Exclusion of the Branchio-Oto-Renal Syndrome Locus (EYA1) From Patients With **Branchio-Oculo-Facial Syndrome**

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In addition to craniofacial, auricular, ophthalmologic, and oral anomalies, the distinctive phenotype of the branchio-oculo-facial (BOF) syndrome (MIM 113620) includes skin defects in the neck or infra/supra-auricular region. These unusual areas of thin, ervthematous wrinkled skin differ from the discrete cervical pits, cysts, and fistulas of the branchio-oto-renal (BOR) syndrome (MIM 113650). Although the BOF and BOR syndromes are sufficiently distinctive that they should not be confused, both can be associated with nasolacrimal duct stenosis, deafness, prehelical pits, malformed pinna, and renal anomalies. Furthermore, a reported father and son [Legius et al., 1990, Clin Genet 37:347-500] had features of both conditions. It was not clear whether they had an atypical presentation of either BOR or BOF syndrome, or represented a private syndrome. In light of these issues, we selected the BOR locus (EYA1) as a possible gene mutation for the BOF syndrome. In five BOF patients, there were no mutations detected in the EYA1 gene, suggesting that it is not allelic to the BOR syndrome. Am. J. Med. Genet. 91:387-390, 2000.

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(BOR) syndrome; chromosome 8q; MCA syndrome

INTRODUCTION

The branchio-oculo-facial (BOF) (MIM 113620) syndrome is a distinctive multiple congenital anomaly syndrome with variable severity [Lin et al., 1995]. Branchial skin defects are accompanied by a variety of eye anomalies (nasolacrimal duct stenosis/atresia, coloboma, true microphthalmia or apparently small palpebral fissures, upslanted palpebral fissures, hypertelorism) and characteristic craniofacial anomalies (dolichocephaly, sparse hair, prematurely gray hair, high forehead, malar hypoplasia, small chin, wide nasal bridge, malformed nose, cleft lip with or without cleft palate, "pseudocleft," low-set and posteriorly rotated ears, cupped pinna with uplifted lobules). Less common features include renal, skeletal, brain, and cardiac anomalies.

Some similarities to the branchio-oto-renal (BOR) (MIM 113650) syndrome have been observed. Both syndromes may have nasolacrimal duct stenosis, deafness, malformed pinnae, prehelical pits, renal anomalies, or development delays (Table I) [Lin et al., 1995]. Despite this apparent overlap, there is little real phenotypic overlap. These conditions should not be confused. Unlike BOF syndrome, patients with BOR syndrome do not have the unusual erythematous, thin wrinkled skin defect of the neck or supra-infra-auricular area, cleft lip or "pseudocleft," and microphthalmia. However, a reported father and son had manifestations of both syndromes, although it was not clear whether they represented an atypical presentation of either condition, or a unique private syndrome [Legius et al., 1990, 1992; Lin et al., 1992]. Aside from this single unusual family, the BOF and BOR syndromes "breed true," and the presence of both conditions in the same family has not been reported.

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Autosomal dominant inheritance due to a single mutant gene is the likely etiology suggested by several instances of vertical transmission, including male-tomale transmission [Lin et al., 1995]. No reported BOF syndrome patients have had a chromosome abnormality. Aside from the superficial overlap with BOR syndrome, other candidate genes have not been proposed. To begin the search for the BOF syndrome gene, we analyzed patients with typical BOF syndrome for presence of mutations in the recently identified BOR gene at 8q13.3 (*EYA1*) [Abdelhak et al., 1997a,b].

MATERIALS AND METHODS Patients

Seven unrelated patients (as well as the unaffected sister of one girl, the affected father of one boy, and 16 unaffected relatives) submitted cheek swab samples. Buccal swabs were obtained after mailing physicians/ families a set of two swabs per person, instructions for sampling, as well as a signed, informed consent, which was returned with the sample in a prepaid mailer. Five patients had been reported previously [patient 1 in Lin et al., 1991; patient 1 in Lin et al., 1992; patient 3 in McCool and Weaver, 1994; and patients 1 and 7 in Lin et al., 1995].

There were two new patients. Patient 980 (AH) is the 4-and-one-half-year- old son of patient JH described by Hall et al.[1983]. He has a typical left-sided cervical skin defect with sinus tract, nasolacrimal duct stenosis, bilateral iris and choroid coloboma, left-sided cataract, and myopia. His facial appearance (Fig. 1) is typical for BOF syndrome and includes protuberant pinnae with uplifted lobules (normal hearing), pseudocleft lip with splayed philtrum, downturned mouth corners, bifid uvula, "fused" lower central incisors, and dermoid cysts of the scalp. There is mild fifth finger clinodactyly, mild syndactyly of toes 2 and 3. Initially, he had mild hypotonia and gross motor developmental delay, but current development is age-appropriate. He is the only BOF syndrome patient with Hirschsprung disease, which was repaired at age 6 months. High resolution chromosomal analysis was normal.

Patient 1061 was a 10-and-one-half-old girl whose family history was not contributory. She had typical bilateral cervical skin defects with hair growth observed anterior and posterior to the defect. Nasolacrimal duct stenosis resolved during infancy. She had a typical BOF facial appearance with malformed pinna, uplifted lobules, bilateral conductive deafness, preauricular left ear pit, unilateral cleft lip with cleft palate, oligodontia, multiple caries, and low frontal hairline. The renal ultrasound was normal. Psychomotor performance was below average, including delayed reading, math, and language. Described as having a "learning disability," but formal IQ testing not available to determine if she has normal cognition. Chromosome analysis was normal.

The DNA of five of these seven patients was successfully amplified forming the basis of our study group. Their clinical manifestations are described in Table I.

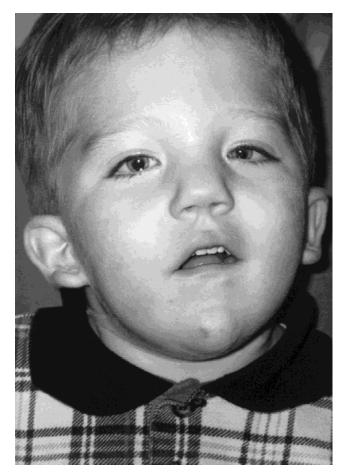


Fig. 1. Patient 980 (AH) at 4 1/2 years with classic BOF facial anomalies including upturned lobules, "splayed" philtrum creating appearance of a pseudocleft, downcurved mouth corners, downslanting palpebral fissures, and bilateral iris coloboma.

None had equivocal clinical manifestations or overlap with the BOR syndrome.

DNA Extraction and Genotyping

DNA was extracted from buccal mucosa cells as previously described [Richards et al., 1993]. Oligonucleotides specific for the EYA1 gene sequences [Abdelak et al., 1997a] were used to amplify all of the exons with the adjacent intron sequences from DNA of individuals with BOF syndrome. Genomic DNA was amplified in a PCR thermocycler (Perkin-Elmer Cetus) with a single 3-minute, 94°C stage followed by 35 cycles comprising three 45-second steps at 94°C, 55°C, and 72°C, respectively, in 10 µl total volume of µl Boehringer 10XPCR buffer, 2.5 pmol of each primer, 2-mM in each dNTP, and 0.25 units of Taq polymerase (Boehringer). For single-strand conformational polymorphism (SSCP), PCR products were heated for 4 minutes at 84°C and electrophoresed for 4 hours at 20 W through a fancooled gel composed of 3.3 ml of 10×TBE, 1.37 ml of glycerol, 13.75 ml of MDE mix (from FMC), 36.6 ml of water, 220 µl of 10% APS, and 22 µl of TEMED. After silver-staining, the gels were visually inspected for bands with altered mobility. Each PCR product from bands showing mobility shifts was sequenced with its

generating primers using an ABI PRISM 373 DNA Sequencer. The sequences were compared with the corresponding normal sequence.

RESULTS

Five unrelated individuals with classic BOF syndrome (Table I) were screened for mutations in the EYA1 gene, previously shown to be responsible for most cases of the BOR syndrome and at least some cases of the branchio-otic (BO) syndrome [Abdelhak et al., 1997b; Vincent et al., 1997]. Each of 16 exons of the EYA1 gene of every affected individual were screened for the presence of point mutations or small deletions/ insertions by PCR-amplification from genomic DNA using SSCP-analysis of the PCR product and direct DNA sequencing of variants. The PCR-amplification was performed using previously published specific primers that were designed outside of each exon in the flanking intron sequences at a distance of at least 60 bp from the corresponding exon-intron junction [Abelhak et al., 1997a] to assure detection of splicing as well as coding region mutations. No abnormal variants were found.

DISCUSSION

The branchial cleft syndromes include the BO (MIM 602588), BOR, and BOF syndromes and share in common cervical skin anomalies. The striking differences between the highly distinctive aplastic wrinkled skin defects in BOF syndrome and the discrete dimples, sinuses, and cysts in BOR and BO have been previously noted [Lin et al., 1995]. The presence of Hirschsprung disease in patient 980 is a new BOF feature and one that does not lend itself to an obvious explanation. Whether it is a fortuitous occurrence or related to altered neural crest migration (beyond the craniofacial developmental field) is speculative. Aside from renal anomalies, visceral anomalies are uncommon in BOF syndrome [Lin et al., 1995].

The BOR syndrome has been well studied. The gene in most cases has been named EYA1 because of its homology to the Drosophila "eyes absent" gene. Although the branchio-otic syndromes (not necessarily the authentic BO syndrome) were not allelic to the BOR syndrome locus at 8q13 in the pedigrees studied by Kumar et al. [1998] and Stratakis et al. [1998], linkage was observed in the classic cases studied by Vincent et al. [1997]. Thus at least some BO families are caused by mutations in the EYA1 gene. Controversy surrounding the definition of what constitutes the "BO syndrome" [Kalatzis and Petit, 1999] is beyond the scope of this report, but illustrates the problem of overlapping manifestations [Kumar et al., 1999]. This current study tested whether the BOF syndrome is allelic to BOR syndrome. In the five patients studied, no mutations were detected using SSCP analysis.

This was a small study, and we readily acknowledge that definitive conclusions cannot be drawn. The families available were too small to test for linkage or even linkage exclusion at the BOR syndrome locus. As demonstrated in the studies of BOR syndrome, genetic heterogeneity may exist. However, with the exclusion of atypical cases [Lin et al., 1995], the phenotype of the vast majority of BOF syndrome cases is remarkably consistent. Other reasons why EYA1 mutations may not have been detected if BOF was truly allelic include SSCP's limitation in detecting 80–90% mutations. If the BOF syndrome gene is a single unique mutation, there is a 10-20% chance of missing it using this technique. If the BOF syndrome can be caused by independent new mutations, then an analysis of five patients would miss all five mutations less than once in 1,000 times. SSCP would also fail to detect the putative BOF syndrome mutation if it is part of the EYA1 gene not yet delineated, such as in regulatory regions embedded in introns or distant noncoding regions. Direct sequencing of the gene was not done because of technical (use of buccal swabs instead of blood sampling) and cost-based reasons. In the future, linkage analysis could be done testing additional candidate genes based on expression patterns of mouse knockouts.

ADDENDUM

After this report was submitted, we became aware of a similar project, which studied the three-general German kindred described by Lin et al. [1995] and reached similar conclusions [Correa-Cerro et al., 1999].

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