A Novel Loss-of-Function Mutation in *HOXB1* Associated with Autosomal Recessive Hereditary Congenital Facial Palsy in a Large Iranian Family

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Keywords

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Abstract

Hereditary congenital facial palsy (HCFP) is a rare congenital cranial dysinnervation disorder, recognisable by non-progressive isolated facial nerve palsy (cranial nerve VII). It is caused by developmental abnormalities of the facial nerve nucleus and its nerve. So far, 4 homozygous mutations have been identified in 5 unrelated families (12 patients) with HCFP worldwide. In this study, a large Iranian consanguineous kindred with 5 members affected by HCFP underwent thorough clinical and genetic evaluation. The candidate gene *HOXB1* was screened and analysed by Sanger sequencing. As in previous cases, the most remarkable findings in the affected members of the family were mask-like faces, bilateral facial palsy with variable sensorineural hearing loss, and some dysmorphic features. Direct sequencing of the candidate gene *HOXB1* identified a novel homozygous frameshift mutation (c.296_302del; p.Y99Wfs*20) which cosegregated with the disease phenotype within the extended family. Our findings expand the mutational spectrum of *HOXB1* involved in HCFP and consolidate the role of the gene

in the development of autosomal recessive HCFP. Moreover, the truncating mutation identified in this family leads to a broadly similar presentation and severity observed in previous patients with nonsense and missense mutations. This study characterises and defines the phenotypic features of this rare syndrome in a larger family than has previously been reported.

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Hereditary congenital facial palsy (HCFP) is a rare condition belonging to the family of congenital cranial dysinnervation disorders (CCDD) and is characterised by the paralysis or weakness of facial muscles due to underdevelopment of the facial branchiomotor nucleus and its nerves (particularly the VI and VII cranial nerves) [Terzis and Anesti, 2011; Gutowski and Chilton, 2015; Chilton and Guthrie, 2016]. Cranial nerve dysfunction often presents at birth with multiple problems, mainly due to the functional and aesthetic consequences such as inadequate sucking and feeding as well as lack of facial expression [Ciorba et al., 2015]. Although HCFP and Moebius syndrome (MBS) share some clinical features, they are different clinical entities with a different pathogenesis [MacKinnon et al., 2014]. MBS (MIM 157900) is

usually sporadic and associated with the involvement of other cranial nerves, commonly the abducens nerve in addition to orofacial and limb malformations as well as defects of the musculoskeletal system [MacKinnon et al., 2014]. De novo mutations in 2 genes, *PLXND1* and *REV3L*, were recently found to be associated with MBS [Tomas-Roca et al., 2015]. Congenital facial palsy (CFP) is classified as acquired or developmental, unilateral or bilateral, and complete (palsy) or incomplete (paresis). A careful diagnostic work-up and differential diagnosis are important in order to determine the aetiology, as the prognosis and the therapeutic approach differs, depending on the underlying pathophysiology [Terzis and Anesti, 2011; Ciorba et al., 2015]. Based on previous studies, genetic heterogeneity and different modes of inheritance have been suggested for this disorder. Two genetic loci, termed HCFP1 (MIM %601471) and HCFP2 (MIM %604185), have been defined in 2 families with autosomal dominant HCFP, but neither gene has been identified [Kremer et al., 1996; Verzijl et al., 1999; Michielse et al., 2006]. The only known autosomal recessive HCFP (HCFP3) is caused by biallelic mutations in *HOXB1* (MIM %142968) in chr17q21.32 (MIM 614744). Recently, 4 mutations (3 missense and 1 nonsense) in the *HOXB1* gene have been identified in 12 patients from 5 unrelated consanguineous families with HCFP3 (Fig. 1) [Webb et al., 2012; Uyguner et al., 2015; Vogel et al., 2016; Sahin et al., 2017]. *HOXB1* is a homeobox transcription factor which is part of a developmental regulatory system expressed in the embryological spinal cord and hind-brain and is essential for rhombomere 4 (r4) development [Webb et al., 2012].

This study presents an Iranian extended consanguineous family in which 5 members suffer from HCFP. Genetic screening of the only currently known candidate gene for the condition revealed a novel homozygous truncating mutation in *HOXB1*.

Patients and Methods

The patient medical records were reviewed and clinical examinations including ophthalmological and auditory exams were performed. A detailed family history was collected and a pedigree was drawn. Blood samples from the 5 affected members of the family as well as their parents and unaffected siblings were obtained for genetic testing.

Genetic Study

Genomic DNA was extracted from blood samples using Qiagen Gentra Puregene DNA isolation kits (Qiagen, Valencia, CA, USA). Mutation analysis was performed by PCR amplifying all exons and

exon-intron boundaries of the *HOXB1* gene, using 2 pairs of primers. All the resulting amplicons were Sanger sequenced. Sequencing results from affected and unaffected individuals were analysed using FinchTV Software. Primer sequences and screening conditions are available upon request.

Results

We investigated a large Iranian consanguineous kindred including 5 affected individuals (Fig. 1A; V-2, 2 years old; V-5, 9 years old; V-7, 11 years old; VI-3, 24 years old, and VI-18, 25 years old) who were initially suspected to have MBS. The initial clinical presentation was congenital non-progressive mask-like faces and bilateral facial palsy. Due to facial muscle weakness and paresis, these individuals could not smile, frown, or cry. They had severe feeding difficulty and inadequate suckling of milk during infancy; however, they could discriminate tastes. Salivation and lacrimation were both intact. Two patients also had congenital mixed, sensorineural and conductive bilateral hearing loss and were using hearing aids. Their hearing impairment was non-progressive and moderate to severe. Additional clinical features of the patients were facial dysmorphism, including a smooth philtrum, low-set ears, external ear malformation (in 3 patients), mid-face retrusion, upturned nasal tip, and tooth abnormalities (Fig. 1C). Brain CT scans performed on 2 affected individuals did not identify any abnormality. The karyotype of one patient was examined and found to be normal. All 5 affected individuals showed typical signs of HCFP with full eye motility and no eye movement abnormalities. Based on the extended 3-generation pedigree, with the presence of consanguinity and healthy parents, an autosomal recessive mode of inheritance was suggested for the disease.

The disease phenotype and family history was not consistent with a diagnosis of MBS; we therefore screened the only known autosomal recessive HCFP-causing gene *HOXB1*. Direct sequencing of the *HOXB1* gene identified a novel 7-bp deletion – (NM_002144.3, hg19) chr17: g.46607965_46607971delAGAGGGT; c.296_302del; Y99Wfs*20 – present in a homozygote state in the first coding exon (Fig. 1B). The mutation resulted in a frameshift predicted to cause premature termination of the protein. This variant has not been reported in a heterozygous or homozygous state in publicly available databases, including dbSNP (build 138), the Exome Sequencing Project databases (<http://evs.gs.washington.edu/EVS/>), genomeAD browser (<http://gnomad.broadinstitute.org/>).

Fig. 1. A Pedigree of the Iranian family with 5 individuals affected by hereditary congenital facial palsy (indicated as solid black symbols). Segregation of the loss-of-function mutation in the family with the mutant allele is shown by + and the wild-type allele is shown by -. The ages of the affected individuals are shown below

the symbols. **B** A section of Sanger sequencing chromatograms for the homozygous mutation (bottom) and wild type (top) are depicted and demonstrate the deletion (box). **C** Pictures of the patients corresponding to the pedigree.

tute.org), the 1000 Genome Project (<http://1000genomes.org/>), The Greater Middle East Variome project (<http://igm.ucsd.edu/gme/index.php>) or our in-house database of 500 Iranian Whole Exome Sequencing. The mutation completely co-segregates with the disease phenotype in this large family (Fig. 1A). Clinical features of the affected family members are summarised in Table 1 and compared to previously reported patients with *HOXB1* mutations.

Discussion

Until now, there are 17 HCFP patients reported worldwide, including 5 patients from this study, in whom causative mutations have been identified (Fig. 2) [Webb et al., 2012; Uyguner et al., 2015; Vogel et al., 2016; Sahin et al., 2017]. Our findings consolidate the role of *HOXB1* in autosomal recessive HCFP and expand the mutational and phenotypic spectrum of *HOXB1* variations in causing CCDD. The mutation we report here would likely result in a null mutation due to nonsense-mediated decay of the

Fig. 2. A schematic representation of *HOXB10* homozygous mutations identified so far indicated by the red arrows.

Table 1. Clinical features of affected members of the Iranian family with HCFP and previously reported cases

Features	Present family	Sahin et al., 2017	Vogel et al., 2016	Uyguner et al., 2015	Webb et al., 2012
Molecular findings					
NM_002144.3	c.296_302del	c.688C>T	c.66C>G	c.620G>A	c.619C>T
NP_002135.2	p.Y99Wfs*20	p.Arg230Trp	p.Tyr22*	p.Arg207His	p.Arg207Cys
Localization	exon 1	exon 2	exon 1	exon 2	exon 2
Protein domain	–	homeodomain	N terminal arm	homeodomain	homeodomain
Origin	Iranian	Turkish	Moroccan	Turkish	German American
Gender	F (2), M (3)	F (2), M (1)	F (1), M (3)	F (1)	F (1), M (3)
Consanguinity	5/5	2/3	4/4	0/1	2/4
Craniofacial findings					
Masked facies	5/5	3/3	4/4	1/1	4/4
Bilateral facial weakness	5/5	3/3	4/4	1/1	4/4
Full oculomotor motility	5/5	4/4	4/4	1/1	4/4
Esotropia/esophoria	1/5, right	0/3	1/4	1/1, left	4/4
Ptosis	1/5, bilateral	0/3	0/4	1/1, left	0/4
Smooth philtrum	2/5	3/3	3/4	0/1	2/4
Midface retrusion	4/5	3/3	4/4	1/1	4/4
Upturned nasal tip	1/5	3/3	4/4	1/1	4/4
Low-set ears	1/5	0/3	1/4	1/1	3/4
Micrognathia	0/5	0/3	0/4	0/1	2/4
External ear malformations	3/5	0/3	4/4	0/1	0/4
Other findings					
Speech delay	0/5	2/3	4/4	0/1	2/4
Feeding difficulties	5/5	3/3	4/4	0/1	2/4
Sensorineural hearing loss	2/5	3/3	2/4	0/1	4/4
Abnormal cochlear function	2/5	3/3	2/4	0/1	2/4
Cochlear structural defect	1/5	n.d.	0/4	n.d.	1/4

F, female; HCFP, hereditary congenital facial palsy; M, male; n.d., not determined.

HOXB1 transcript. A previous report drew parallels between the Arg209Cys point mutation and the *HoxB1*^{-/-} mouse [Webb et al., 2012]. Our data strongly support the conclusion that the key overlapping clinical features – facial weakness and dysmorphia – result from the loss of *HOXB1* function.

Understanding the association of CFP with concomitant esotropia is important in achieving a differential diagnosis of HCFP and MBS. *HOXB1* mutations have not been found in patients with classic MBS in this study or

previous reports, suggesting that it may be a useful diagnostic marker [Ciorba et al., 2015]. Our data also demonstrate that further analysis of the regulation and function of *HOXB1* will help elucidate CCDD pathology.

The loss-of-function mutation in 5 affected members of the current report exhibits a similar clinical presentation to that observed in previous patients with *HOXB1* mutations. Moreover, there is no clear genotype-phenotype correlation with regards to the type of mutations, their location in the protein, and severity of the pheno-

type. However, as seen in Moroccan patients with a loss-of-function mutation, 3 of these Iranian patients showed external ear malformations, which were not observed in the other 8 reported patients with missense mutations or in the *HOXB1*-deficient mouse model [Webb et al., 2012; Uyguner et al., 2015; Vogel et al., 2016; Sahin et al., 2017]. Further cases of *HOXB1*-related HCFP would be necessary before we can confidently establish an association between the genotype and the phenotype of external ear malformation in this rare disease. External ear malformation is not an uncommon occurrence in MBS patients [Suvarna et al., 2006]. Additionally, a combined phenotype of CFP and external ear anomalies has been reported in an unknown autosomal dominant syndrome characterised by conductive deafness due to stapedia anomalies, external ear malformations, and CFP [Sellers and Beighton, 1983].

We identified a novel homozygous truncating mutation in *HOXB1* in a consanguineous kindred with HCFP, which is broadly similar to the clinical phenotype of patients who were previously described. The mutation identified in this family is the first indel described in *HOXB1*

so far. It must be emphasised that in individuals with CFP, hearing loss, dysmorphic facial features, and full eye motility, *HOXB1* mutation screening should be considered to help with an accurate diagnosis.

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Statement of Ethics

The participating family members and their parents provided written informed consent. This study was approved by the local ethical committee of the Shahid Sadoughi University of Medical Sciences in Yazd, Iran.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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