Hereditary Motor and Sensory Neuropathy Type 2C Is Genetically Distinct From Types 2B and 2D
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**Background:** Linkage analysis studies have identified 3 genetically different varieties of hereditary motor and sensory neuropathy type 2 (HMSN 2, also called Charcot-Marie-Tooth disease type 2, or CMT 2): HMSN 2A (linked to 1p35-p36), 2B (to 3q13-q22), and 2D (to 7p14). Hereditary motor and sensory neuropathy type 2C is characterized by diaphragmatic and vocal cord paresis; its disease locus has not been mapped.

**Objective:** To determine whether the HMSN 2C phenotype, previously shown not to be linked to the HMSN 2A locus, is linked to the HMSN 2B or HMSN 2D loci.

**Design:** Linkage analysis.

**Setting and Patients:** Thirty-three subjects, including 12 affected individuals and 11 individuals at risk, in a large family with HMSN 2C.

**Results:** Evidence was found against linkage of HMSN 2C phenotype to either the HMSN 2B or the 2D loci.

**Conclusions:** HMSN 2C is genetically distinct from HMSN 2A, 2B, and 2D. We think that at least 4 genetically distinct varieties of autosomal dominant HMSN 2 exist.

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**HEREDITARY MOTOR and sensory neuropathy (HMSN; also called Charcot-Marie-Tooth disease, or CMT) comprises a group of phenotypically similar inherited disorders of the peripheral nervous system.**

HMSN type 1 (HMSN 1) represents a genetically heterogeneous group of autosomal-dominant demyelinating hypotrophic neuropathies with characteristically low peripheral nerve conduction velocities. The HMSN 1 loci have been mapped to chromosome region 17p11.2-p12 (HMSN 1A) and 1q22-q23 (HMSN 1B).

HMSN type 2 (HMSN 2) is a genetically heterogeneous group of axonal (neuronal) neuropathies with normal or slightly low nerve conduction velocities, also usually inherited as autosomal dominant traits. Hentati et al² and Lopresti et al³ have demonstrated that the autosomal dominant HMSN 2 families that they studied did not have abnormal alleles at the HMSN 1A or HMSN 1B loci. Othmane et al⁴ have reported 3 families with dominantly inherited HMSN 2 linked to chromosome region 1p35-p36 (HMSN 2A) and also 3 other pedigrees with HMSN 2 that did not link to these loci, demonstrating genetic heterogeneity within HMSN 2. Subsequently, Kwon et al⁵ have reported a family with dominantly inherited HMSN 2 linked to markers mapped to chromosome region 3q13-q22 (HMSN 2B). Then Ionasescu et al⁶ described a family with dominantly inherited HMSN 2 linked to 7p14 (HMSN 2D).

Hereditary motor and sensory neuropathy type 2C is a clinical variety of HMSN 2 characterized by motor and sensory involvement of the limbs and progressive weakness of the vocal cords, diaphragm, and intercostal muscles.⁷ We have reported 2 unrelated HMSN 2C families⁸; the larger of these kindreds was genetically distinct from HMSN 2A.⁸ In the present study we tested whether the HMSN 2C phenotype in this family is linked to the HMSN 2B or HMSN 2D loci.

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METHODS

KINSHIP

The clinical and electrophysiological features of the kinship studied here have been described. Autosomal dominant inheritance was evident from at least 1 occurrence of male-to-male transmission based on the clinical examination. Affected persons had weakness and atrophy of peroneal and hand muscles, and decreased or absent deep tendon reflexes of the limbs. Their motor nerve conduction velocities in the median nerve were within the normal range. Sensory involvement of the feet and absent or decreased sensory nerve action potentials occurred in severely affected persons. Of 12 affected subjects, 9 had clinical evidence of diaphragmatic or vocal-cord paresis or paralysis. The severity of clinical symptoms in affected persons was variable; phenotypes did not differ by sex.

In the present study, the neuropathic status of 33 subjects in the larger kinship, including 12 affected individuals and 11 individuals at risk, were investigated (Figure 1). Clinically unaffected offspring who were at risk but younger than 26 years were excluded. After informed consent was given, 20 mL of blood was obtained by venipuncture from the family members. Continuing lymphoblast cell lines were established by Epstein-Barr virus transformation, and genomic DNA was isolated using a nucleic acid extraction kit (Isoquick; Microprobe, Bothell, Wash).

GENETIC STUDIES

Genotyping was performed for DNA microsatellite markers D3S1538, D3S1769, GGAA8B03, D3S1267, D3S1551, D3S1290, GATA4A10, and D3S1744. These markers map to the chromosome region 3q13–q22 and are linked to the HMSN 2B phenotype. Genotyping also was performed for microsatellite markers mapped to 7p14 (D7S1808, D7S1869, D7S435, and D7S1806), linked to the HMSN 2D phenotype, and for markers situated proximally (D7S2201) and distally (D7S526 and D7S1830) to 7p14. The markers (Research Genetics, Huntsville, Ala) were amplified by polymerase chain reaction in a final volume of 15 µL containing 30 ng of genomic template DNA, 2 pmol of each oligonucleotide microsatellite primer, 200 µM concentrations of dATP, dGTP, and dTTP, 50 mM of dCTP (Boehringer Mannheim, Indianapolis, Ind), 0.5 µCi of α-32P-dCTP (Amersham Life Science, Arlington Heights, Ill), and 0.5 U of Taq DNA polymerase (Boehringer Mannheim) in a 1 × Taq buffer containing 10-mmol/L Tris-HCl (pH 8.3 at 20°C), 50-mmol/L potassium chloride, and 1.5-mmol/L magnesium chloride. Amplification was performed for 35 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The results of multipoint linkage analysis with the HMSN 2C locus tested against a fixed genetic map of these markers are presented in Table. No evidence for linkage was seen in this region. The results of multipoint linkage analysis with the HMSN 2C locus tested against a fixed genetic map of these markers are presented in Figure 2. The multipoint LOD scores in the region of these markers were less than −2 between points 9.7 cm proximal to D7S1801 and 10.1 cm distal to D7S435, which excludes the HMSN 2C locus from this region.

RESULTS

The results of pairwise analyses between HMSN 2C phenotype and 8 markers from the region of the HMSN 2B locus are given in the Table: all of the 2-point LOD scores were negative. A multipoint linkage analysis testing with the HMSN 2C locus against the fixed genetic map of chromosome region 3q13–q22 markers is presented in Figure 2. The multipoint LOD scores in the region of these markers were less than −2, excluding the HMSN 2C locus from this region.

Ionescu et al have reported that the HMSN 2D family showed the highest 2-point LOD score (4.83) at D7S435, and the highest multipoint LOD score (6.89) at the point 0.5 cm proximal from D7S435 and 4.6 cm distal to D7S1808. The results of pairwise analysis with 7 markers near the HMSN 2D locus are given in the Table. No evidence for linkage was seen in this region. The results of multipoint linkage analysis with the HMSN 2C locus tested against a fixed genetic map of these markers are presented in Figure 3: the multipoint LOD scores in the region of these markers were less than −2 between points 9.7 cm proximal to D7S1801 and 10.1 cm distal to D7S435, which excludes the HMSN 2C locus from this region.

COMMENT

Three different disease loci have been established for autosomal dominant HMSN 2. Three families in the United States and 2 Japanese families with HMSN 2 have shown linkage with chromosome region 1p35-p36 (HMSN 2A), and an Italian family also has shown evidence suggestive of linkage to this locus. On the other hand, 3 of 6 families in the United States with HMSN 2 and 10 of 11 European families with HMSN 2 have been shown to lack linkage to the HMSN 2A locus; in the aggregate these findings demonstrated genetic heterogeneity in HMSN 2. Subsequently, the disease locus in a family with HMSN 2 in the United States has been mapped to chromosome region 3q13–q22 (HMSN 2B), and then a European family with HMSN 2 has shown suggestive evidence for linkage to the same locus. The third disease locus for HMSN 2 has been mapped to chromosome region 7p14 (HMSN 2D) in a single family in the United States. Clinically HMSN 2A shows a motor-dominant pattern with more pronounced weakness in the lower than the upper extremities. Hereditary motor and sensory neuropathy type 2B is characterized by a marked
sensory disturbance in the distal lower extremities resulting in ulceration in the feet, while HMSN 2D shows more severe motor and sensory disturbance in the upper than in the lower extremities.

In comparison with other forms of HMSN 2, the HMSN 2C family we studied here had unique clinical features in addition to motor and sensory involvement of the limbs, including progressive paresis of the vocal cords, diaphragm, and intercostal muscles. Yoshioka et al have shown this family's abnormality to be genetically distinct from HMSN 2A. In the present study, we found no evidence to support linkage of the HMSN 2C phenotype to either the HMSN 2B locus on chromosome 3 or the HMSN 2D locus on chromosome 7.

Our results provide further evidence of genetic heterogeneity in HMSN 2. At least 4 genetic varieties of HMSN

Two-Point LOD Scores Between CMT 2C Phenotype and HMSN 2B and 2D Markers*

<table>
<thead>
<tr>
<th>Chromosome/Locus</th>
<th>LOD Score at θ</th>
<th>0.00</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
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<td>3q13-q22 (HMSN 2B)</td>
<td>D3S1558</td>
<td>-24.78</td>
<td>-8.80</td>
<td>-4.16</td>
<td>-2.37</td>
<td>-0.91</td>
<td>-0.31</td>
<td>-0.06</td>
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<td></td>
<td>D3S1769</td>
<td>-8.99</td>
<td>-5.45</td>
<td>-2.95</td>
<td>-1.88</td>
<td>-0.85</td>
<td>-0.34</td>
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<td>GGAAB803</td>
<td>-12.82</td>
<td>-4.45</td>
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<td>-0.73</td>
<td>-0.32</td>
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<td>-23.02</td>
<td>-10.91</td>
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<td>-12.56</td>
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<td>7p14 (HMSN 2D)</td>
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</table>

* LOD indicates limit of detection; CMT, Charcot-Marie-Tooth disease; HMSN, hereditary motor and sensory neuropathy; and θ, recombination factor.

extension at 72°C for 15 seconds, with a final elongation cycle at 72°C for 10 minutes. After 10 µL of loading buffer (95% formamide, 10-mmol/L EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol) was added, the amplified products were denatured at 94°C for 10 minutes and then cooled rapidly to 4°C and placed on ice. Three microliters of each sample was electrophoresed on 6% denaturing polyacrylamide sequencing gels at 75 W for 2 to 3 hours. Gels were vacuum dried and autoradiographed overnight at -70°C. Base-pair sizes of the polymerase chain reaction products were calculated using a double-strand DNA cycle sequencing system (Gibco BRL, Grand Island, NY).

**LINKAGE ANALYSIS**

A linkage analysis was carried out between chromosome 3q13-q22 markers and the HMSN 2C phenotype in the large kinship described above. A linkage analysis was also performed between 7p14 markers and the HMSN 2C phenotype. Pairwise limit of detection (LOD) scores (Z values) were calculated, under the assumption of single-gene autosomal-dominant inheritance, using the MLINK option of the computer program LINKAGE (version 5.1; http://linkage.rockefeller.edu/soft/list.html) as described by Lathrop et al. A gene frequency of 0.0001 was assumed for the mutant HMSN 2C allele; no phenocopies were assumed, and penetrance was assumed to be 0.99 for heterozygotes and 1.0 for homozygotes. Male and female recombination fractions were assumed to be equal. Marker allele frequencies for white populations were obtained from the Genome Data Base (accessed June 1999; http://gdbwww.gdb.org). Multipoint location scores for the HMSN 2B locus were determined for the chromosome region 3q13-q22 using the LINKMAP option of LINKAGE, using published genetic map information from the Cooperative Human Linkage Center and Genethon as in a previous report. Multipoint location scores for the HMSN 2D locus were determined for the chromosome region 7p14 using the same computer program, employing published genetic map information from the Genome Data Base. Haldane’s mapping function was used to convert recombination fractions (θ) to genetic distances (centimorgan [cM]). Multipoint LOD scores were computed as the logarithm of the ratio of the likelihood with the disease locus at a specific recombination fraction from the test locus (θ = 0.00-0.49) and the likelihood with the disease locus placed in an unlinked state (θ = 0.50).

To assess how informative the data of our kindred were for linkage studies, simulation calculations were carried out by the computer program SIMLINK. The maximum attainable LOD score at a recombination fraction of 0 with fully informative linkage markers including all persons at risk was 7.7 (average LOD score, 6.1).
2 seem to have been confirmed: HMSN 2A (1p35-p36), HMSN 2B (3q13-q22), HMSN 2D (7p14), and HMSN 2C (not linked to any of these loci). A linkage study employing DNA markers from multiple autosomes will be needed to map the HMSN 2C locus.

![Figure 2. Multipoint linkage analysis with markers spanning chromosome 3q13-22. Limit of detection (LOD) scores are shown on the y-axis and genetic distances (centimorgans [cM]) are shown on the x-axis. Distances between markers are based on maps from the Cooperative Human Linkage Center and Genethon. The points with an LOD score of –2 are 20.7 cM proximal to D3S1558 and 27.8 cM distal to D3S1744. No evidence mapping the HMSN 2C gene to the studied region of chromosome 3 was found. Abbreviation q ter indicates q terminus; cen, centromere.](image)

![Figure 3. Multipoint linkage analysis with markers spanning chromosome 7p14. Ionescu et al. have shown that the hereditary motor and sensory neuropathy (HMSN) type 2D locus is just proximal to D7S435. Limit of detection (LOD) scores are shown on the y-axis and genetic distances (centimorgans [cM]) are shown on the x-axis. Distances between markers are based on maps from the Genome Data Base (accessed June 1999; http://gdbwww.gdb.org/). The points with an LOD score of –2 are 9.7 cM proximal to D7S1801 and 10.1 cM distal to D7S435. No evidence supported mapping of the HMSN 2C gene to the studied region of chromosome 7. Abbreviation cen indicates centromere; p ter, p terminus.](image)

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REFERENCES