

## ELECTRONIC LETTER

# Mutation analysis in the candidate Möbius syndrome genes *PGT* and *GATA2* on chromosome 3 and *EGR2* on chromosome 10

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Möbius syndrome (MBS, MIM 157900) is a rare congenital disorder characterised by paralysis of the facial nerve. This paralysis may be complete or partial and unilateral or bilateral. Other cranial nerves are often implicated, most frequently the abducens and hypoglossal nerve. Limb malformations and facial dysmorphism occur frequently. Features seen less often in MBS are structural anomalies of the ear, defective branchial musculature (Poland syndrome, MIM 173800), and mild mental retardation.<sup>1</sup> Although Möbius syndrome usually occurs in isolated cases, familial recurrence has been reported. Patterns of inheritance observed in affected families suggest different modes of inheritance for the syndrome, ranging from autosomal recessive to autosomal dominant and X linked.

To date, four genetic loci for MBS have been described. In 1977, a reciprocal translocation of 13q12.2-13 was identified, cosegregating with the disease in a three generation MBS family.<sup>2</sup> Slee *et al*<sup>3</sup> reported a MBS patient with a deletion of chromosome 13q12.2 in 1991. Therefore, chromosome 13q12.2-q13 is thought to harbour a gene for MBS (MBS1). We identified two additional loci for MBS, MBS2 at 3q21-q22<sup>4</sup> and MBS3 at 10q21,<sup>1</sup> in two large Dutch families with a mild Möbius phenotype, cosegregating in an autosomal dominant fashion with reduced penetrance. A fourth locus harbouring a gene for MBS on chromosome 1p22 was inferred from two reports. Donahue *et al*<sup>5</sup> identified a t(1;11)(p22;p13) translocation in a patient with Möbius syndrome. The 1p22 locus was confirmed by a t(1;2)(p22.3;q21.1) translocation in a patient with Möbius-like syndrome, reported by Nishikawa *et al*.<sup>6</sup> All these data combined prove genetic heterogeneity for MBS.

Although no conclusive evidence has been gathered so far, two modes of action have been postulated to explain the aetiology of MBS. Firstly, a primary metameric defect in the brainstem nuclei in the region of the tegmentum could result in MBS. Secondly, an ischaemic process resulting from an interruption of the vascular supply of the brainstem and other structures during early fetal development could be causative.<sup>1</sup> In line with these hypotheses, different genetic defects in a broad range of processes underlying neurogenesis, axonal outgrowth, or angiogenesis could result in the features observed in Möbius syndrome.

In order to identify candidate genes for Möbius syndrome, we directed our efforts at genes included in the MBS2 and MBS3 loci, which could play a role in the development of the hindbrain, the guidance of axons, and angiogenesis. Linkage analysis on additional members of the MBS2 linked family<sup>4</sup> enabled us to reduce the MBS2 critical region to a 4.9 cM region between the markers D3S1589 and *ACPP*. Our mutation analysis included the human prostaglandin transporter (*PGT*) gene and the gene encoding the GATA binding protein 2 (*GATA2*) transcription factor at the MBS2 locus, and the early growth response 2 (*EGR2*) gene at the MBS3 locus. Mutation analysis comprised direct sequencing of subjects from the rel-

evant families (either MBS2 or MBS3) on an ABI-PRISM 377 (*EGR2*) or 3700 DNA analyzer (*GATA2*, *PGT*), using BigDye terminator chemistry (Applied Biosystems).

*PGT* (or *SLC21A2*) was analysed because a correlation was suggested between the maternal use of a synthetic prostaglandin, Misoprostol, illegally used as an abortifacient, and an increased incidence of Möbius syndrome in newborns following failed abortion attempts.<sup>7-9</sup> Primers for the amplification and direct sequencing of *PGT* are listed in table 1. Mutation analysis of the *PGT* gene in patients from the MBS2 linked family showed only one nucleotide change, in exon 9. This A to G nucleotide change (base pair 1269) results in the substitution of threonine 396 for alanine (numbering according to sequence data from Genbank accession number NM\_005630). The nucleotide change did not cosegregate with the disease, and several subjects related by marriage also carried the A to G nucleotide change. Therefore, this change in exon 9 can be classified as a polymorphism. No other changes were encountered in the coding sequence (CDs) and flanking intronic sequences of *PGT*. Therefore it is unlikely that the *PGT* gene is involved in MBS2.

The second gene analysed at the MBS2 locus at 3q21-q22 was *GATA2*, a member of the GATA binding protein family of transcription factors.<sup>10</sup> This gene is an interesting candidate for two reasons. (1) *GATA2* expression in the developing hindbrain is limited to rhombomere 4, an important structure for the development of the facial nerve,<sup>11</sup> and (2) *GATA2* expression is regulated by *Hoxb-1*, a homeobox domain protein that functions in patterning of the hindbrain.<sup>12</sup> The *Hoxb-1* knockout mouse shows a defect in the formation of the motor nucleus of the facial nerve, and is considered an appropriate animal model for Bell's palsy and Möbius syndrome.<sup>13</sup> Primers were designed flanking the seven exons of *GATA2* (two alternatively used exons of the 5'UTR and five coding exons), shown in table 1. No nucleotide changes were found in the CDs or splice sites in patients from the MBS2 linked family. This lack of mutations in the *GATA2* gene suggests that this gene is not the causative gene for MBS2.

The early growth response 2 gene, coding for the *EGR2* transcription factor, is located at 10q21.3 near the MBS3 critical region. The *EGR2* gene was included in the mutation analysis based on the function of the mouse orthologue *Krox-20*. *Krox-20* is preferentially expressed in rhombomeres 3 and 5 in the developing hindbrain,<sup>14</sup> embracing rhombomere 4. During brain development, *Krox-20* regulates the expression of several homeobox proteins in rhombomeres 3 and 5 that are important for patterning of the hindbrain. Genes regulated by

**Abbreviations:** MBS, Möbius syndrome; CHN, congenital hypomyelinating neuropathy; CMT1, Charcot-Marie-Tooth disease type 1

**Table 1** Oligonucleotide sequences flanking each of the exons of the *PGT*, *GATA2*, and *EGR2* genes. *GATA2* has two alternatively used, non-coding, exons 1; we named these exon 1a and 1b. Exon 2 of the *EGR2* gene was divided into eight amplicons (a–h) to facilitate PCR and subsequent sequencing. Amplification conditions are available upon request

	Forward 5'→3'	Reverse 5'→3'	Product size (bp)
<i>PGT</i>			
Exon 1	GAGGGAGAGCGCTTCATC	CGGGTGCCCAGCCGAAGG	351
Exon 2	GCTTGTCGTCCAGAACTCAGG	GGATCTTGTTGGTCAGGGTGGTG	240
Exon 3	TTCTCTGTGGGTGGGTGAGGACAG	CCATTCCTTACCCTTGAGGC	267
Exon 4	GTGGAGACCAGCAAGCACTCTC	GCTACATCAGGACCTCACCCATC	310
Exon 5	CCTTTCCAGCCATCTTATTGGCC	TTCTCTGTTCTGATTGGATGAGGG	192
Exon 6	AAATCAGTAACTCCCCTGATC	TCCCTTCTTCAACTCCATCAGG	130
Exon 7	CGTGAACACTCACCCCTTATTG	GTCACTGTGCCAGCCCACC	149
Exon 8	GGGGTTCTGCCAGAGCTTGAC	GGGTGGTGGAGTGCCTG	268
Exon 9	TAGCCACATGCAGTCCCAG	AGTTAAGGAAGCAGGAAGGAAGATG	337
Exon 10	TGGTCTTGCTTTGTTGATTCCC	GCCTATCCTGGAGCCGAGAAAC	306
Exon 11	AATTTCCCTTTTCCCTCC	AGAAGCCACGCCCCAGACTC	238
Exon 12	CTGCTGATTCTGTCTCTTTTC	TTCTTGGGAAGAGGGGTCTAGAC	122
Exon 13	GCAGTCCACCTGACCAGGG	TGGGACACACATACATGATGGG	193
Exon 14	AGAGCCCCCTCCCTACCAG	CAAGGTCCACTCTCTGGAGCAG	201
<i>GATA2</i>			
Exon 1a	CGAGGCGCACTACCCCA	CAGAGTCCCCTCAAAGCTAG	207
Exon 1b	CAGGCGCTGGACCTGGTAG	GCAAACGGACCACAGCATTCCG	532
Exon 2	CACCTCGTGGTGGGACTTTG	GATTCCTGGGATCCTACATC	430
Exon 3	GAGTCGTATCTCAATGTCTG	GAAACCAACTGCCACCTC	837
Exon 4	TCCTGCCAGGCTGTGCAG	AAATCTGGCCCGAAGAATCTG	383
Exon 5	GATTAGCCCTCCTGACTG	CAAGCTGGATCTTGTGGCTG	314
Exon 6	CTCAGCTGACCTGCCTCTG	GTGTCGGCTTCGGGAAATGC	460
<i>EGR2</i>			
Exon 1	AAGTGTGGAGGGCAAAGGA	ACGCGGCTTACCTCCGGC	299
Exon 2a	TCCCCTCCCCAGATGGCATG	TGACGCTGGATGAGGCTGTG	248
Exon 2b	GCTACCCAGAAGGCATAATC	CGGAAGGAGGTGGTGGGTAG	300
Exon 2c	CCCTTCTGCGTTCCTGTCAG	CAGGGTAAAGTTACGGATTG	273
Exon 2d	ACCCAGGTGTCTCCCAATG	GCAGGTGGTGTGGGTATAG	306
Exon 2e	CAATCCGTAACCTTACCCTG	GGATGTGCCGTGTCAGCTCG	286
Exon 2f	CGAGCTGACACGGCACATCC	CTCCGCCAAGACTGTGCTG	326
Exon 2g	CGCAAGTACCCCAACAGACC	GGTGGTCACTGCGGCTGAAG	186
Exon 2h	ATCGGTGCCAGCCCCTCTA	TAGGTGAAAGGGGGCAGTG	287

*Krox-20* include *Hoxa-2*, *Hoxb-2*, and *Hoxb-3*.<sup>15-17</sup> As rhombomeres 4 and 5 are the site of origin of the majority of the cells that eventually make up the facial nerve,<sup>11</sup> failure to express functional *EGR2* could give rise to a Möbius phenotype. Although mutations in the *EGR2* gene have been described in patients with congenital hypomyelinating neuropathy (CHN), and Charcot-Marie-Tooth disease type 1 (CMT1),<sup>18</sup> allelism with MBS3 cannot be excluded. Primers were designed for amplification and direct sequencing of the *EGR2* coding sequence and splice sites (table 1). No mutations were found in the coding sequence and splice sites of the *EGR2* gene in patients from the MBS3 linked family. This suggests that the *EGR2* gene is not involved in MBS3.

In summary, the lack of pathogenic mutations in the coding sequences and splice sites of the three genes investigated justifies the exclusion of these genes as candidate genes in MBS2 (*PGT* and *GATA2*) or MBS3 (*EGR2*).

In a future positional candidate gene approach, new candidate genes need to be considered for mutation analysis in MBS2 or MBS3 patients. As Möbius syndrome is a rare disorder, familial cases are hard to find. Additional familial Möbius syndrome patient material would be very useful, as this increases the chances of identifying causative mutations.

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