RESEARCH ARTICLE

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Genotype–Phenotype Analysis of the Branchio-Oculo-Facial Syndrome

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Branchio-oculo-facial syndrome (BOFS; OMIM#113620) is a rare autosomal dominant craniofacial disorder with variable expression. Major features include cutaneous and ocular abnormalities, characteristic facies, renal, ectodermal, and temporal bone anomalies. Having determined that mutations involving *TFAP2A* result in BOFS, we studied a total of 30 families (41 affected individuals); 26/30 (87%) fulfilled our cardinal diagnostic criteria. The original family with the 3.2 Mb deletion including the *TFAP2A* gene remains the only BOFS family without the typical CL/P and the only family with a deletion. We have identified a hotspot region in the highly conserved exons 4 and 5 of *TFAP2A* that harbors missense mutations in 27/30 (90%) families. Several of these mutations are recurrent. Mosaicism was detected in one family. To date, genetic heterogeneity has not been observed. Although the cardinal criteria for BOFS have been based on the presence of each of the core defects, an affected family member or thymic remnant, we documented *TFAP2A* mutations in three (10%) probands in our series without a classic cervical cutaneous defect or ectopic thymus. Temporal bone anomalies were identified in 3/5 patients investigated. The occurrence of CL/P, premature graying, coloboma, heterochromia irides, and ectopic thymus, are evidence for BOFS as a neurocristopathy. Intrafamilial clinical variability can be marked. Although there does not appear to be mutation-specific genotype–phenotype correlations at this time, more patients need to be studied. Clinical testing for *TFAP2A* mutations is now available and will assist geneticists in confirming the typical cases or excluding the diagnosis in atypical cases.

Key words: branchio-oculo-facial syndrome; cleft lip/palate; mutation analysis; neurocristopathy; *TFAP2A*

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INTRODUCTION

Branchio-oculo-facial syndrome (BOFS; OMIM#113620) is a rare, distinctive, autosomal dominant developmental disorder with variable manifestations [Lin et al., 1995]. The name of the condition reflects involvement of the three major systems. The classic features are thinned erythematous cutaneous defects in the cervical or infra- and/or supra-auricular region, ocular anomalies (microphthalmia or anophthalmia, coloboma, strabismus, cataract, ptosis), and nasolacrimal duct obstruction. The characteristic craniofacial features include dolichocephaly, malformed pinnae, thick nasal tip, upslanted eyes, and cleft lip (CL) (including lesser forms, such as microform, "pseudocleft," or abnormal philtrum [Lin et al., 2009]) with or without cleft palate (CP). Additional findings include conductive/mixed/sensorineural hearing loss, ectodermal anomalies (small teeth, dysplastic nails, sparse and prematurely gray hair), ectopic dermal thymus, and scalp cysts. Growth restriction, renal anomalies (dysplastic, multicystic or absent kidneys, vesicoureteral reflux), upper-lip pits and mild mental retardation are reportedly infrequent findings. More recently, inner ear malformations (incomplete partition type II, enlarged vestibule, and enlarged vestibular aqueduct) and temporal bone anomalies (stenosis of the round and oval windows, malformations of the stapes, and hypoplastic long process of the incus) [Stoetzel et al., 2009; Tekin et al., 2009] are of diagnostic and clinical importance.

We originally described five families with BOFS that had heterozygous mutations or a deletion of the *TFAP2A* gene [Milunsky et al., 2008]. Four research groups have confirmed our original findings [Gestri et al., 2009; Stoetzel et al., 2009; Tekin et al., 2009; Reiber et al., 2010a]. This article extends ongoing clinical and molecular research to include 30 BOFS families (41 affected individuals), the largest series involving this syndrome.

MATERIALS AND METHODS

Patients

Families were enrolled under a protocol at the Boston University School of Medicine, or submitted as individual patients for clinical genetic testing. Permission for publication of those photos that appear was signed by each participant and/or parent. Pictures of selected patients appear in Figure 1. The diagnostic criteria used for BOFS are summarized in Table I. We also reviewed previously described patients [Gestri et al., 2009; Stoetzel et al., 2009; Tekin et al., 2009; Reiber et al., 2010a].

Methods

In the original family we identified a 3.2 Mb deletion at chromosome 6p24.3, utilizing a 500K Microarray (Affymetrix, Santa Clara, CA). We then employed Multiplex ligation-dependant probe amplification (MLPA) to confirm that the deletion included the *TFAP2A* gene. Details of both of these methods are previously published [Milunsky et al., 2008, Patients 1a and b]. For *TFAP2A* sequencing analysis, genomic DNA was processed with the Autopure automated DNA extractor according to manufacturer's instructions (Gentra Systems, Minneapolis, MN). The seven coding exons and intron/exon boundaries of the *TFAP2A* gene were amplified with PCR using appropriate primers. Primer sequences for the generation of amplicons were derived from the NCBI gene website. The method used to sequence the gene utilizes the ABI

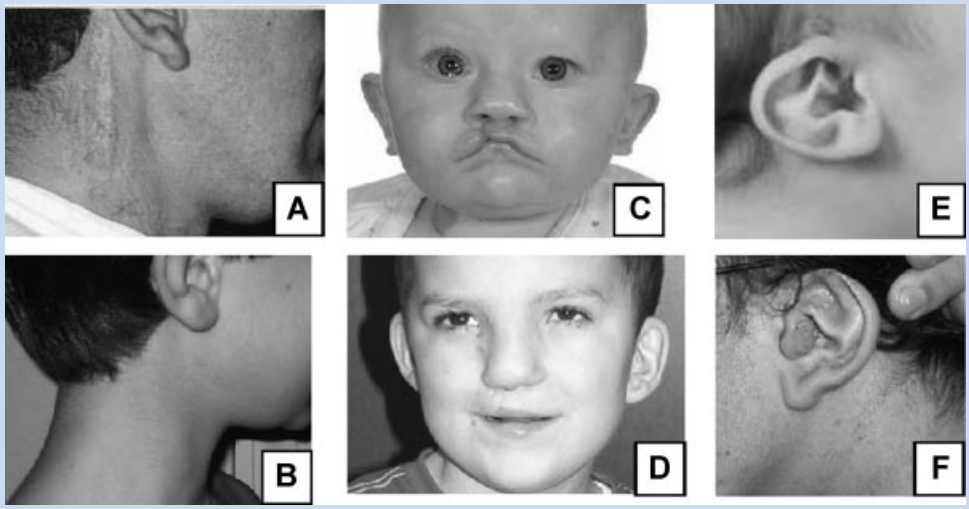

FIG. 1. A: Illustrates the typical "B," the cutaneous defect scar in an adult. In striking contrast, (B) (Patient 27) lacked a typical cutaneous defect, but had a very fine hair extension in the characteristic distribution. Though difficult to illustrate in a photograph, this feature was documented over the years by his pediatrician, mother and barber. C (Patient 12): Typical mildly up-slanted palpebral fissures, full nasal tip, low set protruding ears and lesser form cleft lip prior to surgical repair. The tears pooling in the right eye illustrate nasolacrimal duct atresia. D (Patient 15c): Mildly low-set pinnae, hypertelorism, high nasal bridge, thick nasal tip, following repair of cleft lip and palate. E,F: Variation of the characteristic pinnae anomalies.

VariantSEQr Resequencing System (Applied Biosystems, Foster City, CA). Mutation analysis was performed with the Mutation Surveyor program (SoftGenetics, State College, PA). Several of the mutations were further confirmed using restriction digestion with the appropriate enzyme (New England Biolabs, Beverly, MA), specific to each mutation. In addition, more than 300 normal control individuals were sequenced (see Supplementary Online Version for detailed methodology).

RESULTS

The current BOFS diagnostic criteria (Table I) were derived from Lin et al. [1995] and Milunsky et al. [2008], with the addition of an independently verified first-degree relative or distinctive defect (ectopic thymus). The molecular and clinical features of 41 patients, 20 males and 21 females (6 months–69 years, mean 18 years) are summarized in Table II. Twenty families were enrolled using our Institutional Review Board research informed consent process. An additional 10 families/individuals are reported after consent was obtained following clinical genetic testing. We also reviewed 16 cases of BOFS reported in the literature (Table II).

TABLE I. Diagnostic Criteria for the Branchio-Oculo-Facial Syndrome (BOFS)*

(1) All three of the main features present:
Branchial (cutaneous) defect
Ocular anomaly
Facial (characteristic facial anomalies)
(2) Two of the three main features plus one of the following
Affected first-degree relative, independently diagnosed
Ectopic thymus (dermal)

*Adapted from Lin [2009].

Molecular Analysis

The molecular data presented in Table II include the exon where the mutation was found, the protein consequence of the mutation, and whether the mutation was de novo. The original family with the 3.2 Mb deletion including the *TFAP2A* gene is the only BOFS family without the typical CL/P and our only family with a gene deletion. One proband (Family 29) had a single base pair insertion (c. 376_377 Ins G [p.Asp126GlyfsX43]) in exon 2 that resulted in a stop codon in exon 3, 43 amino acids downstream from the insertion. This was the only frameshift mutation in our series. All other mutations were missense; the majority clustered in exon 4 (23/28, 82%). There were six recurrent mutations (five in exon 4 and one in exon 5; Table III). All missense mutations involved highly conserved amino acids (Fig. 2). Family 22 was the only one with a missense mutation outside of this hotspot region (H384Y) in exon 7. Family 7 was interesting in that there was classic BOFS phenotype in the 11-year-old daughter (R254G mutation in exon 4) and a milder phenotype in her 45-year-old father, who had premature gray hair, preauricular pits, and a supernumerary nipple. On sequencing and restriction digestion (Fig. 3), the father appeared to be a mosaic for the mutation in blood. Further studies from other tissues have been requested.

TABLE II. Molecular and Clinical Findings in Patients (30 Probands, in Bold) With BOFS

Patient	**1a**[1]	**1b**[1]	**1c**	**2**[1]	**3**[1]	**4**[1]	**5**[1]	**6**	**7a**	**7b**	**8a**[2]	**8b**[2]	**9**	**10**	**11**[3]	**12**	**13**
Exon				4	4	4	5	4	4	4	5	5	4	4	4	4	5
Protein consequence	Whole gene del	Whole gene del	Whole gene del	R255G	L249P	R254W	G262E	R254W	Mos R254G	R254G	A256V	A256V	R254W	R236P	G251E	R254W	A256V
De novo	No	No	U	Yes	Yes	Yes	Yes	U	U	No	U	No	U	U	Yes	Yes	Yes
Sex	F	M	F	F	M	M	F	M	M	F	M	F	M	F	M	F	F
Age (years)	25	1	46	18	2	14	17	11	45	11	52	13	9	7	22	2	5
FH	+	+	+	−	−	−	−	−	−	+	−	+	−	+	−	−	−
Branchial anomalies																	
Cervical cutaneous anomaly	R	B	L	B	R	B	B	B	−	B	B	B	B	B	B	B	B
Ectopic thymus	U	U	U	+	−	−	−	U	U	U	U	U	U	U	+	+	U
Ocular anomalies																	
Coloboma	−	R	−	R	−	R	B	−	−	−	−	B	B	B	−	−	B
An/microphthalmia	−	R	−	R	−	R	B	−	−	B	−	−	−	B	R	−	−
NLDS/A	−	−	−	B	B	B	−	−	−	B	−	−	B	B	+	+	B
Cataract	−	−	−	−	−	−	−	−	−	−	−	−	U	U	−	−	U
Facial anomalies																	
Auricular anomaly	B	B	−	B	B	B	B	−	−	B	−	−	B	B	B	B	B
Characteristic face	−	+	−	+	+	+	+	+	−	+	−	+	+	+	+	+	+
Chin dimple/cleft	−	−	−	+	+	−	+	−	+	−	−	−	−	U	−	−	−
CL/P	−	− Abn philtrum	−	B CL/P	B CL	B CL	B CL	B CL	−	B Pseudo CL	+	Pseudo CL	Pseudo CL	Pseudo CL	CL/P	B CL	B Pseudo CL
Lip pits, upper	−	−	−	−	−	−	−	−	−	−	−	−	U	U	−	−	+
Facial nerve weak/palsy	−	−	−	−	+	−	−	−	−	R	−	−	R	−	−	L	−
Preauricular pits	−	−	−	−	+	+	+	−	+	−	+	+	−	U	−	−	−
Ectodermal anomalies																	
Dental anomalies	−	−	−	+	−	+	+	−	U	+	−	−	+	+	−	U	−
Nail anomalies	−	−	−	−	−	+	+	−	−	−	−	−	+	U	−	−	+
Premature gray hair	+	−	−	−	−	+	−	−	+	−	+	+	U	U	+	−	−
Cysts	−	+ Scalp	−	−	−	−	−	−	−	+ Scalp	−	−	+ Scalp	U	+ Upper chest, R eyebrow	−	−
Sparse hair	−	−	−	−	−	−	−	−	−	−	−	−	U	U	−	−	+
Additional features																	B PAP
Temporal bone anomalies	U	U	U	U	U	U	U	U	U	+	U	U	U	U	U	U	U
Hearing loss	−	B	+	B	+	+	B	Severe Cond	−	B, SN	−	+	B Mixed	B Cond	Cond	−	B Cond
Growth restriction	−	−	−	+	−	+	+	−	−	+	−	+	+	+	−	+	−
Kidney anomalies	−	−	U	−	−	U	+	U	−	L	−	L	R	+	−	−	−
Supernumerary nipples	−	−	U	−	−	−	−	−	+	+	−	−	U	U	U	−	−
CHD	U	U	U	−	−	U	−	U	−	U	−	U	U	U	U	U	U
Psychiatric symptoms	−	−	−	+	−	−	+	−	−	−	−	−	U	+	−	−	−
Cancer	−	−	−	−	−	−	+	−	−	−	−	−	U	U	−	−	−

TABLE II. (*Continued*)

Patient	**14**	**15a**	**15b**	**15c**	**16[4]**	**17a**	**17b**	**18**	**19a**	**19b**	**19c**	**19d**	**20**	**21**	**22a**	**22b**
Exon	4	4	4	4	4	4	4	4	4	4	4	4	5	4	7	7
Protein consequence	L253P	R217C	R217C	R217C	L218R	R255G	R255G	R237G	G251E	G251E	G251E	G251E	A256V	E242K	H384Y	H384Y
De novo	Yes	Yes	No	No	U	U	No	U	U	No	No	No	U	Yes	U	No
Sex	F	F	M	M	M	F	M	M	F	F	F	F	M	F	F	F
Age (years)	69	40	9	7	17	37	12	8	61	33	18	5	2	9	31	0.5
FH	−	+	+	+	−	−	+	−	+	+	+	+	+	−	+	+
Branchial anomalies																
Cervical cutaneous anomaly	B	B	B	B	B	L	R	R	−	−	L	−	B	−	−	−
Ectopic thymus	U	U	U	−	−	−	U	U	−	−	+	−	+	−	U	−
Ocular anomalies																
Coloboma	Other: heterochromia irides B	B	B	−	−	−	B	−	−	U	−	R	U	R	+	+
An/microphthalmia	−	−	−	−	−	−	−	−	−	R	−	+	−	R	−	+
NLDS/A	B	B	−	B	B	−	B	+	U	U	+	+	B	+	−	+
Cataract	B	R	−	B	−	−	−	−	−	−	−	U	U	−	+	−
Facial anomalies																
Auricular anomaly	−	B	B	B	B	B	B	B	U	−	−	+	−	+	−	−
Characteristic face	+	+	+	+	+	+	+	+	−	−	+	+	+	+	−	− Broad nasal tip
Chin dimple/cleft	−	−	U	U	−	−	−	+	U	−	−	U	−	−	−	−
CL/P	B CL	CL/P	Pseudo CL	CL/P	B CL	CL	CL/P	Pseudo CL	−	−	CL/P	CL/P	CL	B Pseudo CL	−	Pseudo CL
Lip pits, upper	−	−	−	−	+	−	−	U	U	−	−	−	−	−	−	−
Facial nerve weak/palsy	−	−	−	−	B	−	−	−	−	−	−	−	−	−	−	−
Preauricular pits	−	−	−	−	−	−	−	+	+	−	+	+	−	−	+	−
Ectodermal anomalies																
Dental anomalies	−	−	−	−	+	U	+	−	+	+	+	+	U	−	−	U
Nail anomalies	−	−	−	−	−	+	+	+	−	−	−	U	−	−	−	−
Premature gray hair	+	+	+	−	−	+	−	−	+	−	U	−	U	−	+	+
Cysts	+ Scalp	−	−	−	−	−	−	U	−	+	−	−	−	−	−	−
Sparse hair	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	U
Additional features																
Temporal bone anomalies	−	U	U	U	U	U	U	+	U	U	U	−	U	U	U	U
Hearing loss	B Mixed	B SN	B SN	−	B Cond	R SN	Cond	B Mixed	−	L Cond	B Cond	L Cond	−	−	−	−
Growth restriction	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−
Kidney anomalies	−	R	−	R	−	−	R	−	−	U	−	−	−	−	U	−
Supernumerary nipples	+	−	−	+	−	U	−	−	U	−	−	−	−	−	−	−
CHD	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	−
Psychiatric symptoms	−	−	−	−	−	U	+	+	U	−	−	−	−	−	−	U
Cancer	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−

Patient	23	24	25	26	27	28	29	30	New # probands (%), 30 (100)	New # pts (%), 41 (100)	Literature[5–8] # pts (%), 16 (100)
Exon	4	4	4	4	4	4	2	4			See text
Protein consequence	E242K	R237P	R237P	R246E	S247L	E242K	p.Asp126GlyfsX43	R254P			See text
De novo	U	Yes	U	U	U	U	Yes	U	11/18 (61)	12/23 (52)	8/16 (50)
Sex	F	M	F	M	M	M	M	M	M 17/30 (57)	F 21/41 (51)	F 8/16 (50)
Age (years)	29	2	14	1	9	1	13	19	Range 0.5–69 years, mean 11.6 years	Range 0.5–69 years, mean 18 years	Range 1–57 years, mean 17.2
FH	–	–	–	+	–	–	–	–	10/30 (33)	18/41 (44)	8/16 (50)
Branchial anomalies											
Cervical cutaneous anomaly	B	B	B	L	+ Faint hair only	–	R	B	27/30 (90)	33/41 (81)	10/16 (63)
Ectopic thymus	U	–	–	U	U	U	U	+	6/14 (43)	6/19 (32)	0/16 (0)
Ocular anomalies									27/30 (90)	32/41 (78)	5/6 (83)
Coloboma	–	–	–	L	B	U	L	B	17/28 (61)	20/38 (53)	10/14 (71), 1 heterochromia iris
An/microphthalmia	–	–	–	B	R	–	L	–	12/30 (40)	14/41 (34)	11/15 (73)
NLDS/A	–	B	B	B	B	+	B	+	24/30 (80)	27/39 (69)	9/14 (64)
Cataract	–	–	–	–	–	U	–	+	2/25 (8)	5/35 (14)	1/7 (14)
Facial anomalies									30/30 (100)	39/41 (95)	16/16 (100)
Auricular anomaly	+	+	+	+	+	–	B	B	23/30 (77)	28/40 (70)	15/16 (94)
Characteristic face	+	+	+	+	+	+	+	+	29/30 (97)	33/41 (81)	5/6 (83)
Chin dimple/cleft	–	–	–	–	–	–	+	–	5/28 (18)	5/36 (14)	0/16 (0)
CL/P	CL/P	B Pseudo CL	B Pseudo CL	B Pseudo CL:	Psuedo CL	B Psuedo CL, bifid uvula	B pseudo CL	B CL/P	Any 29/30 (97), CL 8/30 (27), CL/P 6/30 (20), pseudo 15/30 (50); 1 abn philtrum	Any 34/41 (83), CL 7/41 (17), CL/P 9/41 (22), pseudo 15/41 (37); 1 abn philtrum, not stated 1/41 (2)	Any 5/16 (31), CL 3/16 (19), CL/P 2/16 (13), pseudo 1/16 (6)
Lip pits, upper	–	–	–	–	+	+	–	–	4/27 (15)	4/37 (11)	0/3 (0)
Facial nerve weak/palsy	–	–	B	–	–	–	+	U	7/29 (24)	7/40 (18)	1/11 (9)
Preauricular pits	+	+	–	+	–	–	–	–	9/29 (31)	14/40 (35)	3/11 (27)
Ectodermal anomalies											
Dental anomalies	–	+	+	–	+	U	–	+	13/26 (46)	16/35 (46)	6/13 (46)
Nail anomalies	–	–	–	–	–	–	–	+	7/29 (24)	8/39 (20)	0/5 (0)
Premature gray hair	–	–	–	–	–	–	–	+	7/26 (27)	14/37 (38)	6/9 (67)
Cysts	–	U	+	–	–	–	U	+	7/26 (27)	8/37 (21)	1/5 (20)
Sparse hair	–	+	–	–	–	+	–	–	5/27 (18)	5/38 (13)	1/16 (6)
Additional features						B PAP					

(Continued)

Temporal bone anomalies	U	+	–	U	U	U	–	U	3/6 (50)	3/7 (42)	4/16 (25)
Hearing loss	B Mixed	–	B Cond	L SN	U	–	R SN	+	23/29 (79)	28/40 (70)	6/16 (38)
Growth restriction	–	–	–	–	+	+	–	+	12/30 (40)	13/41 (32)	1/13 (8)
Kidney anomalies	U	+	–	R	U	R	–	+	10/26 (39)	12/34 (35)	3/7 (43)
Supernumerary nipples	+	–	–	–	–	–	– Inverted	–	3/27 (11)	5/35 (14)	1/16 (6)
CHD	U	ASD	U	–	–	–	–	–	1/11 (9)	1/12 (8)	2/16 (13)
Psychiatric symptoms	–	U	–	U	–	U	–	–	5/25 (20)	5/34 (14)	0/16 (0)
Cancer	–	–	–	–	–	–	–	–	1/28 (4)	1/39 (5)	0/16 (0)

Abn, abnormal; ASD, atrial septal defect; B, bilateral; B PAP, bilateral post-axial polydactyly; CL/P, cleft lip/palate; Cond, conductive; CHD, congenital heart defect (determined by echocardiography); del, deletion; FH, family history; L, left; mos, mosaic; LDs, learning disabilities; NLDS/A, nasolacrimal duct stenosis/atresia; pts, patients; Pseudo CL, pseudocleft (includes mini-microform); R, right; Sev, severe; SN, sensorineural; U, unknown.

[1] Milunsky et al. [2008]; [2] Mégarbané et al. [1998]; [3] Lin et al. [1995]; [4] Lin et al. [2009]; [5] Gestri et al. [2009]; [6] Stoetzel et al. [2009]; [7] Tekin et al. [2009]; [8] Reiber et al. [2010]; [9] the senior authors (J.M.; A.L.) determined the status of certain features from published photos if not specifically stated in text.

TABLE III. Recurrent *TFAP2A* Mutations Reveal Hotspot of Mutable Amino Acid Residues in BOFS

Exon	Mutation of amino acid residue (# individuals or families)
4	R254G/W/P (6)
4	R237G/P (3)
4	E242K (3)
5	A256V (3)
4	G251E (2)
4	R255G (2)

Clinical Features

The frequency and pattern of defects in our patients and in the literature is similar to that reported in both clinical [Lin et al., 1995] and molecular series [Milunsky et al., 2008], but includes several new findings. Importantly, the typical cervical or supra-auricular ("branchial") cutaneous defects were not ubiquitous (90% and 62% in our series and the literature, respectively). The most minimal expression was a patch of faint hair located in the same linear cervical distribution [Patient 27; Stoetzel et al., 2009, Patient 1.2]. Two patients had heterochromic irides [Patient 14; Stoetzel et al., 2009, Patient 1.2]. Congenital heart defects were rare (~8% among total patients), including two atrial septal defects [Patient 24; Gestri et al., 2009 Patient 2], and one tetralogy of Fallot [Reiber et al., 2010a, Patient SP2, previously reported by Bennaceur et al., 1998]. Two probands in our series had bilateral post-axial polydactyly that was not present in their families. This finding may be related to BOFS (rarely), as preaxial polydactyly, type A has been reported [Fujimoto et al., 1987, Patient 1].

Psychomotor development is usually normal, despite frequent visual and hearing handicaps, but, there are two patients reported with autism spectrum disorder and a patient with severe mental retardation [Reiber et al., 2010a, Patient SP2; Gestri et al., 2009; Reiber et al., 2010b].

DISCUSSION

The research families presented had a clinical diagnosis of BOFS based on probands demonstrating all three BOFS features (cervical cutaneous defects, ocular anomalies, facial anomalies) or two features and a first-degree affected relative. Aims of the study included determining genetic heterogeneity, degree of clinical variability, and whether there are any mutation-specific genotype–phenotype correlations. We do not have complete clinical data on all affected individuals, as most have not had temporal bone CT scans or echocardiograms, and some have not had audiology evaluations, or renal ultrasonography. The details of each patient's clinical findings are summarized in Table II. One of our original patients (Patient 5) remains the only affected individual with cancer (medulloblastoma) [see further discussion in Milunsky et al., 2008].

Both Tekin et al. [2009] and Stoetzel et al. [2009] highlighted the inner ear and temporal bone anomalies that had been reported previously in BOFS [Raveh et al., 2000] as well as in other disorders, such as CHARGE syndrome [Amiel et al., 2001] and branchio-

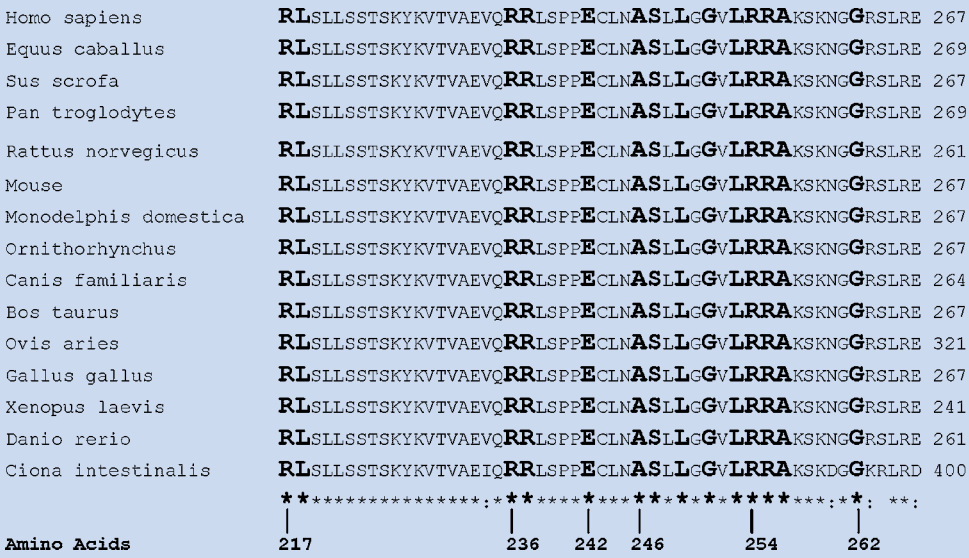

FIG. 2. Evolutionary conservation of amino acids in exons 4 and 5 of the *TFAP2A* gene. Bolded residues are altered in BOFS patients. Select amino acids are numbered for reference.

oto-renal syndrome [Ceruti et al., 2002]. In our cohort, 3/6 probands investigated had temporal bone anomalies, whereas CHARGE syndrome is associated with semicircular canal aplasia/hypoplasia, which is nearly diagnostic [Glueckert et al., 2010; Zentner et al., 2010]. In contrast, BOR is associated with mostly cochlear anomalies [Ceruti et al., 2002]. This finding is likely to be under-diagnosed; further large clinical studies are needed to determine the frequency of temporal bone anomalies in BOFS.

The probands in every family had some form of CL, with or without CP. The one exception, without an overt cleft, was our deletion Family 1, who had a short-tented prominent philtrum. Gestri et al. [2009] reported a BOFS family with a paternally inherited whole gene deletion. The brother and sister with "unaffected" parents had an unusual phenotype with orbital cysts who were diagnosed as having BOFS [Fielding and Fryer, 1992], but viewed as "possibly affected" and "atypical" in a large series [Lin et al., 1995]. Recent molecular analysis confirmed the diagnosis and examination of their father showed premature graying and abnormal philtrum [Gestri et al., 2009]. None of the family members had an overt labial cleft, but rather were characterized by having a distinctly abnormal philtrum or a "pseudocleft." Further data come from Davies et al. [1999] and Misceo et al. [2008], who both reported chromosome 6 interstitial deletions, including *TFAP2A* that do not result in overt clefting, but rather a

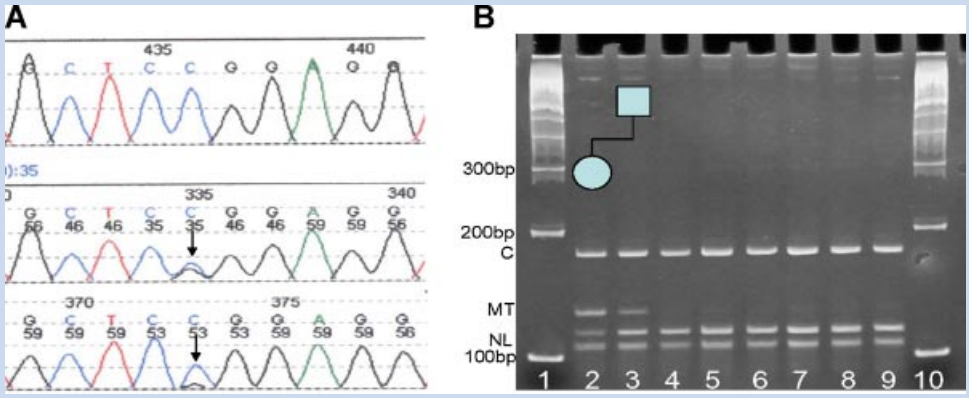

FIG. 3. A: Chromatogram of R254G mosaic mutation from Family 7 indicated by arrow. Top panel is the reference sequence. Middle panel is the female proband. Lower panel is the father's mosaic mutation. B: Restriction digest of the R254G mutation. The mutation destroys a Msp1 site. C = constant restriction site; MT = mutant band; NL = normal band. Lanes 1 and 10: 100 bp marker; lane 2: affected daughter's sample; lane 3: father's sample; lanes 4–9 normal control samples.

prominent philtrum. Lin et al. [2009] discussed that the abnormally short philtrum and bilateral notched vermilion-mucosa border are on the spectrum of microform CL and noted the absence of isolated CP in BOFS.

''Branchial'' or pharyngeal arch involvement, typically manifesting as the cervical cutaneous anomaly is a classic finding in patients with BOFS [Lin et al., 1995]. Nevertheless, three described probands from Families 21, 22, and 28 did not have this anomaly (examined by geneticists A.R, [London], A.D., and M.W.). Two additional patients were viewed as having a very minor expression in the form of faint hair on the neck [Patient 27; Stoetzel et al., 2009, Patient 1.2]. Interestingly, Family 21 and 28 have the same mutation (E242K); however, the mutation was also found in another classic BOFS patient (Family 23) with bilateral cervical cutaneous anomalies. The third family (Family 22) had a missense mutation in the helix-span-helix region of the DNA binding and dimerization domain. Further families without this classic cervical anomaly should be genotyped in order to establish a more precise genotype–phenotype correlation, if one exists. Nevertheless, the lack of the cervical cutaneous anomaly further broadens the variability of the BOF diagnosis, as has recently been reported by Reiber et al. [2010a].

Twenty-eight of 40 (70%) patients investigated had hearing loss, mostly bilateral and categorized as sensorineural, conductive, or mixed. Additional details are not readily available from the majority of patients regarding hearing frequency, progression and effectiveness of amplification. Further studies to address these questions would be helpful. Echocardiography is uncommonly performed in BOFS, and the occurrence of atrial septal defect (one ach in our series and the literature) may not reflect true occurrence. Nevertheless, tetralogy of Fallot [Reiber et al., 2010a] may have some importance as a conotruncal defect in the analysis of the developmental mechanism (see below).

A large percentage of patients (12/34; 35%) that were investigated had various renal anomalies, including dysplasia, agenesis, multicystic kidneys, and vesicoureteral reflux. These data support obtaining renal ultrasonography in the diagnostic evaluation of possible BOFS patients.

Premature graying of hair or poliosis is likely another underreported frequent finding in BOFS families. In our series, 14/37 (38%) individuals had this feature; it was especially prevalent in the more mildly affected older generations who harbored the familial deleterious mutation. Mégarbané et al. [1998] initially reported poliosis in a daughter and her father with BOFS (Family 8). This underscores that clinical screening of first-degree relatives of BOFS patients should include some of the less obvious phenotypes (as some individuals dye their hair). Interestingly, Patient 14, in addition to her premature graying and characteristic BOFS features, also had heterochromia of the irides, another feature of neurocristopathies. This is not surprising as *TFAP2A* is known to be expressed in premigratory and migratory neural crest cells [Hilger-Eversheim et al., 2000; Li and Cornell, 2007]. Thus, premature graying hair, sensorineural deafness, inner ear/temporal bone anomalies, coloboma, heterochromia, facial nerve weakness, tetralogy of Fallot, and ectopic thymus provide clinical support for neural crest involvement in BOFS. We had originally reported that Patients 2 and 5 had anxiety and depression [Milunsky et al., 2008]. In surveying the rest of the BOFS patients, we found another three who had psychiatric symptoms. It is difficult to determine if the psychiatric symptomatology is related to the psychosocial context of having a craniofacial disorder or related to their genotype. As previously reported, the AP-2 family may be involved in the regulation of the monoaminergic systems in the adult brain, resulting in neuropsychiatric disorders [Damberg, 2005]. It would seem prudent to inquire about such symptoms in the diagnostic and follow-up evaluations of BOFS patients.

The molecular spectrum in 30 families with 41 affected individuals with BOFS includes heterozygous missense mutations, a frameshift mutation, and a complete deletion of the *TFAP2A* gene. The frequency of partial or whole gene *TFAP2A* deletions remains unknown, but appears to be low. More deletion cases need to be identified to determine their frequency and whether lesser forms of CL are present in these patients. The data from Gestri et al. [2009] clearly demonstrate that a deletion of the *TFAP2A* gene results in a variety of ophthalmologic anomalies that have been reported in BOFS, including coloboma (typically posterior segment, but also iris), microphthalmia, nasolacrimal duct stenosis, and cataract. These ocular anomalies have also been described in patients with larger deletions including the *TFAP2A* gene [Davies et al., 1999; Misceo et al., 2008].

The intrafamilial clinical variability was marked in Families 1, 7, 8, and 19 (see Table II). This variability could be explained in Family 7 by the apparent mosaicism detected on sequencing and restriction digestion. Additional tissues from the father in Family 7 have been requested to further demonstrate the likely mosaicism. Given this marked intrafamilial variability, testing of parents of a molecularly confirmed case of BOFS is recommended for more accurate recurrence risk counseling. In addition to mosaicism, modifier genes are likely playing a role in the clinical variability seen in families. Once a mutation has been established, genetic counseling and prenatal diagnosis would be available.

We have thus far not demonstrated any genetic heterogeneity for those cases fulfilling the clinical criteria previously discussed.

We have identified a hotspot region in the highly conserved exons 4 and 5 (basic region of the DNA binding and dimerization domain) of *TFAP2A* that harbors missense mutations in 27/30 (90%) (Fig. 2) families. Several of these mutations are recurrent and are listed in Table III. One family harbors a mutation in exon 7 of the *TFAP2A* gene (helix-span-helix region of the DNA binding and dimerization domain). The proband in this family did not have obviously different clinical findings than our other patients. Not surprisingly, the proband of Family 29 did not have patently different clinical findings than our other patients; harboring a frameshift mutation in exon 2 leading to disruption of the DNA binding and dimerization domain.

In addition to the whole gene deletion cases reported by Gestri et al. [2009], they also described an individual with classic BOFS with a deletion between amino acid residues 233 and 236. Tekin et al. [2009] discovered a complex *TFAP2A* allele (deletion of 18 and insertion of 6 nucleotides) between amino acids 276 and 281 that altered amino acids in the basic DNA binding and dimerization domains. That patient also had a phenotype characteristic of BOFS. Stoetzel et al. [2009] reported one family and two sporadic cases with missense mutations in exon 4 (S239P, L249P, and

L218P). We had previously found the L249P in Patient 3 [Milunsky et al., 2008], and noted another change (L218R) of the same amino acid residue in this report. Reiber et al. [2010a] published five patients with BOFS (two familial and three sporadic) with mutations in exons 4–6. They noted the recurrent R255G mutation in a familial and sporadic case [mutation seen in Case 2 in Milunsky et al., 2008] and a mutation of amino acid residue 237 that was found in three patients in this report (see Tables II and III). They also found two novel missense mutations in exon 5 (L269P) and exon 6 (E296K).

Implications for Management

BOFS is a multisystem disorder that requires multidisciplinary care, including genetic counseling for affected families. This article emphasizes that absence of the "B" feature (cutaneous defect) does not exclude the diagnosis, and thus, patients who may have been followed as "possible BOFS" should be reconsidered with the advantage of molecular analysis. Management recommendations are summarized in Table IV. Ophthalmologic evaluation has always been essential because of the various ocular anomalies and the potential for visual limitation. Craniofacial surgery should be performed by a plastic surgeon experienced in treating children with orofacial clefts and congenital facial anomalies, preferably in the setting of a CP or craniofacial center. Lesser forms of CL, formerly known as "pseudocleft" may need surgical repair although seemingly minor defects, and may represent a minimal expression of the BOFS gene [Lin et al., 2009]. The recent reports of temporal bone anomalies are compelling evidence that aggressive evaluation of hearing loss should be accompanied by CT imaging. Given the frequency of kidney anomalies, renal ultrasonography is recommended in the initial diagnostic evaluation of BOFS patients. Congenital heart defects are probably rare, but the detection of a murmur should prompt referral for echocardiography. Although intelligence is usually normal, challenges to learning mean that an educational interventional program and formal psychometric testing should be arranged. Given the potential risks for psychiatric problems and predisposition to cancer, although routine testing is not recommended, continued monitoring seems prudent. The intrafamilial variability of BOFS appears significant, and warrants molecular testing of parents for more accurate recurrence risk counseling. Finally, the results of this study indicate that although genotyping does not predict a specific phenotype, further investigation is needed.

CONCLUSIONS

This is the largest series of patients with BOFS with genotyping of *TFAP2A*. Identification of a hotspot region and recurrent mutations remain important to the molecular diagnostic strategy in clinical laboratories. The diagnosis of the BOFS remains clinical in most patients. Nevertheless, molecular testing for *TFAP2A* mutations will assist geneticists in confirming apparently typical cases or excluding the diagnosis in atypical cases. Aside from the data presented on the patients with a deletion, there does

TABLE IV. Management of Individuals With BOFS

Clinical issue	Guideline
General	Ideally, follow children in a multidisciplinary cleft palate or craniofacial clinic setting
Branchial (cutaneous) defects	When superficial or small, these may heal spontaneously. Usually, require surgical excision; a sinus tract must be dissected. This should be done only by an experienced pediatric plastic surgeon. Exploration for a thymic remnant may be necessary, and if detected, this should be sent for histopathologic examination
Ophthalmologic defects	Complete ophthalmologic examination is needed because of visual limitation and strabismus, as well as an evaluation for nasolacrimal duct patency
Facial anomalies: orofacial clefts	Surgical treatment should be done only by a pediatric plastic surgeon experienced in treating children with cleft lip. Lesser forms of cleft lip, formerly known as "pseudocleft" may need surgical correction
Nose	In addition to the nasal tip flattening or asymmetry that may be associated with cleft lip, there may be a characteristic full, flat nasal tip which may need a corrective procedure
Teeth	Hypoplastic or absent teeth should be carefully monitored
Ears	Malformed protruding pinnae may require surgical correction. If diagnosed in early infancy, auricular molding may be indicated
Hearing	In addition to the newborn hearing screen, a referral to an audiologist is essential. The recent reports of temporal bone anomalies are compelling evidence that CT imaging should be done to anticipate optimal hearing correction
Heart defect	Echocardiogram indicated if there is a murmur or symptomatic
Kidney defect	Renal ultrasonography recommended at the time of diagnosis
Development	Speech therapy is likely and learning challenges are common if there are visual and hearing special needs
Psychologic issues	Monitor
Cancer	Monitor
Genetic issues	Confirmation of the *TFAP2A* mutation should be performed by a CLIA-approved laboratory. Parental targeted molecular analysis recommended given significant phenotypic variability. Genetic counseling to review the inheritance and reproductive risks should be provided

not appear to be mutation-specific genotype–phenotype correlations at this time, but more patients need to be studied.

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