Okihiro syndrome and acro-renal-ocular syndrome: clinical overlap, expansion of the phenotype, and absence of *PAX2* mutations in two new families

K Becker, PL Beales, D M Calver, G Matthijs, S N Mohammed

J Med Genet 2002;**39**:68–71

The Okihiro syndrome consists of Duane anomaly, radial ray defects, and deafness. There are similarities with the acro-renal-ocular syndrome in which there are radial ray and renal abnormalities and colobomas which mostly involve the optic nerve. Both malformation syndromes are dominantly inherited. We report two families with an overlapping phenotype, suggesting a common aetiology. The combination of optic nerve coloboma and renal disease is also seen in families with mutations of the *PAX2* gene. We did not find any evidence of *PAX2* involvement in our families.

The main features of the autosomal dominant Okihiro syndrome are radial ray defects of variable severity and Duane anomaly.[1 2] Sensorineural hearing loss and spinal and other skeletal abnormalities also occur, and polydactyly, hemifacial microsomia with skin tags, cardiac defects, and Hirschsprung disease have also been reported.[1 3–5] The radial ray defects may be associated with vascular abnormalities such as hypoplasia of the radial artery[6] and abnormal nerve conduction studies with reduced or absent motor response from the median nerve.[1 6] The acro-renal-ocular syndrome, which is also autosomal dominant, consists of radial ray defects, renal anomalies, and ophthalmological abnormalities, mainly colobomas, but also microphthalmia, ptosis, and Duane anomaly.[7–12] There is considerable overlap between the two syndromes, and it has been suggested previously that they are one clinical entity.[8] In addition, mutations in the *PAX2* gene have been documented in patients with optic nerve colobomas and renal malformation.[13–15] There are also similarities to Wildervanck syndrome, Goldenhar syndrome, and thalidomide embryopathy. This can lead to considerable difficulties with classification in individual families. We describe four affected subjects in two new families with features of both Okihiro syndrome and the acro-renal-ocular syndrome and investigate the possibility of *PAX2* involvement.

Figure 2 Arms of patient 2.

FAMILY 1

Patient 1

The female proband was the second child of unrelated parents. She was born with severe upper limb defects, had a preaxial extra digit surgically removed from the right hand, and was diagnosed as having bilateral Duane anomaly in early childhood. She was referred to the Genetics Centre at the age of 24 years. On examination, she had a longer arm on the right with an elbow, a shortened forearm, and a hand with four digits. The left upper arm was severely shortened and three digits were directly attached to the shortened forearm. The thumbs were absent bilaterally. Both humeri were shortened and the radii were absent. Ophthalmological examination showed bilateral latent fine nystagmus and slightly dysplastic optic discs, and orthoptic assessment showed bilateral Duane anomaly with severe limitation of eye abduction (fig 1A, B, C). ECG and renal ultrasound did not show any abnormalities and an audiogram showed mild bilateral conductive hearing loss only. Her karyotype was 46,XX.

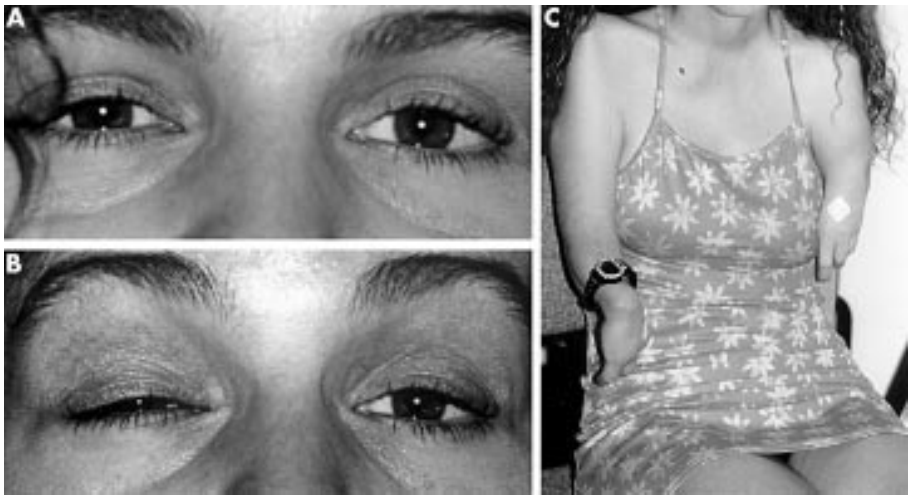

Figure 1 (A) Eyes of patient 1 in neutral position. (B) Duane anomaly on left lateral gaze. (C) Arms of patient 1.

Patient 2

The mother of case 1 was noted to have absent thumbs and shortened forearms at birth (fig 2). She was otherwise well and ophthalmological examination was normal. A renal ultrasound showed a normal right kidney and a smaller left kidney in pelvic position, situated in the left iliac fossa.

An audiogram showed bilateral, moderate, low frequency conductive loss and significant bilateral, high frequency sensorineural hearing loss, of which the patient had previously been unaware. An ECG was normal and chromosomes were 46,XX. Her parents had died and no other family members were known to have any limb abnormalities.

FAMILY 2

Patient 3

The female proband was the second child of unrelated parents. She presented as a neonate with absent left thumb and hypoplastic right thumb (fig 3A, B). Visual inattention and abnormal eye movements were noted by the parents in the first few weeks of life. Ophthalmological examination showed a right "morning glory" optic disc (a major dysplastic disc with a deep central pit) and a left dysplastic optic disc with a large inferior retinal coloboma. In the Genetics Clinic, unilateral, left Duane anomaly was noted, which was subsequently confirmed by detailed ophthalmological and orthoptic assessment.

She has since then developed bilateral nystagmus secondary to the visual impairment. Her development has otherwise been age appropriate to date.

An EEG, ERG/VER, cranial ultrasound, and MRI scan of the brain were all normal. A renal ultrasound showed mild left pelvicalceal dilatation and an MCUG showed grade 1 vesicoureteric reflux. Audiometry showed bilateral moderate hearing impairment which appeared to be conductive in origin, as there was middle ear effusion on the left and right total meatal occlusion with wax; a sensorineural component, however, cannot be excluded at present. The karyotype was 46,XX.

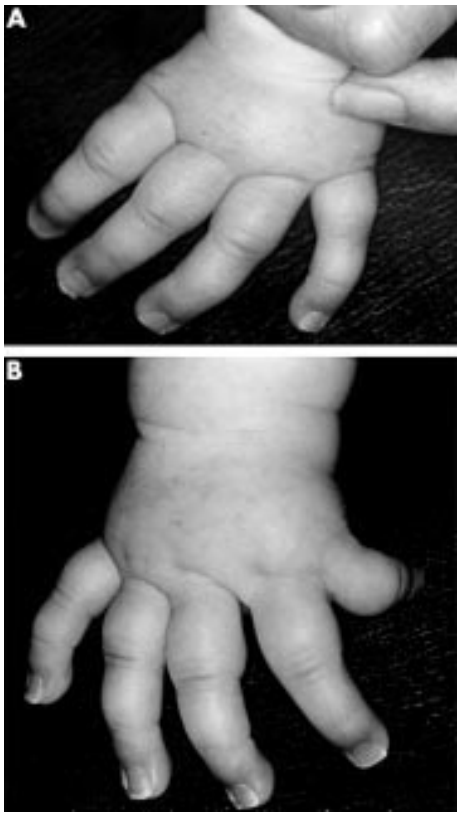

Figure 3 (A) Left hand of patient 3. (B) Right hand of patient 3.

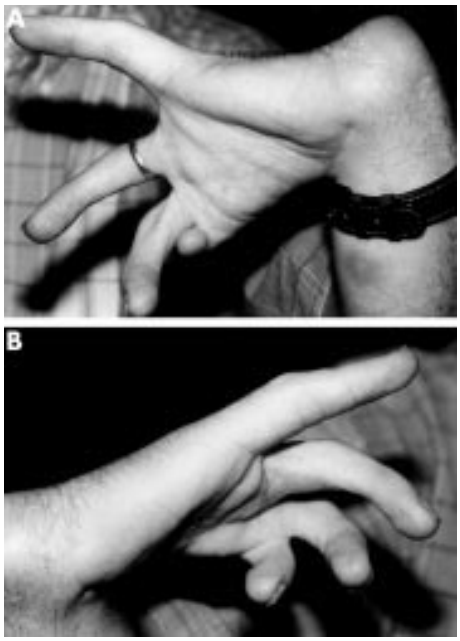

Figure 4 (A) Left hand of patient 4. (B) Right hand of patient 4.

Case 4

The father of case 3 presented with severe upper limb defects at birth which were thought to be the result of thalidomide exposure in utero (fig 4A, B). His mother had taken one single thalidomide tablet during the pregnancy. He was noted to have unilateral, right Duane anomaly in the Genetics Clinic, which was later confirmed by the orthoptist. Ophthalmological examination showed bilateral segmental optic disc hypoplasia. A renal ultrasound was normal. The patient declined a hearing test. His mother had a hearing impairment from an early age, and a female cousin had a left hypoplastic optic disc, but did not have the Duane anomaly.

MUTATIONAL ANALYSIS OF THE *PAX2* GENE

Exons 2, 3, and 4 (containing the paired box domain) and exon 5 (containing the octapeptide sequence) of the *PAX2* gene were investigated for mutations with a combination of single strand conformation polymorphism analysis and direct sequencing as previously described.[15] No mutations in the two functional domains were found in patients 2 and 4.

DISCUSSION

Six families with Okihiro syndrome and six families with acro-renal-ocular syndrome have been reported. Radial ray defects, vertebral anomalies, pre- and postaxial polydactyly, Duane anomaly, and deafness occur in families with either syndrome. Urinary tract anomalies have been described in two families with some features of Okihiro syndrome. One family presented with upper limb defects, Duane anomaly, renal agenesis, and malrotation, but the report preceded delineation of the Okihiro syndrome and the acro-renal-ocular syndrome.[7] In the second family, the affected family members were found to have a supernumerary bisatellited marker chromosome derived from chromosome 22. In addition to renal agenesis, the proband in this family showed other features such as absence of the cervix and uterus with blind ending fallopian tubes, which have not been reported in other Okihiro syndrome families.[16] The lack of reports of renal abnormalities in Okihiro syndrome families may be a reflection of the lack of renal investigations in this patient cohort. Urinary tract abnormalities are a defining feature of the acro-renal-ocular syndrome. Colobomas of the iris, choroid, and optic nerve and other ophthalmological findings

such as optic nerve hypoplasia, microphthalmia, microcornea, cataract, ptosis, and nystagmus are only mentioned in connection with the acro-renal-ocular syndrome, but retinal abnormalities may not have been specifically looked for in the Okihiro patients. The "morning glory" optic disc anomaly has not previously been reported in either syndrome; the association of the Duane anomaly and the "morning glory" anomaly is rare, but has been reported previously.[17] The "morning glory" optic disc anomaly also occurs in renal-coloboma syndrome, which is caused by mutations in the *PAX2* gene, in combination with renal disease. A locus for isolated autosomal dominant Duane anomaly has been mapped to chromosome 2q31.[18 19] It also occurs as part of a contiguous gene syndrome in patients with interstitial deletions of chromosome 8q.[20 21]

Both our families show a combination of radial ray defects, Duane anomaly, renal abnormalities (malrotation, vesicoureteric reflux), and abnormal retinal findings (optic nerve hypoplasia or dysplasia, retinal coloboma, and "morning glory" anomaly of the optic nerve). One of four affected subjects had a significant sensorineural hearing loss and two had preaxial polydactyly. Radiological examinations of the spine were not carried out in our families. No cardiac lesions were identified.

Our two families are the first to show an overlapping phenotype clinically, which suggests that the two syndromes are one clinical entity. To date, no mapping studies have been published and molecular confirmation of this clinical hypothesis is awaited. It has been pointed out by Naito *et al*[9] and Aalfs *et al*[11] that the pattern of malformations is consistent with a disturbance of embryonic development around the fourth to eighth week. The phenotypic resemblance to the pattern of malformations induced by thalidomide is interesting. The mode of action of this well known teratogen is still poorly understood, but as in chondrodysplasia punctata and warfarin embryopathy[22] teratogens and genes can act through common pathways.

Diagnostic difficulties often arise when not all features are present in an affected subject or within a family. In Okihiro syndrome, subjects with radial ray defects only[3 4] or Duane anomaly only[1 2 4] have been described. Clinically normal obligate gene carriers also occur.[4] In the acro-renal-ocular syndrome, renal abnormalities are frequent, but radial ray defects and/or ophthalmological findings may be absent.[8 9] The main features distinguishing Goldenhar syndrome from Okihiro syndrome and the acro-renal-ocular syndrome are the hemifacial microsomia and epibulbar dermoid; in Wildervanck syndrome, it is the absence of limb abnormalities. Epibulbar dermoid and preauricular tags have been described in acro-renal-ocular syndrome[8] and facial asymmetry and microtia have been reported in a subject with Okihiro syndrome.[4] Goldenhar-like features including Duane anomaly can occur in subjects with Townes-Brocks syndrome and mutations in the *SALL1* gene.[23] Renal anomalies, sensorineural hearing loss, and thumb abnormalities are also part of this syndrome, but our families did not show any anal anomalies, and the limb involvement was more extensive. Until molecular studies are carried out to identify the gene(s) involved in the pathogenesis of these syndromes, careful clinical examination and further investigation of affected subjects and family members are the only means of attempting classification. We have excluded a coding sequence mutation in exons 2-5 of the *PAX2* gene in our families by single strand conformation polymorphism analysis and direct sequencing. Most of the mutations reported to date in *PAX2* are within exons 2-5 for as yet unknown reasons.[24 25]

Renal and retinal anomalies need to be specifically looked for in subjects presenting with Duane anomaly and/or radial ray defects.

ACKNOWLEDGEMENTS

We thank Dr Keith Pohl and Dr P N P Devlin for referring the families to us, Liz Tomlin for orthoptic evaluation, and Professor Michael Eccles for his helpful comments.

Authors' affiliations

K Becker, P L Beales, S N Mohammed, Genetics Centre, Guy's and St Thomas's NHS Trust, 7th Floor New Guy's House, Guy's Hospital, London SE1 9RT, UK
D M Calver, Department of Ophthalmology, Guy's Hospital, London SE1 9RT, UK
G Matthijs, Centre for Human Genetics, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium
P L Beales, Molecular Medicine Unit, Institute of Child Health, University College, London WC1N 1EH, UK

Correspondence to: Dr K Becker, The Kennedy-Galton-Centre, Level 8V, Northwick Park Hospital, Watford Road, Harrow, Middlesex HA1 3UJ, UK; k.becker@ic.ac.uk

REFERENCES

1 **Okihiro MM**, Tasaki T, Nakano KK, Bennett BK. Duane syndrome and congenital upper-limb anomalies. *Arch Neurol* 1977;**34**:174-9.
2 **Collins A**, Baraitser M, Pembrey M. Okihiro syndrome: thenar hypoplasia and Duane anomaly in three generations. *Clin Dysmorphol* 1993;**2**:237-40.
3 **Ferrell RL**, Jones B, Lucas RV. Simultaneous occurrence of the Holt-Oram and the Duane syndromes. *J Pediatr* 1966; **69**:630-4.
4 **Hayes A**, Costa T, Polomeno RC. The Okihiro syndrome of Duane anomaly, radial ray abnormalities, and deafness. *Am J Med Genet* 1985;**22**:273-80.
5 **MacDermot KD**, Winter RM. Radial ray defect and Duane anomaly: Report of a family with autosomal dominant transmission. *Am J Med Genet* 1987;**27**:313-19.
6 **Rashad F**, Keith MW, Shields R, Barre PS, Sobel M. Congenital vascular abnormalities in Okihiro's syndrome - a case report. *Angiology* 1987;**38**:642-6.
7 **Temtamy S**, McKusick VA. *The genetics of hand malformations.* New York: Alan R Liss for the National Foundation-March of Dimes. Birth Defects 1978;**XIV**(3):133-5.
8 **Halal F**, Homsy M, Perreault G. Acro-renal-ocular syndrome: autosomal dominant thumb hypoplasia, renal ectopia, and eye defect. *Am J Med Genet* 1984;**17**:753-62.
9 **Naito T**, Kida H, Yokoyama H, Abe T, Takeda S, Uno D, Hattori N. Nature of renal involvement in the acro-renal-ocular syndrome. *Nephron* 1989;**51**:115-18.
10 **Pierquin G**, Hall M, Vanhelleputte C, Van Regemorter N. A new case of acro-renal-ocular (radio-renal-ocular) syndrome with cleft palate and costo-vertebral defects? A brief clinical report. *Ophthal Paediatr* 1991;**12**:183-6.
11 **Aalfs CM**, van Schooneveld MJ, van Keulen EM, Hennekam CM. Further delineation of the acro-renal-ocular syndrome. *Am J Med Genet* 1996;**62**:276-81.
12 **Guillén-Navarro E**, Wallerstein R, Reich E, Zajac L, Ostrer H. Acro-renal-ocular syndrome: expansion of the phenotype. *Clin Dysmorphol* 1998;**7**:243-8.
13 **Sanyanusin P**, Schimmenti LA, McNoe LA, Ward TA, Pierpont MEM, Sullivan MJ, Dobyns WB, Eccles MR. Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. *Nat Genet* 1995; **9**:358-64.
14 **Schimmenti LA**, Cunliffe HE, McNoe LA, Ward TA, French MM, Shim HH, Zhang YH. Further delineation of the renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations. *Am J Hum Genet* 1997;**60**:869-78.
15 **Devriendt K**, Matthijs G, Van Damme B, Van Caesbroeck D, Eccles M, Vanrenterghem Y, Fryns JP, Leys A. Missense mutation and hexanucleotide duplication in the PAX2 gene in two unrelated families with renal-coloboma syndrome. *Hum Genet* 1998;**103**:149-53.
16 **Cullen P**, Rodgers CS, Callen DF, Connolly VM, Eyre H, Fells P, Gordon H, Winter RM, Thakker RV. Association of familial Duane anomaly and urogenital abnormalities with a bisatellited marker derived from chromosome 22. *Am J Med Genet* 1993;**47**:925-30.
17 **Kawano K**, Fujita S. Duane's retraction syndrome associated with morning glory syndrome. *J Pediatr Ophthalmol Strabismus* 1981;**18**:51-4.
18 **Appukuttan B**, Gillanders E, Juo SH, Freas-Lutz D, Ott S, Sood R, Van Auken A, Bailey-Wilson J, Wang X, Patel RJ, Robbins CM, Chung M, Annett G, Weinberg K, Borchert MS, Trent JM, Brownstein MJ, Stout JT. Localization of a gene for Duane retraction syndrome to chromosome 2q31. *Am J Hum Genet* 1999;**65**:1639-46.
19 **Evans JC**, Frayling TM, Ellard S, Gutowski NJ. Confirmation of linkage of Duane's syndrome and refinement of the disease locus to an 8.8-cM interval on chromosome 2q31. *Hum Genet* 2000;**106**:636-8.
20 **Vincent C**, Kalatzis V, Compain S, Levilliers J, Slim R, Graia F, de Lurdes Pereira M, Nivelon A, Croquette MF, Lacombe D, Vigneron J, Helias J, Broyer M, Callen DF, Haan EA, Weissenbach J, Lacroix B,

Bellane-Chantelot C, Le Paslier D, Cohen D, Petit C. A proposed new contiguous gene syndrome on 8q consists of branchio-oto-renal (BOR) syndrome, Duane syndrome, a dominant form of hydrocephalus and trapeze aplasia; implications for the mapping of the BOR gene. *Hum Mol Genet* 1994;**3**:1859-66.
21 **Calabrese G**, Stuppia L, Morizio E, Guanciali Franchi P, Pompetti F, Mingarelli R, Marsilio T, Rocchi M, Gallenga PE, Palka G, Dallapiccola B. Detection of an insertion deletion of region 8q13-q21.2 in a patient with Duane syndrome: implications for mapping and cloning a Duane gene. *Eur J Hum Genet* 1998;**6**:187-93.
22 **Franco B**, Rappold GA, Andria G, Petit C, Ballabio A, Meroni G, Parenti G, Levilliers J, Bernard L, Gebbia M, Cox L, Maroteaux P, Sheffield L. A cluster of sulfatase genes on Xp22.3: mutations in chondrodysplasia punctata (CDPX) and implications for warfarin embryopathy. *Cell* 1995;**81**:15-25.
23 **Kohlhase J**, Taschner PEM, Burfeind P, Pasche B, Newman B, Blanck C, Breuning MH, ten Kate LP, Maaswinkel-Mooy P, Mitulla B, Seidel J, Kirkpatrick SJ, Pauli RM, Wargowski DS, Devriendt K, Proesmans W, Gabrielli O, Coppa GV, Wesby-van Swaay E, Trembath RC, Schinzel AA, Reardon W, Seemanova E, Engel W. Molecular analysis of SALL1 mutations in Townes-Brocks syndrome. *Am J Hum Genet* 1999;**64**:435-45.
24 **Eccles MR**, Schimmenti LA. Renal-coloboma syndrome: a multi-system developmental disorder caused by PAX2 mutations. *Clin Genet* 1999;**56**:1-9.
25 **Amiel J**, Audollent S, Joly D, Dureau P, Salomon R, Tellier AL, Auge J, Bouissou F, Antignac C, Gubler MC, Eccles MR, Munnich A, Vekemans M, Lyonnet S, Attie-Bitach T. PAX2 mutations in renal-coloboma syndrome: mutational hotspot and germline mosaicism. *Eur J Hum Genet* 2000;**8**:820-6.

Branchio-oculo-facial syndrome and branchio-otic/ branchio-oto-renal syndromes are distinct entities

T Trummer, D Müller, A Schulze, W Vogel, W Just

J Med Genet 2002;**39**:71–74

Branchio-oculo-facial syndrome (BOF, MIM 113620[1]) is a rare autosomal dominant disorder. The symptoms of this disorder include bilateral postauricular cervical branchial sinus defects with haemangiomatous, scarred skin, cleft lip with or without cleft palate, pseudocleft of the upper lip, nasolacrimal duct obstruction, low set ears with posterior rotation, a malformed, asymmetrical nose with a broad bridge and flattened tip, and, occasionally, prematurely grey hair. Father to son transmission of this disorder has been observed,[2] which indicate autosomal dominant inheritance. Another disorder with hearing loss resulting from bilateral branchial cleft fistulas is branchio-oto-renal syndrome (BOR, MIM 113650). Common features of both syndrome are summarised in table 1. Some characteristics of both BOR and BOF syndromes have been reported in a father (BOF) and his son (BOR), but the constant features of BOF syndrome were not observed in either of them. This observation led to the conclusion that BOF and BOR might be allelic variants of the same gene.[3] It was suggested that, in both syndromes, penetrance and expression could be variable, and it was concluded that BOF and BOR syndromes are the variable results of mutations in the same autosomal gene.[3] However, it was pointed out later that both subjects in fact should be considered as BOF syndrome rather than BOF and BOR syndrome, and that these syndromes are distinct entities and may not be allelic.[4] Another related disorder is branchio-otic syndrome (BO, MIM 602588), which comprises branchial fistulas, preauricular pits, and hearing impairment, but lacks renal anomalies (table 1).

The first candidate gene for BOR has been mapped. This gene, *EYA1* ("eyes absent-like", a human homologue of the *Drosophila eyes absent* gene), was found by positional cloning[5] and maps to chromosome 8q13.3. Mutations in *EYA1* have been described,[6–8] which made it a candidate gene for BOR syndrome. The authors of the first report[8] concluded that BO and BOR syndromes are allelic. The hunt for a candidate gene in BOF syndrome was more difficult, because only a few familial cases exist[9] that could be studied. Since an allelic variant of BOF and BOR syndromes was not dispelled conclusively, several independent attempts have been undertaken to study the *EYA1* gene region as a candidate gene region for BOF syndrome. By sequence analysis, no mutations were found in the *EYA1* gene in five BOF syndrome patients.[7] This suggests once more that BOR syndrome might not be allelic to BOF syndrome. *EYA1* is a member of a gene family comprising at least four genes (*EYA1-EYA4*). *EYA1* is expressed during embryogenesis in the branchial arches and the somites and during limb development in connective tissue precursors.[10] At the tailbud stage of zebrafish, its expression is confined to cranial placodal precursors and, thereafter, to the anterior pituitary, olfactory, and otic placodes.[11] The expression of the other members of the *EYA* gene family, *EYA2-3*, is similar to the *EYA1* expression pattern.[10–12] The *EYA4* pattern, however, is confined to the dermamyotome and the limb, and expression was not found in the branchial arches.[13] The expression patterns in early embryogenesis together with the developmental defects in BOF syndrome prompted a segregation analysis for these four genes in a large pedigree with BOF syndrome, but no cosegregation of the disorder with genetic markers was found.[14] The latter study excluded the *EYA* genes as candidates for BOF syndrome.

Recently, a second gene locus (BOR2) for a BOR syndrome-like phenotype was mapped to human chromosome 1q31.3-q32.1.[15] Linkage between the BOR syndrome related disorder branchio-otic syndrome (BO) and marker D1S2757 was observed with a maximum lod score of 4.81 at a recombination fraction of zero.[16] The variability of the clinical phenotype of BOF syndrome and the overlapping symptoms with BOR or BO syndromes prompted us to perform a segregation analysis in this second candidate gene region of BO/BOR syndrome in order to verify whether the clinical differences reflect allelic variants of the same gene. In the present study, we show that BOF syndrome is not allelic to BOR/BO syndromes at this locus. This, taken together with previous reports, is a second proof based on genetic studies that BOR and BOF syndromes are distinctive entities. Our findings firmly support former hypotheses on the distinctiveness of these syndromes, which were based solely on the clinical phenotype.

MATERIAL AND METHODS

The analyses were performed on DNA of patients with BOF syndrome from one family, which represents the largest published BOF pedigree with five affected and two unaffected members (fig 1). The family studied here was described earlier in a review including photographs of the patients with the characteristic features of BOF syndrome[9] (ID 10-14). DNA was extracted from peripheral blood lymphocytes by standard techniques.[17] Screening for mutations in the candidate gene