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Clinical report

A new hereditary congenital facial palsy case supports arg5 in HOX-DNA binding domain as possible hot spot for mutations

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ABSTRACT

Moebius syndrome (MBS) is a rare congenital disorder characterized by rhombencephalic mal development, mainly presenting with facial palsy with limited gaze abduction. Most cases are sporadic, possibly caused by a combination of environmental and genetic factors; however, no proven specific associations have been yet established. Hereditary congenital facial palsy (HCFP) is an autosomal dominant congenital dysinnervation syndrome, recognizable by the isolated dysfunction of the seventh cranial nerve. Mutant mice for *Hoxb1* were reported to present with facial weakness, resembling MBS. Recently a homozygous mutation altering *arg*5 residue of HOXB1 homeodomain into *cys*5 was identified in two families with HCFP. We screened 95 sporadic patients diagnosed as MBS or HCFP for mutations in *HOXB1.* A novel homozygous alteration was identified in one HCFP case, affecting the same residue, resulting to *his*5. In silico protein analysis predicted stronger HOXB1-DNA binding properties for *his*5 than *cys*5 that resulted to milder phenotype. It should be noted that, inclusive of the previous report, only two mutations revealed in *HOXB1* associated with HCFP involved the same amino acid *arg*5 in *HOXB1* residing in HOXB1-DNA-PBX1 ternary complex.

1. Introduction

A high percentage of MBS cases are sporadic and no specific genes are yet identified related to this condition. Two nested factors may delineate the fruitless efforts to identify the causative genes for MBS. First, linkage algorithms are generally powerless for sporadic occurrences due to genetic heterogeneity. Second, uncharacterized heredibility patterns may complicate the linkage analysis, since autosomal dominant with incomplete penetrance, autosomal recessive with homozygous or compound heterozygous mutations, or de novo heterozygous mutations, could all be under possibility. With improvements in diagnostic assessments, leading to the subdivision of patients according to their specific clinical features, may facilitate both the identification and the association of novel genes extracted from exome or whole genome sequencing data (MacKinnon et al., 2014).

In 1996, it was reported that null mutant mice for *Hoxb1,* with disrupted development of the facial nerve, originating from the fourth rhombomere, had features resembling Moebius syndrome in humans (Goddard et al., 1996, Studer et al., 1996).

Recently, a homozygous mutation in *HOXB1*, c.619C > T, altering arginine to cysteine at 207 (p.Arg207Cys), corresponding to arg5 > cys5, identified in two unrelated patients of German-American descent with bilateral facial weakness, feeding difficulties and hearing loss. Both affecteds share the same infrequent haplotype suggestive for a founder mutation implicated that the families can be distantly related (Webb et al., 2012). Thereupon, *HOXB1* entitled to be the first gene associated with HCFP, addressed to HCFP3 (MIM#614744) at 17q21.32.

HOXB1 is a homeodomain containing sequence-specific transcription factor of the HOX family, under the HOXL subgroup of ANTP-class (Holland et al., 2008). It has a characteristic helix-turn-helix DNA binding motif with three alpha helical regions (α1, α2, α3) where the specificity may be contemplated by heterodimerization with PBX1 (Piper et al., 1999). Yeast two-hybrid assays showed that Pbx homeodomain (TALE class) is necessary but not adequate for cooperation, which required conserved amino acids (Chang et al., 1995). Nuclear Magnetic Resonance (NMR) studies demonstrated that the conserved hexapeptide of Hoxb1 (TFDWMK) stabilizes binding of Pbx1 and Hoxb1 to DNA (Carolyn et al., 2001). The fifth amino acid of HOXB1, arginine (*arg*5),

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demonstrated to confer DNA binding specificity to the first thymidine in the DNA recognition motif of 5′-^{1}T-A-A-T-T/G-A/G-3′ by hydrogen bonding and also by hydrophobic packing interactions (Wilson et al., 1996, Noyes et al., 2008).

We report here a HCFP case with a novel homozygous mutation in the *HOXB1* gene and present the results of in silico protein modeling for the prediction of protein function to investigate if compatible with phenotype.

2. Material and methods

2.1. Patients

A total of 39 sporadic MBS/HCFP patients were enrolled for candidate gene screening. The investigation was approved by the Institutional Review Board of Istanbul Medical Faculty, Istanbul University. Written informed consent was obtained from patients and from their parents before sampling commenced. Additionally, DNA samples from 56 Moebius syndrome patients provided as Dutch cohort referred from Department of Human Genetics, Radboud University, Nijmegen Medical Centre, Netherlands, were also included.

2.2. Molecular analysis

Genomic DNA was extracted from 2 ml of K_3EDTA blood samples using DNA isolation kit (Mammalian Blood, Roche). Primers covering 5′ and 3′ of coding exons plus flanking introns designed for *HOXB1* (NM_002144, NP_002135) gene and PCR for each region was performed in 50 μL reaction mixture, composed of 0.3 μl primer pairs, 1X reaction buffer, 2 mM $MgCl_2$, 0.2 mM dNTP mix in 0.5 unite taq polymerase enzyme (Fermentas), in two step cycle (±Tm of primers) program in thermal cycler (Bio Rad), starting with 10 min initial denaturation, followed by 30 s denaturation, 30 s annealing and 1 min/1 kb elongation periods. PCR products were purified by spin colon kit (High Pure PCR product purification kit, Roche) and sequenced (Macrogen, Seoul, Korea). Electropherograms were analyzed for sequence variants by using publicly available BLAST program (NCBI, USCS). DNA samples from 100 healthy control individuals were screened for c.620G > A alteration in *HOXB1* gene by PCR and mutation specific restriction digestion.

2.3. Protein models

Three-dimensional models for the full HOXB1 protein and mutants were created based on multiple-threading alignments by Local Meta-Threading-server (LOMET) by using in silico tool, I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER). HOXB1-HD domains for normal and mutants were created by a homology modeling tool, SWISS-MODEL (http://swissmodel.expasy.org). A HOXB1-DNA-PBX1 model was downloaded from the Protein Data Bank in Europe-EBI website for the examination of P14653 (http://www.ebi.ac.uk/pdbe-srv/view/entry/1b72/downloads.html) (Piper et al., 1999, Zhang, 2008, Roy et al., 2010). I-Tasser builds five predictions with individual confidence scores estimating the quality of the models based upon the capacity of the threading template alignments and the convergence parameters of the structure assembly simulations. Typically, the confidence score of 3D models in this system ranges between −5 and +2, where the higher value signifies greater confidence. Among the five strongest 3D models created by the server, the highest confidence score recorded was −3.40 for *arg5*, −3.48 for *cys5* and −3.58 for *his5*. This was the model selected and used for the analysis. Quality structure estimation of protein models built by the Swiss Model is stated by the Qualitative Model Energy Analysis 4 (QMEAN4) scoring system consisting of four statistical potential terms: C-beta interaction energies; all atom pair wise energy; solvation energy, and; torsion angle energy. Reliable values range between 0 and 1 (Arnold et al., 2006; Benkert et al., 2011). The created models recorded a QMEAN4 score of 0.544 for *arg5*, 0.5 for *cys5* and 0.536 for *his5*.

3. Results

3.1. Clinical

The patient, 23 months old female, was referred from pediatric neurology outpatient clinics due to facial paralysis with a preliminary diagnosis of Moebius syndrome. She was the only child of first-degree cousins, once removed, born at term via Caeserian section due to cephalo pelvic disproportion, with a weight of 3320 g (−0.19SD). The child's length and occipito frontal circumference (OCF) at birth were not recorded. Developmental milestones were achieved normally; the patient sat without support at seven

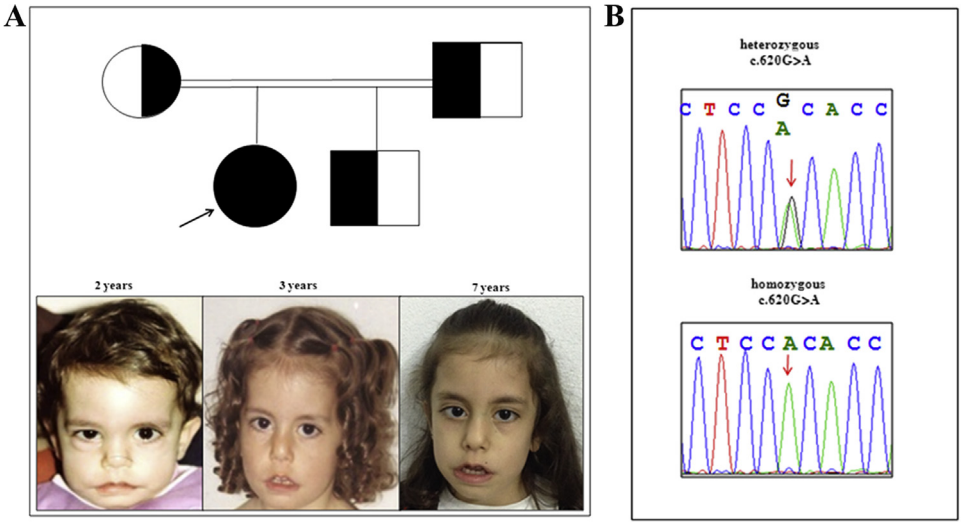

Fig. 1. **(A)** Pedigree of the family and facial appearance of the patient at the age of 23 months, 38 months and seven years of age, respectively (Note bilateral facial paralysis). **(B)** Partial electropherogram showing the mutation in *HOXB1*gene.