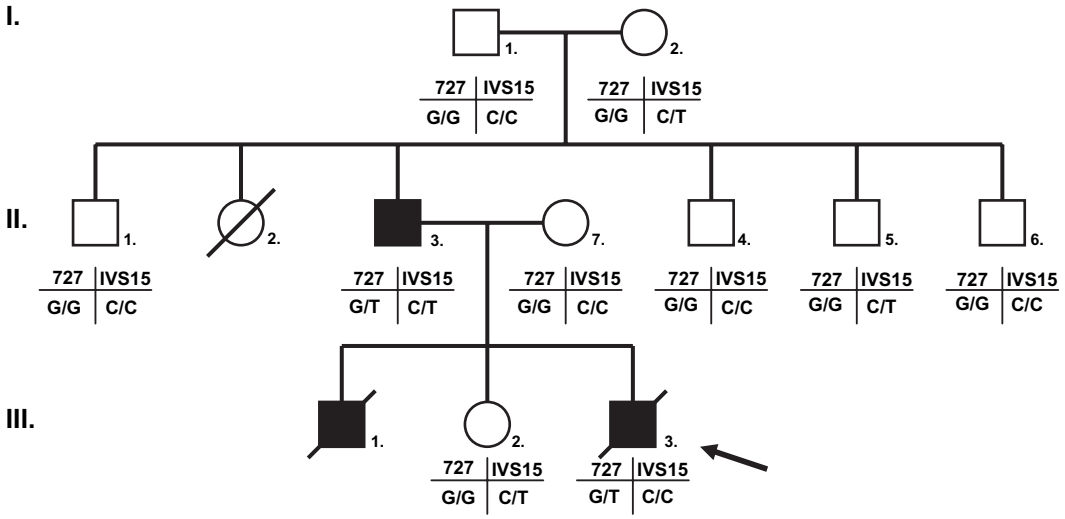

Fig. 3. Complete pedigree of family JCA7. Family members with a branchio-oto-renal/branchiootic (BOR/BO) syndrome phenotype are denoted by blackened symbols. Haplotypes of the *EYA1* gene at nucleotide position 727 and IVS15-3 are included for each individual available for analysis. Individual II.2 died at 9 months of age from unknown causes and individual III.1 presented with bilaterally absent kidneys at birth. Samples from these individuals were unavailable for analysis. Only family members with a BOR/BO syndrome phenotype who were available for analysis possessed the 727G>T mutation. The IVS15-3 C>T single nucleotide polymorphism is detectable in all three generations in this pedigree beginning with I.2, and it is not associated with a BOR/BO syndrome phenotype.

is in frame, the first stop codon is observed at IVS7 + 138, and the first 1 kb of the intron contains 22 stop codons.

Discussion

BOR/BO syndrome is an autosomal dominant developmental disorder that features a wide intrafamilial variability, variable penetrance and a prevalence of 1:40,000 live births (2, 7). We have characterized a novel mutation in exon 7 of the *EYA1* gene that leads to BOR/BO syndrome in a South African Afrikaner kindred. We conclude that the E243X mutation in this family would result in a truncated protein product lacking the critical EYAhr (Fig. 1a). Our findings demonstrate that the causative EYA1 727G>T mutation results in both a BOR and a BO syndrome phenotype in this pedigree. This finding is consistent with other reports (3–6) and is supportive of the hypothesis that the variable BOR/BO syndrome phenotype is likely influenced by other genetic modifiers.

The unique renal phenotype observed in III.3 indicates that the metanephric mesenchyme was not maintained past the inductive stage and subsequently had undergone a dysgenic process either by apoptosis or by other mechanism. A similar atypical phenotype has been reported in a new model of *Eya1*-deficient mice by Sajithlal et al. (13). Using offspring generated from intercrosses between *Eya1* heterozygous null ($Eya1^{+/-}$) and *Eya1* hypomorphic ($Eya1^{bor/+}$) mice, the authors were able to determine that a minimum of ~20% of normal *Eya1* expression is required to induce the metanephric mesenchyme, but is insufficient to maintain normal branching of the ureteric bud. While the $Eya1^{+/-}$ mice with ~48% functional *Eya1* have a low incidence of renal anomalies (10, 13), the compound heterozygote mutants ($Eya1^{bor/-}$) with ~20% functional *Eya1* presented with bilateral absence of the kidneys 100% of the time (13). In humans, individuals with BOR syndrome only present with severe renal abnormalities in approximately 6% of the cases (7). The rare occurrences and inconsistency of severe renal abnormalities in humans with BOR syndrome and $Eya1^{+/-}$ mice are suggestive that other factors such as modifying genes may play a role in the severity of the BOR/BO syndrome phenotype, and perhaps as to the degree that the urogenital system will be affected. We expect that these modifying genes would have a temporal and spatial relationship with the *EYA1* expression pattern (e.g. metanephric mesenchyme) and that they either may or may not directly interact with EYA1.

We were unable to determine the exact function of the 727G>T mutation. Translation of the mutation does result in a termination codon at this position. However, we have also demonstrated that the same end result could also be obtained if the 5′ss of intron 7 was sufficiently weakened and

translation were allowed to continue into intron 7. Additionally, because of the variable phenotypes in this family, it appears that there are other factors involved. One possibility is that the IVS15-3 C>T SNP could influence the expression of the opposite allele as II.3 is seemingly unaffected by renal anomalies. However, we are unable to confirm this, and more comprehensive gene expression studies would be required to assess these influences. Mutations in *EYA1* are only detectable in approximately 40% of those with a BOR/BO syndrome phenotype (16), and in some cases an affected individual without a mutation in *EYA1* was found to have mutations in the transcription factor SIX1 – an EYA1-binding partner (20). The EYA-SIX-PAX developmental network does not appear to have a dogmatic hierarchy, and expression of any one gene in this network is multifactorial (8). Further characterization of this network, as well as of the various BOR/BO syndrome phenotypes in humans and mice, may provide information useful to elucidating these additional influencing factors.

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