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Localization of a gene for Möbius syndrome to chromosome 3q by linkage analysis in a Dutch family

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Möbius syndrome (MIM no. 157900) consists of a congenital paresis or paralysis of the Vth cranial nerve, frequently accompanied by paralysis of other cranial nerves, orofacial and limb malformations, defects of the musculoskeletal system and mental retardation. Although most patients are sporadic cases, familial recurrence is not rare. Different pedigrees suggest different modes of inheritance. We performed linkage analysis in a large family with autosomal dominantly inherited Möbius syndrome, consisting essentially of asymmetric bilateral facial pareses. After exclusion of the candidate region for Möbius syndrome on 13q12.2–q13, we localized the gene to chromosome 3q21–22, indicating genetic heterogeneity of Möbius syndrome. This heterogeneity is further proven by the exclusion of both loci in a second family with Möbius syndrome.

INTRODUCTION

Möbius syndrome has been defined as a congenital paresis or paralysis of the facial nerve that can be accompanied by paralysis or dysfunction of other cranial nerves, either unilaterally or bilaterally. The abducens nerves are most frequently involved and, often also, the hypoglossal nerve. Less commonly affected are the oculomotor, trochlear, trigeminal, vestibulocochlear or glossopharyngeal nerves. Besides the cranial nerve dysfunction, orofacial and limb malformations and defects of the musculoskeletal system are often present in Möbius syndrome. Orofacial malformations can consist of bifid uvula, microglossia, microstomia, micrognathia, malformations of the jaws, teeth and ears, hypertelorism and epicanthus. Limb malformations associated with Möbius syndrome are club foot, syndactyly, brachydactyly, ectrodactyly and absent or deformed fingers and toes. Finally, symptoms of the musculoskeletal system are defects of ribs, Klippel-Feil anomaly and aplasia of muscles (1–7).

In one study mental retardation was found in about 10% of the patients (8), while in another study it was said to be present in at least 50% of the patients (5).

Pathological data on Möbius syndrome are scarce. Most cases showed aplasia or hypoplasia of the affected cranial nerve nuclei and/or nerve trunks (9–13). Based on the symptoms and the limited pathological observations, rather divergent mechanisms have been proposed as the cause of the syndrome such as agenesis, hypoplasia or degeneration of the nuclei of the sixth and seventh cranial nerves (1,2,13,14).

Möbius syndrome is a rare disorder for which the incidence in the population has not been determined. Most cases are sporadic but familial occurrence has been described as well. Different pedigrees suggest different modes of inheritance: autosomal dominant, autosomal recessive as well as X-linked recessive inheritance have been proposed (15).

Based on the deletion of 13q12.2 in a sporadic patient (16) and on the co-segregation of a reciprocal translocation with the syndrome in a three-generation family (17) chromosome 13q12.2–q13 is thought to harbour a locus for Möbius syndrome.

As a first step toward the elucidation of the genetic defect(s), we performed linkage analysis in a large Dutch family with autosomally segregating Möbius syndrome (Fig. 1). A branch of the family was described before by Van der Wiel (18). We were able, after exclusion of the candidate region on chromosome 13, to localize the Möbius gene in this family to the long arm of chromosome 3.

RESULTS

Linkage of Möbius syndrome to chromosome 3q

Linkage analysis was performed in the Dutch family described in part by Van der Wiel (18). Thirty-one persons were investigated, 20 of whom were affected and one of whom was an obligate carrier (Fig. 1). The candidate region on chromosome 13 [13q12.2–q13 (16,17)] was excluded (Table 1). Subsequently, a genome scan was started with polymorphic markers spaced 10–15 cM. After exclusion of about 15% of the genome, mainly

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of chromosomes 1 and 3, an indication for linkage was obtained with marker D3S196 derived from the long arm of chromosome 3. The highest lod score with this marker was 2.04 at θ = 0.13. A substantial decrease of the lod score with markers proximal to D3S196 (Table 2) pointed to a more proximal location of the disease gene.

**Fine mapping of the gene for Möbius syndrome**

For fine mapping of the Möbius gene, 12 markers located proximal to D3S196 were tested. Two-point lod scores are given in Table 2. The highest lod score was 5.76 at θ = 0.0 with the marker D3S1292.

Haplotypes were constructed to determine the borders of the critical region of the Möbius gene (Fig. 1). Recombinations visible in the individuals III.14 and IV.6 demarcated the chromosomal fragment co-segregating with the syndrome to a region of about 10 cM between the markers D3S1589 and D3S1576. Individual IV.7 is interesting because for the relevant interval she carries the same genotype as her unaffected brother. On examination, she had minimal signs considered by two independent neurologists as compatible with the diagnosis of Möbius syndrome. If this clinical diagnosis is correct, it can be explained in various ways. The first explanation is that a double cross-over has occurred in the paternal chromosome either between two adjacent markers or in the intervals in which markers in the patient's father are not informative. The order of the markers D3S1290, D3S1273, D3S1587 is not clearly resolved (19,20). If the markers D3S1290 and D3S1273 are assumed to be located distal to D3S1292, there is a coherent interval of about 4 cM in which the father is non-informative. In this larger interval a double recombination is more likely to occur. Additional polymorphic markers are necessary to detect this double recombination. Alternative explanations are that individual IV.7 is a phenocopy or that gene conversion has occurred.

**Figure 1. Pedigree of the family and haplotypes of persons available for this study.** Lines beside the haplotypes mark the mutation carrying chromosome.

Table 2: Two-point lod scores at markers proximal to D3S196.

<table>
<thead>
<tr>
<th>Marker</th>
<th>LOD Score</th>
<th>Marker</th>
<th>LOD Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1290</td>
<td>2.04</td>
<td>D3S1273</td>
<td>1.67</td>
</tr>
<tr>
<td>D3S1292</td>
<td>1.76</td>
<td>D3S1200</td>
<td>1.45</td>
</tr>
<tr>
<td>RHO</td>
<td>1.20</td>
<td>D3S1576</td>
<td>1.15</td>
</tr>
<tr>
<td>D3S1541</td>
<td></td>
<td>D3S1236</td>
<td></td>
</tr>
<tr>
<td>D3S1288</td>
<td></td>
<td>D3S1238</td>
<td></td>
</tr>
<tr>
<td>ACP3</td>
<td></td>
<td>D3S1587</td>
<td></td>
</tr>
<tr>
<td>D3S1576</td>
<td></td>
<td>D3S1576</td>
<td></td>
</tr>
</tbody>
</table>

Note: The table includes markers tested for two-point lod scores with D3S196. The table is not exhaustive, and additional markers in the region may be informative for fine mapping.

Additional comments and considerations are essential for a comprehensive understanding of the genetic basis of Möbius syndrome.
Table 1. Two-point lod scores between the polymorphic markers derived from the candidate locus on chromosome 13 and Möbius syndrome

<table>
<thead>
<tr>
<th>Locus</th>
<th>0.00</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
</tr>
</thead>
<tbody>
<tr>
<td>D13S283 (13q12.1)</td>
<td>-11.26</td>
<td>-3.80</td>
<td>-2.57</td>
<td>-1.31</td>
<td>-0.62</td>
</tr>
<tr>
<td>D13S221 (13q12.1)</td>
<td>-25.66</td>
<td>-4.04</td>
<td>-2.31</td>
<td>-0.81</td>
<td>-0.23</td>
</tr>
<tr>
<td>FLTI (13q12)</td>
<td>-6.64</td>
<td>-2.52</td>
<td>-1.53</td>
<td>-0.61</td>
<td>-0.24</td>
</tr>
<tr>
<td>D13S217 (13q12)</td>
<td>-23.40</td>
<td>-3.03</td>
<td>-1.62</td>
<td>-0.48</td>
<td>-0.10</td>
</tr>
<tr>
<td>D13S220 (13q12-1q13)</td>
<td>-12.23</td>
<td>-1.15</td>
<td>0.16</td>
<td>0.24</td>
<td>0.16</td>
</tr>
<tr>
<td>D13S218 (13q13-1q14.1)</td>
<td>-4.67</td>
<td>-1.04</td>
<td>-0.71</td>
<td>-0.39</td>
<td>-0.21</td>
</tr>
</tbody>
</table>

Table 2. Two-point lod scores between the polymorphic markers and Möbius syndrome

<table>
<thead>
<tr>
<th>Locus</th>
<th>0.00</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>Zmax (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1303</td>
<td>-8.26</td>
<td>0.41</td>
<td>0.78</td>
<td>0.88</td>
<td>0.70</td>
<td>0.94 (0.17)</td>
</tr>
<tr>
<td>D3S1551</td>
<td>-8.06</td>
<td>2.65</td>
<td>2.92</td>
<td>2.53</td>
<td>1.80</td>
<td>2.92 (0.10)</td>
</tr>
<tr>
<td>D3S1589</td>
<td>-10.24</td>
<td>2.21</td>
<td>2.38</td>
<td>2.05</td>
<td>1.38</td>
<td>2.38 (0.10)</td>
</tr>
<tr>
<td>D3S1541</td>
<td>2.38</td>
<td>2.12</td>
<td>1.82</td>
<td>1.17</td>
<td>0.57</td>
<td>2.38 (0.01)</td>
</tr>
<tr>
<td>RHO</td>
<td>4.61</td>
<td>4.15</td>
<td>3.67</td>
<td>2.64</td>
<td>1.52</td>
<td>4.61 (0.00)</td>
</tr>
<tr>
<td>ACPP</td>
<td>3.17</td>
<td>2.89</td>
<td>2.58</td>
<td>1.94</td>
<td>1.23</td>
<td>3.17 (0.00)</td>
</tr>
<tr>
<td>D3S1273</td>
<td>0.21</td>
<td>5.44</td>
<td>5.13</td>
<td>4.13</td>
<td>2.84</td>
<td>5.46 (0.04)</td>
</tr>
<tr>
<td>D3S1900</td>
<td>-1.68</td>
<td>4.51</td>
<td>4.23</td>
<td>3.28</td>
<td>2.08</td>
<td>4.53 (0.04)</td>
</tr>
<tr>
<td>D3S1587</td>
<td>2.85</td>
<td>2.59</td>
<td>2.31</td>
<td>1.72</td>
<td>1.07</td>
<td>2.85 (0.00)</td>
</tr>
<tr>
<td>D3S1292</td>
<td>5.76</td>
<td>5.27</td>
<td>4.77</td>
<td>3.66</td>
<td>2.44</td>
<td>5.76 (0.00)</td>
</tr>
<tr>
<td>D3S1238</td>
<td>1.29</td>
<td>1.22</td>
<td>1.13</td>
<td>0.92</td>
<td>0.65</td>
<td>1.29 (0.00)</td>
</tr>
<tr>
<td>D3S1576</td>
<td>-1.80</td>
<td>3.71</td>
<td>3.78</td>
<td>3.22</td>
<td>2.27</td>
<td>3.80 (0.08)</td>
</tr>
<tr>
<td>D3S196</td>
<td>-9.07</td>
<td>1.53</td>
<td>1.94</td>
<td>1.80</td>
<td>1.26</td>
<td>2.04 (0.13)</td>
</tr>
<tr>
<td>D3S1265</td>
<td>-22.39</td>
<td>-3.90</td>
<td>-2.25</td>
<td>-0.85</td>
<td>-0.29</td>
<td>0.10 (0.50)</td>
</tr>
</tbody>
</table>

Location of the markers and marker order are derived from Guapay et al. (20) and Washington et al. (44).

The marker order, given from centromere to telomere, is derived from the recent YAC contig map (19), the genetic map described by Guapay et al. (20) and the chromosome 3 data base.

Five-point linkage analysis was performed in order to determine the location of the gene for Möbius syndrome with the highest likelihood (Fig. 2). The analysis resulted in the highest probability (lod score 4.59) for a location at D3S1292 with an odds ratio of 1.4 between this locus and the next most likely location between D3S1587 and D3S1292 (Fig. 2).

**DISCUSSION**

Linkage analysis in a Dutch family enabled us to localize a gene for Möbius syndrome to the long arm of chromosome 3. The critical region is delimited by the markers D3S1589 and D3S1576 and spans about 10 cM at chromosome 3q21-q25.2 (GDB). However, from the recent data on physical mapping the more precise location of 3q21-q22 can be deduced (19). As soon as new markers become available, the critical region might be reduced. In that case the presence and extent of the co-segregating chromosomal fragment in individual IV.7 can be established.

Together with the data indicating a gene for Möbius syndrome on chromosome 13 (16,17), the localization of the present gene argues for genetic heterogeneity. Genetic heterogeneity has been suggested before based on the clinical variability of the syndrome and the segregation of the disorder in families (7,15 and references therein). Linkage analysis performed by us in another family, which was described in part by Fortanier and Speyer (21), suggests the existence of a third locus involved in Möbius syndrome as we excluded both the locus on chromosome 3 and the candidate locus on chromosome 13q12-13 in this family.

At the moment about 25 genes have been mapped to an interval overlapping with the Möbius critical region. The absence of detailed information on the expression pattern of most of these genes makes it difficult to decide whether they are true candidates for the Möbius gene. Four of them have or may have a function in development: the ZNF9 gene encoding zinc finger protein 9, two genes encoding a retinol binding protein, CRBP1 and CRBP2, and PBX2, a homeobox gene. It has been suggested that the ZNF9 protein is involved in regulation of cellular cholesterol levels (22) which makes the gene a less likely candidate. The same holds true for CRBP2, the expression of which seems to be confined to fetal liver and adult small intestinal mucosa (23). Also PBX2 is not very likely to be the Möbius gene. Analysis of genomic DNA of the PBX2 copy suggests that this copy is a processed pseudogene (24).

CRBP1 is located very close to D3S1576 (20,25). Analysis of the TaqI polymorphism in this gene (26) gave no further clues on its location either distal or proximal to D3S1576. In mouse development, the gene is widely expressed with a differential spatio-temporal pattern (27-29) which includes the migrating nucleus of the facial nerve and the mesencephalic nucleus of the fifth cranial nerve (29). This induced us to screen the protein coding exons of the CRBP1 gene for mutations by single strand conformation polymorphism (SSCP) analysis. There were no indications for a mutation.

One of the genes for Blepharophimosis- Ptosis-Epicanthus inversus Syndrome (BPES), an autosomal dominant disorder of craniofacial development (for review see 30), has been localized to the same chromosomal region as Möbius syndrome, by both linkage analysis (31,32), allelic loss in deletion patients (33) and cytogenetic studies (34). Although the syndromes both affect craniofacial development they have no symptoms in common and a common primary defect seems unlikely. In a patient with a balanced translocation one of the breakpoints could be placed between D3S1316 and D3S1615 which is distal to D3S1576 (33), and thus outside of the Möbius critical region. However, effects of chromosomal aberrations on genes more than 100 kb away from the aberration are known (35,36). Therefore, based on their location allelism of both conditions cannot be excluded.

**MATERIALS AND METHODS**

**Patients**

On examination, the proband (IV.2, Fig. 1) had asymmetric weakness of the facial muscles and unequal involvement of the muscles of the three branches of the facial nerve. He was reported to have been born with facial weakness similar to his maternal grandmother and many of her sibs. His mother and his brother...
were said to be without facial weakness. On clinical examination his brother showed slight asymmetric weakness of the orbicularis oculi muscles but his mother had no hint of weak facial muscles. Electromyography of the facial muscles of the mother revealed normal action potentials. The right blink reflex was absent and there was a conduction block of the facial nerve to the right orbicularis oculi muscles and a prolonged distal motor latency to the orbicularis oculi muscles. The facial muscles on the left were not tested. Electromyography was considered compatible with a neuropathic lesion. Family examination revealed minimal involvement in all but one case. Individual IV-7 was examined by two neurologists independently and was found to be minimally affected by both.

**Typing of DNA markers**

Genomic DNA was isolated as described by Miller et al. (37). Amplification of the locus-specific DNA fragments and their analysis was performed according to Kremer et al. (38).

**Statistical analysis of linkage data**

Two-point lod scores were calculated with the subroutine Mlink of the program Linkage (version 5.1, 3£M-1). Statistical analysis of linkage data was performed according to Kremer (38).

**ACKNOWLEDGEMENTS**

We are very grateful to the members of the family who participated in this study. We thank Prof. H.-H. Ropers for advice and discussion and L. Boender-van Rossum, S. van de Velde-Visser and Drs H.J. van der Wiel, K. Cuenelaere and J. van Deutekom for their contribution to this study.

**REFERENCES**