Autosomal dominant Charcot-Marie-Tooth axonal neuropathy mapped on chromosome 7p (CMT2D)

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Clinical, electrophysiological and genetic linkage studies were performed on a large autosomal dominant family with Charcot-Marie-Tooth axonal neuropathy type 2 (CMT2) with 38 members of which 14 were affected. Onset of the disease was between 16 and 30 years of age with weakness and atrophy of the hands more severe than of the feet with slow progressive course in 12 patients. Deep tendon reflexes were absent in the upper extremities and decreased in the lower extremities. There was distal hypesthesia for touch, proprioception and vibration sense for the hands more than for the feet. Motor nerve conduction velocities showed normal values (48–53 M/s) with normal latencies (2–3 msec) and electromyography revealed signs of denervation. Genetic linkage analysis used 167 short tandem repeat markers (STRPs) spaced throughout the 22 autosomes. Linkage to the short arm of chromosome 7 at 7p14 was found using the marker D7S435 (Z = 4.83 at θ = 0). Flanking markers were D7S1808 and D7S1806 and the genetic distance between them was 6.8 cM. The multipoint linkage analysis gave a peak multipoint lod score of 6.89 between the markers D7S1808 and D7S435. Linkage analysis showed significantly negative lod scores (with values less than –2) with markers of chromosomes 1 and 3 where CMT axonal forms have been previously mapped. PFGE analysis indicated the absence of the CMT1A duplication. Our findings are consistent with a new genetic type of axonal CMT neuropathy designated by us as CMT2D. Potential candidate genes are multiple T-cell gamma receptor genes which map to the same cytogenetic interval as CMT2D neuropathy.

INTRODUCTION

Clinical and molecular genetic studies have shown that Charcot-Marie-Tooth (CMT) neuropathies are heterogeneous. Two major types of CMT, demyelinating (CMT1) and axonal (CMT2), were individualized on the basis of electrophysiological and neuro-pathological features (1,2). CMT1 is characterized by reduced motor nerve conduction velocities (MNCVs) with values between 9 to 40 M/s with a mean of 25 M/s. Nerves are hypertrophic and sural nerve pathology shows frequent ‘onion bulbs’. The primary defect in CMT1 affects myelin, which accounts for the nerve conduction impairment. There are several gene mutations responsible for demyelinating neuropathy, mapped to chromosome 17p11.2-p12 (CMT1A) (3–5), 1q22 (CMT1B) (6), and undetermined autosome (CMT1C) (7). The average frequency of CMT1 is around 60% versus 20% for CMT2 (8) of all dominantly inherited CMT neuropathies. CMT2 is characterized by normal MNCVs, EMG with signs of denervation and by neuropathological findings of axonal loss (1,2). CMT2 is genetically distinct from CMT1. A CMT2 locus was assigned by linkage studies to 1p36 and designated as CMT2A (9). A second locus was assigned to the 3q13-q22 region and named CMT2B (10). CMT type 2C with diaphragm and vocal cord weakness is still only a clinical entity and its locus, different from 1p36, has not yet been determined (11,12). In the present report we examined a large autosomal dominant axonal CMT family and found linkage to chromosome 7p14. We designated this new genetic type CMT2D.

RESULTS

Clinical, MNCV and EMG findings

The family studied by us has 14 CMT patients and 24 unaffected members as shown by the pedigree (Fig. 1). Male-to-male transmission was apparent excluding the possibility of X-linked inheritance. Onset of the disease was between 16 and 30 years of age with weakness of the hands. Affected persons examined by different neurologists had severe weakness and atrophy of the hands and mild to moderate weakness of the feet, except two patients who showed severe weakness of both hands and feet and also used lower leg braces. Deep tendon reflexes were absent in the upper extremities and decreased in the lower extremities, except two patients who showed generalized areflexia. There was distal hypesthesia for touch, proprioception and vibration sense more evident for the hands than for the feet. Variable pes cavus deformity and/or hammer toes were present in all the patients. Mild to moderate balance impairment was present in five patients with Romberg sign being positive. Gowers and Trendelenburg signs were present in only two patients. Scoliosis was present in

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four patients. There was no evidence of nerve enlargement. Tremor of the hands, feet ulcers, paralysis of vocal cords and/or diaphragm were absent. Coordination and cranial nerves were normal. The disease had a mild progressive course in 12 patients.

Motor nerve conduction velocities (MNCVs) showed normal values (48–53 m/s) with normal latencies (2–3 msec). Electromyograms (EMGs) revealed signs of denervation with large motor unit potentials, fibrillation potentials and positive sharp waves.

**Molecular genetic results**

Duplication analysis by pulsed field gel electrophoresis was negative in all patients. Linkage to the short arm of chromosome 7 at 7p14 was found using the marker D7S435 (Z = 4.83 at θ = 0) (Table 1) using the full pedigree. Flanking markers were D7S1808 (Z = 2.37 at θ = 0.053) and D7S1806 (Z = 0.95 at θ = 0.091) with a genetic distance of 6.8 cM between them. Estimates for the allele frequencies for D7S435 were derived from the spouses of family members. An analysis using only the affected members of the pedigree with the same allele frequencies for D7S435 as described above yielded a peak lod score of 3.20 at a θ = 0. To test the sensitivity of the pairwise lod score analysis to allele frequency estimation, we analyzed the data using different estimates of the allele frequencies of D7S435. In practice, we varied the frequency of the linked allele while dividing the remainder among the remaining alleles. We found that with an estimate as high as 0.60 for the linked allele, the pairwise lod score remained above 3. Since the estimate of the heterozygosity for D7S435 is 0.69 (13), we feel that this result provides strong evidence in favor of linkage of CMT2D to D7S435. The multipoint linkage analysis gave a peak multipoint lod score of 6.89 between the markers D7S1808 and D7S435. The multipoint graph is illustrated in Figure 2. Linkage analysis showed significantly negative lod scores (lod < -2) with markers of chromosomes 1 and 3 where CMT2A and CMT2B axonal forms have been previously mapped.

**DISCUSSION**

Clinical heterogeneity is not easily detectable in axonal CMT neuropathies, Ben Othmane et al. (9) believe that CMT2 and CMT1 are clinically indistinguishable when examining any single individual. However, Yoshioka et al. (12) consider that later onset and the absence of palpably enlarged nerves suggest CMT2. Kwon et al. (10) report foot sores that were slow to heal or amputated limbs related to the poor healing of foot ulcers in CMT2B. Dyck et al. (11) consider that diaphragm weakness and vocal cord paralysis are characteristic findings of CMT2C. The clinical picture of our patients with CMT2D is also different from other axonal CMT2 types. The weakness and atrophy are more severe for the hands than for the feet. The gait is usually normal. The sensory impairment has the same prevalence as the motor involvement. The disease has a slow progressive course.

**Table 1. Two point Lod scores for CMT2D**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Recombination fraction (θ)</th>
<th>Z max</th>
<th>θ max</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7S1808</td>
<td>0.00</td>
<td>2.37</td>
<td>0.53</td>
</tr>
<tr>
<td>D7S1869</td>
<td>3.20</td>
<td>2.37</td>
<td>0.00</td>
</tr>
<tr>
<td>D7S435</td>
<td>4.83</td>
<td>2.37</td>
<td>0.00</td>
</tr>
<tr>
<td>D7S1806</td>
<td>0.88</td>
<td>2.37</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The molecular genetic results also reveal characteristic findings in our reported axonal CMT family. Linkage to the short arm of chromosome 7 markers at 7p14 reached significant lod scores and was not previously reported in other axonal CMT types. Our analysis brings further documentation for genetic heterogeneity of CMT axonal neuropathies.

Review of potential candidate genes mapping to the linked region reveals no genes of the same functional class as PMP22, a gene known to cause CMT1A neuropathy (14–16). Of interest is the fact that multiple T-cell gamma receptor genes map to the same cytogenetic interval as CMT2D neuropathy. An autoimmune mechanism for CMT pathogenesis has been previously suggested by Williams et al. based on significantly increased CD26+ T-cell activation found sequentially in CMT patients (17,18).
MATERIALS AND METHODS

Materials

Restriction enzymes were from New England Biolabs or Boehringer Mannheim Biochemicals and used according to the manufacturer’s instructions. Agarose was genetic technology grade from FMC BioProducts. Radioactive labeling was done with a Random Primed labeling kit from Boehringer Mannheim Biochemicals.

Pulsed field gel electrophoresis and duplication analysis were done as previously described (19).

Linkage analysis

One hundred and sixty-seven short tandem repeat markers (STRPs) spaced throughout the 22 autosomes were used. Two point and multipoint linkage were performed using the MLINK and LINKMAP programs of the LINKAGE package (version 5.1) as described by Lathrop et al. (20). Equal male and female recombination rates were assumed. Multipoint lod scores were defined as the log_{10} difference between the disease locus at a specific recombination fraction (θ = 0.00 to 0.49) from the test locus and the disease locus placed in an unlinked state (θ = 0.50). Log_{10} differences greater than or equal to 3 are taken as significant evidence for linkage (same as >10^3, 1000:1 odds). Values between –2 and 3 are indecisive and values equal to or below –2 indicate exclusion from that region. The 95% confidence limits were defined as the θ values at which the lod score equaled the maximum lod score minus 1.

For both the pairwise and multipoint analysis, allele frequencies were derived from the spouses of members of the pedigree. A penetrance value of 0.9 was used for the analysis of the full pedigree, while a penetrance value of 1.0 was used for the analysis involving only the affected pedigree members. For the multipoint linkage analysis, the map and distances for the markers were obtained from the CHLC (ftp access: ftp.chlc.org).

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REFERENCES