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Original article

Molecular genetic screening of MBS1 locus on chromosome 13 for microdeletions and exclusion of FGF9, GSH1 and CDX2 as causative genes in patients with Moebius syndrome

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ABSTRACT

Moebius syndrome is a rare disorder primarily characterized by congenital facial palsy, frequently accompanied by ocular abduction anomalies, and occasionally associated with orofacial, limb and musculoskeletal malformations. Abnormal development of cranial nerves V through XII underlines the disease pathogenesis. Although some investigations suggested that a causative gene may lie on 13q12.2–q13, there have been no molecular studies targeting possible microdeletions in this region to date. In the present study, we performed microdeletion analyses on 13q12.11–q13 in nine patients, and sequenced three candidate genes in nineteen patients for functional relevance and further resolution of our screening. We ruled out microdeletions on the critical region as a common cause of Moebius syndrome and excluded FGF9, GSH1 and CDX2 genes.

1. Introduction

Moebius syndrome (MBS) is a rare genetic disorder characterized by congenital uni- or bilateral facial palsy due to disruption of facial cranial nerve (CN), frequently accompanied by the involvement of other CNs, especially abducens and hipoglossal CNs, and associated with orofacial, limb, and musculoskeletal malformations [29,31]. Although the pathological mechanism is not yet completely understood, complex regional maldevelopment of the brainstem was proposed to underlie MBS [32].

Conventionally proposed vascular, teratogenic, and genetic causes for the etiology of MBS remain elusive [3,31]. Ziter et al. [35] reported a reciprocal translocation between chromosomes 1p34 and 13q13 co-segregating with congenital facial diplegia and flexion finger contractures in seven family members over three generations. In addition, Slee et al. [27] described a 2.5-year-old female MBS patient with deletion of chromosome 13q12.2. Therefore, it was suggested that a responsible gene for MBS could be located on 13q12.2–q13. To our knowledge, there have been no molecular genetic studies delineating this point, which would otherwise help to identify the causative gene(s). In this study, we screened MBS patients for microdeletions via high-density microsattelite marker genotyping on 13q.

2. Material and methods

2.1. Clinical data

Nineteen MBS patients (eighteen cytogenetically normal and one 46,XX[13]/47,XXX[19] mosaic [MBS-12]) and twenty-two healthy relatives (parents of eight patients and mother, sister, paternal aunts and uncles of one patient [MBS-6]) were included. All clinical findings in the patients are presented in Table 1.

Detailed pedigree analyses showed that none of our MBS patients were born to a consanguineous marriage but that some parents were originated from the same villages and some grandparents were consanguineous (Supplementary Table 1). To obtain the highest heterozygosity, only the families with affected

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Table 1
Clinical findings of Moebius syndrome patients included in the present study.

Patients	MBS-1	MBS-2	MBS-3	MBS-4	MBS-5	MBS-6	MBS-7	MBS-8	MBS-9	MBS-10	MBS-11	MBS-12	MBS-13	MBS-14	MBS-15	MBS-16	MBS-17	MBS-18	MBS-19
Gender	F	F	M	F	M	M	M	F	F	M	F	F	F	F	M	M	F	M	M
Age (years + months)	4 + 0	3 + 3	1 + 3	2 + 1	2 + 7	5 + 7	5 + 10	3 + 0	0 + 10	0 + 7	1 + 6	1 + 7	7 + 0	3 + 8	8 + 8	3 + 7	9 + 0	0 + 10	13 + 7
Prenatal teratogenic exposure	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CN VII palsy (R/L)	+/+	–/+	+/–	+/–	+/+	+/+	+/+	+/–	+/+	+/–	+/+	+/+	+/+	–/+	+/+	–/+	–/+	–/+	+/+
CN VI palsy (R/L)	+/+	–/+	+/+	+/+	–	+/+	+/+	+/–	+/+	–	+/+	–	–	–	+/+	+/+	–	–	+/+
CN XII palsy (R/L)	+/+	–	+/–	–	–	–/+	–	–	–	–	–	–	–	–/+	+/+	–	–	–	+/+
CN III palsy (R/L)	+/+	+/–	–	–	+/+	–	+/–	–	+/+	+/+	–	–	–	–	+/+	–	–	–	–
CN IV palsy (R/L)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+/+	–	–	–
CN V palsy	+	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
CN IX/X palsies	+	–	+	–	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–
Orofacial dysmorphism	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Limb deformities	–	+	+	+	+	+	–	+	+	+	+	–	–	–	+	+	–	+	+
Poland anomaly	–	–	–	–	–	+	–	–	+	–	–	–	–	–	+	–	–	+	+
Hypogonadism	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cranial MRI findings	ACMCF	NF	NA	NA	CCA	BCPP	NA	HRCH, HVAP, NPF	NF	NF	HABPM	NA	CM-I	LVD, TCC	ALV	CCA	NF	NF	NA

Abbreviations: ACMCF, arachnoid cyst on middle cranial fossa; ALV, asymmetry in left ventricules; BCPP, bilateral choroid plexus papilloma; CCA, corpus callosum agenesis; CM-I, Chiari malformation type I; CN, cranial nerve; F, female; HABPM, hypoplasia/atrophy of bulbus, pons and mesencephalon; HRCH, hypoplasia of right cerebellar hemisphere; HVAP, hypoplasia of vermis anterior and posterior; L, left; LVD, left ventricular dilatation; M, male; NA, not available, NF, no finding; NPF, narrow posterior fossa; R, right; TCC, thin corpus callosum.

individuals, whose parents were not consanguineous and were from different geographical regions, were selected for microdeletion screenings (nine families; MBS-1 to MBS-8 and MBS-13; Group A). The remaining ten patients (MBS-9 to MBS-12 and MBS-14 to MBS-19; Group B) were included in candidate region/gene screening.

2.2. DNA isolation

Peripheral blood samples were collected following the approval of the informed consent by the family members. Genomic DNA was isolated using standard procedures.

2.3. STR markers

Twenty known STR markers were taken from UCSC Genome Browser Database (March 2006 [hg18] Assembly; http://www.genome.ucsc.edu). Thirty-seven novel STR markers, D13S1855 through D13S1891, were designed for this study and submitted to the Human Genome Database (http://www.gdb.org). The primer sequences of known and novel STR markers are presented in Supplementary Table 2.

2.4. Candidate gene screening

Upon initial STR analyses, three critical regions were identified in Group A and confirmed in Group B. Candidate genes (FGF9, GSH1, and CDX2) in these critical regions were selected by functional relevancy and sequenced in all patients. Deep intronic primers for the amplification of FGF9, GSH1 and CDX2 genes covering coding exons and exon–intron boundaries were designed on the genomic sequences retrieved from ENSEMBL (Release 49 – March 2008; http://ww.ensembl.org). Primer sequences are presented in Supplementary Method.

2.5. Molecular methods

All relevant DNA fragments were amplified from genomic DNA by touch-down PCR (see Supplementary Method for details). All PCR products of fifty-seven STR markers were run on 10% (19:1) polyacrylamide gels. The alleles were genotyped independently by two co-workers. Haplotypes were constructed manually. Sequence analyses were carried out using an automated sequencer (Applied Biosystems 3730xl, Macrogen Inc., Seoul, Korea).

3. Results

Fifty-seven STR markers located on 13q12.11–13.1 were genotyped in a total of thirty-one individuals, twenty-four of which were patient–parent trios of the eight families. The genotype of the father of the MBS-6 patient, who had deceased prior to our study, was indirectly predicted via the genotypes of the patient himself, the patient's healthy sister, and the father's four siblings (Supplementary Fig. 6) (data not shown). Nine of the thirty-seven novel STR markers (D13S1859, D13S1861, D13S1876, D13S1878, D13S1879, D13S1882, D13S1884, D13S1885, and D13S1889) did not show polymorphism in the study sample and therefore, were excluded from the final haplotypes, which were constructed using the genotypes obtained from forty-eight STR markers (Supplementary Figs. 1–9).

The haplotypes covered a region of almost 12.8 Mb flanked by D13S1856 located at 18.5 Mb on 13q12.11 and D13S260 at 31.3 Mb on 13q13.1. Thus, the resolution of the haplotypes was ~272 kb (ranging from 15 kb between D13S292 and D13S1867 to 793 kb between D13S1243 and D13S283). Higher resolutions were