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Report

Genomewide Search and Genetic Localization of a Second Gene Associated with Autosomal Dominant Branchio-Oto-Renal Syndrome: Clinical and Genetic Implications

Shrawan Kumar,[1] Karen Deffenbacher,[1] Henri A. M. Marres,[2] Cor W. R. J. Cremers,[2] and William J. Kimberling[1]

[1]Department of Genetics, Center for Hereditary and Communication Disorders, Boys Town National Research Hospital, Omaha; and [2]Department of Otorhinolaryngology, University Hospital Nijmegen, Nijmegen, The Netherlands

Branchio-oto-renal (BOR) syndrome is characterized by ear malformations, cervical fistulas, hearing loss, and renal anomalies. It is an autosomal dominant disorder with variable clinical manifestations. The most common features of BOR syndrome are branchial, hearing, and renal anomalies. However, many affected subjects have been observed with branchial-cleft anomalies and hearing loss but without renal anomalies, a condition called "branchio-otic" (BO) syndrome. It is logical to question whether the BOR and BO syndromes are allelic or whether they represent distinct genetic entities. We identified a very large extended family whose members had branchial and hearing anomalies associated with commissural lip pits that segregated in an autosomal dominant fashion. Using a genomewide search strategy, we identified genetic linkage, with a maximum LOD score of 4.81 at recombination fraction 0, between the BO phenotype and polymorphic marker D1S2757 in the genetic region of chromosome 1q31. This is the first report of linkage for a second gene associated with BOR syndrome. The findings have important clinical implications and will provide insight into the genetic basis of BOR syndrome.

Branchio-oto-renal (BOR [MIM 113650]) syndrome is a developmental disorder characterized by branchial cleft cysts, renal anomalies, hearing loss, and other otologic manifestations. It is an autosomal dominant disorder, with ear pits, branchial clefts, and hearing anomalies being the symptoms that are expressed most frequently. The association between ear anomalies and cervical fistula was first described in the late 19th century (Asherson 1834; Heusinger 1864; Paget 1878). However, the syndrome was not well recognized until the publication of the genetic mode of inheritance of branchial and hearing anomalies (Precechtel 1927; Fourman and Fourman 1955). Since then, several other researchers have published reports about BOR syndrome, and more-precise clinical definition has emerged (Fraser et al. 1978; Melnick et al. 1978; Cremers and Fikkers-Van Noord 1980). Many other clinical features—such as abnormalities of the face, palate, ureters, and bladder, dysfunction of the lacrimal system, otitis media, and shoulder abnormalities—have also been associated with BOR syndrome (Fitch and Srolovitz 1976; Cremers and Fikkers-Van Noord 1980; Preisch et al. 1985; Heimler and Lieber 1986; Pennie and Marres 1992). However, the issue of genetic heterogeneity associated with clinical variations such as branchio-otic (BO), branchio-renal (BR), branchio-oculo-urethral (Fraser et al. 1983), and branchio-oculo-facial (McCool and Weaver 1994; Lin et al. 1995) syndromes, remains unresolved.

The incidence of BOR syndrome is ~1:40,000 and has been reported to occur in 2% of profoundly deaf children (Fraser et al. 1980). The first BOR syndrome gene has been mapped to chromosome 8q (Kumar et al. 1992, 1996; Smith et al. 1992); recently, the EYA1 gene (homologous to the *Drosophila* developmental gene denoted as "eyes absent") underlying this syndrome has been identified, and several mutations have been reported (Abdelhak et al. 1997; Kumar et al. 1998*a*, 1998*b*). The clinical expression of the BOR-syndrome gene is extremely variable from one family to the next

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Address for correspondence and reprints: Dr. Shrawan Kumar, Department of Genetics, Boys Town National Research Hospital, 555 North 30th Street, Omaha, NE 68131. E-mail: Kumar@boystown.org

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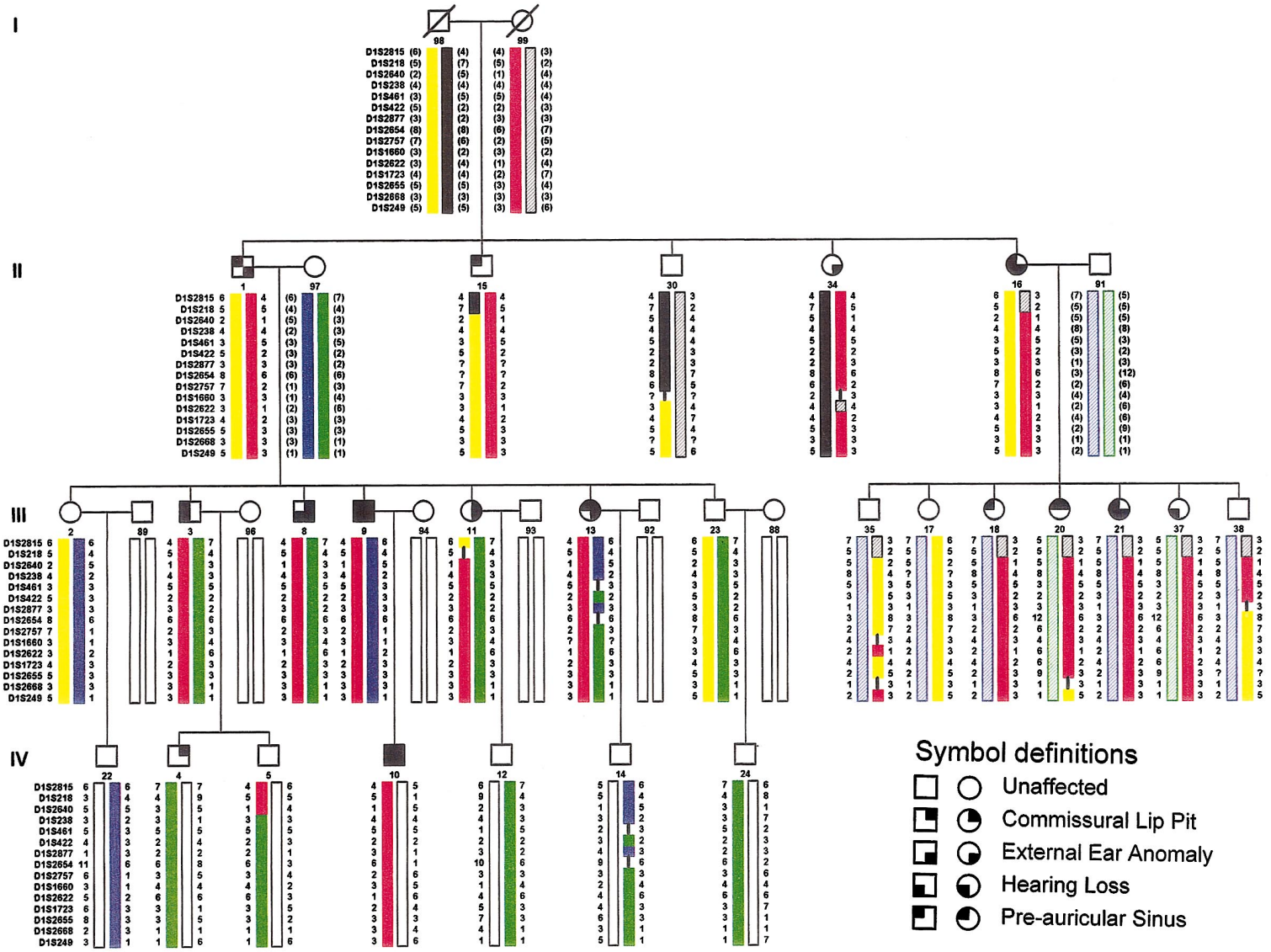

Figure 1 Genotypic data for 1q markers, listed in centromere-to-telomere order, for the kindred with BO. Haplotype results that are associated with affected status are indicated by a red bar. The genotypes shown in parentheses are inferred from their children.

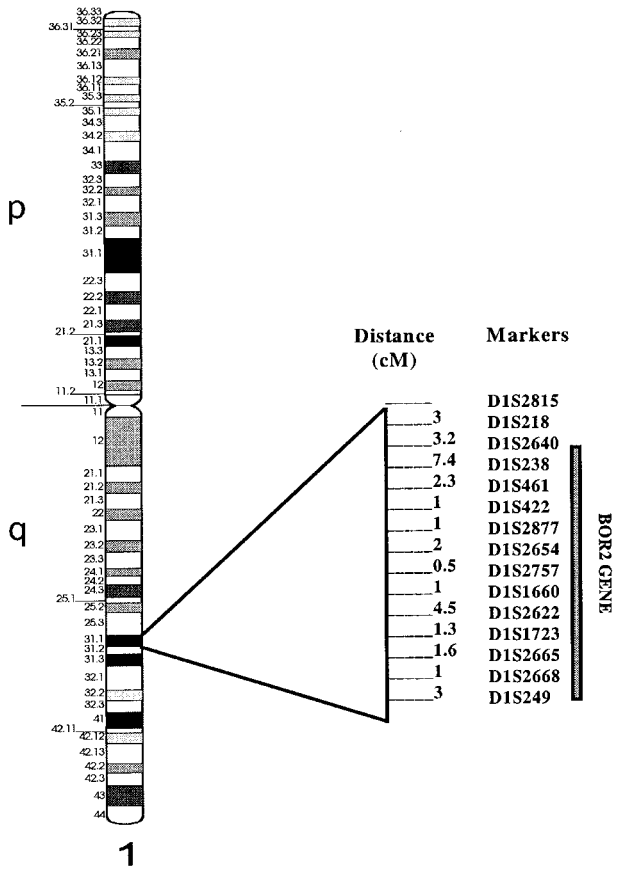

Figure 2 Ideogram of chromosome 1. The genetic distance between markers is presented, and the approximate location of the BO gene is indicated by a vertical bar.

(Fraser et al. 1978; Melnick et al. 1978; Cremers and Fikkers-Van Noord 1980; Heimler and Lieber 1986). Clinical studies suggest that there are at least three distinct syndromes involved in the branchiogenic disorders: the first, BOR dysplasia, is associated with renal anomalies; but the second, BO dysplasia, lacks renal anomalies; and the third, BR anomalies, results in branchial and renal disorders with no associated hearing loss. The question arises as to whether BOR syndrome, BO, and BR are due to (*a*) different alleles at the same locus, (*b*) mutations at totally separate loci, or (*c*) "normal" variation in a highly diverse phenotype.

Identification of a second genetic locus associated with BOR syndrome would provide important understanding about the spectrum of defects associated with branchial and hearing anomalies. The familial disorder described in the present study resembles the clinical phenotypes of BOR syndrome. We have identified a very large family in whose members affected with BO the disease gene segregates in an autosomal dominant fashion, with no evidence of linkage to markers in the chromosome 8q region (Kumar et al. 1998*c*). As a part of ongoing study, the purpose of the present investigation was to identify the genetic locus in this family, by linkage, as a first step toward identification of the gene defect responsible for BO. Conclusive evidence of genetic linkage with markers on chromosome 1q31 was obtained, establishing a genetic heterogeneity associated with BOR syndrome.

A multigenerational BO family was identified when the proband visited the clinic at the University Hospital Nijmegen, The Netherlands. Subsequently, several family members were contacted, and blood samples were collected. A total of 55 individuals, consisting of 32 male subjects, 7 of whom had BO, and 23 female subjects, 8 of whom have BO, were studied in this large family. The family members are scattered throughout The Netherlands; they have been consistent in undergoing medical investigation or treatment. Recently, ⩽35 individuals have undergone clinical evaluation at a hospital in The Netherlands. All individuals received a clinical examination, which included general medical and detailed otorhino-laryngological evaluations. Pure-tone audiometry was performed on all persons of age >3.5 years. If this consistently showed conductive or mixed hearing loss, an impedance test also was performed. Renal examination included analysis of blood and urine chemistry, renal ultrasonography, and intravenous pyelography. The details of clinical evaluation have been described elsewhere (Marres et al. 1994).

There is a high degree of variability in the phenotypic expression of BOR syndrome, observed both among patients with both typical (classic) and among those with atypical manifestations. This is observed both within and between families. In typical BOR syndrome, many

Table 1

Two-Point LOD Scores of Different Polymorphic Markers on Chromosome 1q, for BO Syndrome

	LOD Score at θ =[a]					
Marker	.0	.05	.1	.2	.3	.4
D1S2815	$-\infty$	-.95	-.03	.55	.56	.29
D1S218	$-\infty$	-1.15	-.58	-.10	.05	.07
D1S2640	$-\infty$	2.58	2.53	2.07	1.40	.60
D1S238	1.28	1.16	1.04	.79	.52	.26
D1S461	1.87	1.88	1.74	1.31	.78	.25
D1S422	3.91	3.58	3.23	2.46	1.62	.70
D1S2877	.65	.58	.51	.36	.22	.10
D1S2654	3.59	3.28	2.95	2.47	1.46	.61
D1S2757	4.81	4.41	3.99	3.08	2.06	.94
D1S1660	1.42	1.30	1.17	.91	.63	.32
D1S2622	$-\infty$	3.13	3.04	2.48	1.69	.76
D1S1723	4.81	4.41	3.99	3.08	2.06	.94
D1S2665	3.59	3.28	2.95	2.24	1.46	.61
D1S2668	1.20	1.09	.97	.72	.46	.20
D1S249	$-\infty$	1.85	2.08	1.87	1.32	.60

[a] θ values for males were assumed to be equal to those for females.

organs are involved, and there are branchial, hearing, and renal anomalies, whereas an atypical case might include involvement of only one or a few organs (Fraser et al. 1978, 1980; Melnick et al. 1978), such as in the case of BO or BR. The reduced penetrance associated with BOR syndrome might pose a problem in linkage analysis. Therefore, our criteria for inclusion of affected individuals in the analysis were very stringent, to avoid any spurious linkage. The disease in affected members of this family is characterized by ear anomalies, preauricular sinuses, deafness, and commissural lip pits.

For purposes of linkage analysis, individuals with two or more of these major findings were considered to be affected. People with only one component of the disorder were characterized as "unknown." Members who did not undergo clinical evaluation also were characterized as being of "unknown" status. All people who had married into the family were assumed to be unaffected. The analysis was performed with full penetrance, as well as with 0.8-adjusted penetrance. Phenotypically, affected members of the family had the branchial and otic abnormalities typical of BO syndrome, with the addition of commissural lip pits. After localization of the BOR-syndrome gene on chromosome 8q, we investigated members of this family by use of 8q markers and did not find cosegregation with either the BO phenotype or mutations in the EYA1 gene (Kumar et al. 1998*c*). This result indicates that BO syndrome is not allelic to BOR syndrome. Both the portion of the pedigree relevant to genetic-linkage study and the haplotype data are shown in figure 1.

Fluorescently labeled microsatellite markers from all 27 panels of the ABI Prism Linkage mapping set, version 1, were typed for the family members with BO. These panels span all of the chromosomes, with a ~10-cM resolution between markers. Additional markers were also obtained from Integrated DNA Technologies, to refine the BOR2 region. Resulting genotype data were analyzed by use of GENESCAN 2.1 and GENOTYPER 2.0 software from ABI. Linkage analysis was performed on a personal computer using the LINKAGE computer package, version 5.1. Two-point linkage analysis was performed by use of FASTLINK, version 3.0 (Cottingham et al. 1993), and the multipoint analysis was done by use of the memory-efficient computer program VITESSE (O'Connell and Weeks 1995). LOD scores were calculated by use of a model of an autosomal dominant mode of inheritance. The marker-allele frequency was assumed to be uniformly distributed. Distances between markers are shown in figure 2.

With use of ABI panel markers, a genomewide search was performed on the selected informative individuals in the family. Initial linkage analysis with ABI panel markers yielded a maximum two-point LOD score of 1.2, at recombination fraction (θ) .05, for marker D1S249. More family members were then included in the next analysis, to increase the linkage information. Several other neighboring markers also were genotyped. A linkage with a maximum LOD score of 4.8, for marker D1S2757, was then observed. This establishes the presence of a second genetic locus associated with BOR syndrome; henceforth, it will be referred to as "BOR2." Once the linkage was established, a high-density map of additional closely spaced markers was obtained from the human-genome database for the BOR2 critical region. Two-point LOD scores, calculated under an autosomal dominant model, for several markers spanning the candidate interval region are summarized in table 1.

We repeated the multipoint analysis of the critical region, using 15 polymorphic markers. Map distances used in the analysis are presented in figure 1. The analysis was done by use of two penetrance models. Under the model of reduced penetrance (with a penetrance of .8), the maximum LOD score of 4.32 was observed between markers D1S2654 and D1S2757, whereas the full-penetrance model yielded a maximum LOD score of 4.91 at a position between markers D1S2757 and D1S1660. The results of the multipoint analysis suggest that the likely location of the BO gene is within the 23.6-cM interval between D1S2640 and D1S2668 (fig. 3). In light of the marker haplotype segregating with BO (fig. 2), it can be seen that a critical recombination event that limits the assignment of BO to the region distal to D1S2640 was observed in individual II-16. The pedigree also displays crossover in individual III-20, limiting the assignment of BO to a position proximal to D1S249. Individual 35 in generation III provides provisional data,

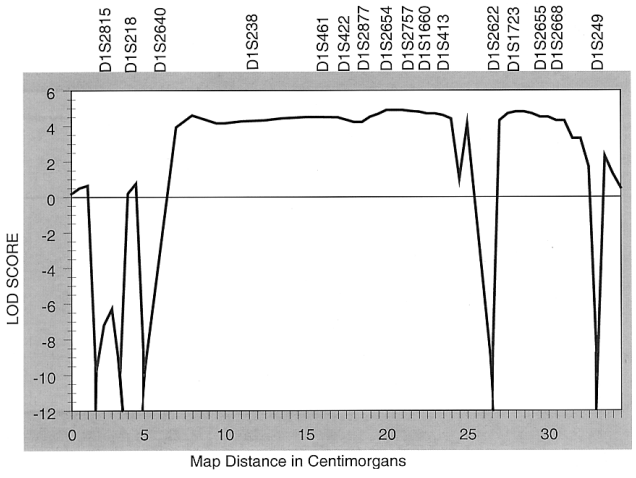

Figure 3 Multipoint analysis. Multipoint LOD scores observed between the BO phenotype and markers on the chromosome 1q31 region are presented on the *X*-axis, and the positions of markers loci are located on the *Y*-axis. The order of markers and the genetic θ used in the analyses are as in figure 1.

analysis of which suggests that the gene might be proximal to D1S2622. This conclusion must be considered tentative, because of the problem regarding penetrance in this family. Individual III-38 is not included in the analysis, since the clinical data were inconclusive.

The 1q31 region is known to contain the locus for van der Woude syndrome (VWS), a highly penetrant craniofacial disorder with an autosomal dominant mode of inheritance. VWS maps (1q32-41) close to the BOR2 region and is characterized by lip pits, clefting of the primary or secondary palate, and hypodontia. Since, in the family that we studied, several members with BO had commissural lip pits, we decided to investigate the 1q31 region, for any possible relationship. Markers D1S2692, D1S245, D1S505, D1S419, and D1S2629 have been mapped to the region of VWS (Sander et al. 1995). Therefore, in the family under study, we have typed this marker in the members with BO. The two-point result suggests that the BO gene is not within the VWS region.

In the present analysis, only individuals who had distinctive clinical features (i.e., presence of more than one clinical phenotype) were included in the analysis; for example, family members 15 and 34 in generation II and family members 18 and 37 in generation III (fig. 2) had only one clinical phenotype, and these individuals were excluded from the linkage analysis, to avoid any spurious results in the initial screening process. However, it is interesting to note that individual 4 in generation IV has only commissural lip pits, which we initially considered to be one of the clinical features strongly indicative of this syndrome. Clearly, this individual did not inherit from his parents the affected segment of chromosome 1. This result suggests that, in members of this family, the commissural lip pits are perhaps not associated with BO and are segregating independent of this syndrome. Further studies will be needed to confirm this possibility.

In this report, we have presented evidence of a second genetic locus for BO syndrome. Linkage was performed by use of two different models, one with a reduced penetrance level and the other with complete penetrance. Both models gave essentially similar results and demonstrated evidence that a gene in this region of chromosome 1q is involved in the etiology of branchial and hearing anomalies. The genetic-linkage analysis was limited to this large, multigenerational family. Indeed, we have a large data set of families, both large and small, whose members have BOR syndrome. Since there is a great deal of clinical variability associated with BOR syndrome, it is quite possible that several genes are involved in this developmental disorder, and it is difficult to evaluate the possibility of linkage in members of the smaller families. Therefore, our first attempt was to screen all families for mutations in the EYA1 gene, to determine the proportion of families without linkage to the chromosome 8q region. Surprisingly, ~70% of our large data set of families whose members have BOR syndrome do not show mutations in the EYA1 gene (Kumar et al. 1998*a*, 1998*b*). This further complicates the issue and suggests either that most of the mutations lie in the untranslated region or that several genes are involved in the branchiogenic disorder. At present, it is difficult to evaluate what percentage of families will have linkage to 1q or 8q, since most of the families were not sufficiently large to give conclusive evidence of linkage. However, identification of the second gene will help resolve this issue. Given the fact that the BOR syndrome phenotype is highly heterogeneous and that other families with BO have been shown not to have linkage to the 8q region (Stratakis et al. 1998), it is possible that there is a third responsible locus in branchiogenic disorders.

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Electronic-Database Information

The accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for BOR syndrome [MIM 113650])

References

Abdelhak S, Kalatzis V, Heilig R, Compain S, Samson D, Vincent C, Weil D, et al (1997) A human homologue of the *Drosophila* eyes absent gene underlies branchio-oto-renal (BOR) syndrome and identifies a novel gene family. Nat Genet 15:157–164

Asherson FM (1834) De fistulis collii congenitalis. Phobus Preuss ver. Zeitung, Berlin

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–263

Cremers CW, Fikkers-Van Noord M (1980) The earpits-deafness syndrome: clinical and genetic aspects. Int J Pediatr Otorhinolaryngol 2:309–322

Fitch N, Srolovitz H (1976) Severe renal dysgenesis produced by a dominant gene. Am J Dis Child 130:1356–1357

Fourman P, Fourman J (1955) Hereditary deafness in a family

with ear-pits (fistula auris congenital). Br Med J 2:1354–1356

Fraser FC, Ayme S, Halal F, Sproule J (1983) Autosomal dominant duplication of the renal collection system, hearing loss, and external ear anomalies: a new syndrome. Am J Med Genet 14:473–478

Fraser FC, Ling D, Clogg D, Nogrady B (1978) Genetic aspects of the BOR syndrome—branchial fistulas, ear pits, hearing loss, and renal anomalies. Am J Med Genet 2:241–252

Fraser FC, Sproule JR, Halal F (1980) Frequency of the branchio-oto-renal (BOR) syndrome in children with profound hearing loss. Am J Med Genet 7:341–349

Heimler A, Lieber E (1986) Branchio-oto-renal syndrome: reduced penetrance and variable expressivity in four generations of a large kindred. Am J Med Genet 25:15–27

Heusinger CF (1864) Hals Kiemen Fisteln von noch nicht beobachteter Form. Virchow Arch Pathol Anat 29:358

Kumar S, Deffenbacher K, Cremers CW, Van Camp G, Kimberling WJ (1998*a*) Branchio-oto-renal syndrome: identification of novel mutations, molecular characterization, mutation distribution, and prospects for genetic testing. Genet Test 1:243–251

Kumar S, Kimberling WJ, Kenyon JB, Smith RJ, Marres HA, Cremers CW (1992) Autosomal dominant branchio-oto-renal syndrome—localization of a disease gene to chromosome 8q by linkage in a Dutch family. Hum Mol Genet 1:491–495

Kumar S, Kimberling WJ, Lanyi A, Sumegi J, Pinnt J, Ing P, Tinley S, et al (1996) Narrowing the genetic interval and yeast artificial chromosome map in the branchio-oto-renal region on chromosome 8q. Genomics 31:71–79

Kumar S, Kimberling WJ, Weston M, Schaefer BG, Anne Berg M, Marres HAM, Cremers CWRJ (1998*b*) Identification of three novel mutations in human EYA1 protein associated with branchio-oto-renal syndrome. Hum Mutat 11:443–449

Kumar S, Marres HAM, Cremers CWRJ, Kimberling WJ (1998*c*) Autosomal dominant branchio-otic (BO) syndrome is not allelic to the branchio-oto-renal (BOR) gene at 8q13. Am J Med Genet 76:395–401

Lin AE, Gorlin RJ, Lurie IW, Brunner HG, van der Burgt I, Naumchik IV, Rumyantseva NV, et al (1995) Further delineation of the branchio-oculo-facial syndrome. Am J Med Genet 56:42–59

Marres HAM, Cremers CWRJ, Huygen PLM, Joosten FBM (1994) The deafness, pre-auricular sinus, external ear anomaly and commissural lip pits syndrome—otological, vestibular and radiological findings. J Laryngol Otol 108:13–18

McCool M, Weaver DD (1994) Branchio-oculo-facial syndrome: broadening the spectrum. Am J Med Genet 49: 414–421

Melnick M, Hodes ME, Nance WE, Yune H, Sweeney A (1978) Branchio-oto-renal dysplasia and branchio-oto-dysplasia: two distinct autosomal dominant disorders. Clin Genet 13:425–442

O'Connell JR, Weeks DE (1995) The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. Nat Genet 11:402–408

Paget J (1878) Cases of branchial fistulae in the external ears. Rev Med Chir Soc Lond 61:41

Pennie BH, Marres HAM (1992) Shoulder abnormalities in association with branchio-oto-renal dysplasia in a patient who also has familial joint laxity. Int J Pediatr Otorhinolaryngol 23:269–273

Precechtel A (1927) Pedigree of anomalies in the first and the second branchial cleft, inherited according to laws of Mendel and contribution to technique of extirpation of congenital lateral fistulae colli. Acta Otolaryngol 11:23–30

Preisch JW, Bixler D, Ellis FD (1985) Gustatory lacrimation in association with the branchio-oto-renal syndrome. Clin Genet 27:506–509

Sander A, Murray JC, Scherpbier-Heddema T, Buetow KH, Weissenbach J, Zingg M, Ludwig K, et al (1995) Microsatellite-based fine mapping of the Van der Woude syndrome locus to an interval of 4.1 cM between D1S245 and D1S414. Am J Hum Genet 56:310–318

Smith RJH, Coppage KB, Ankerstjerne JKB, Capper DT, Kumar S, Kenyon J, Kimberling WJ (1992) Localization of the gene for branchiootorenal syndrome to chromosome 8q. Genomics 14:841–844

Stratakis CA, Lin J-P, Rennert OM (1998) Description of a large kindred with autosomal dominant inheritance of branchial arch anomalies, hearing loss and ear pits, and exclusion of the branchio-oto-renal (BOR) syndrome gene locus (chromosome 8q13.3). Am J Med Genet 79:209–214