Evidence for Linkage of the Central Core Disease Locus
to the Proximal Long Arm of Human Chromosome 19

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Central core disease of muscle (CCD; MIM 117000) is a rare inheritable myopathy that is frequently found in association with susceptibility to malignant hyperthermia (MHS). This observation has prompted us to perform a linkage study in CCD families using various chromosome 19q probes that are linked to the MHS locus and map close to the ryanodine receptor gene (RYR1), a strong MHS candidate gene. Our genetic linkage data support a location of the CCD gene on proximal 19q13.1 and thus suggest that CCD and MHS may be allelic.

INTRODUCTION

Central core disease of muscle (CCD; MIM 117000) is a rare inheritable myopathy which was first described as a separate entity by Shy and Magee in 1956. Patients with central core disease show a wide variation in the clinical spectrum of muscle involvement. Hypotonia may be evident in the neonatal period and attainment of motor milestones can be markedly delayed in infancy. Muscle weakness of the lower extremities frequently is the leading complication, but the severity of symptoms may vary from almost normal to severe. Because the clinical course is slow or nonprogressive, many patients are diagnosed only later in life. The differential diagnosis from other forms of congenital myopathies is based on muscle histology: more than 90% of type I muscle fibers should show well-demarcated centrally located "cores" for which the disease was named by Greenfield et al. (1958). Identification of cores is facilitated by staining for oxidative enzyme activity. As the cores are depleted of mitochondria they appear as negative areas within the normal activity of the surrounding muscle fiber. The biochemical nature of the cores and the underlying biochemical defect are currently unknown.

Genetic analysis of several large pedigrees was compatible with autosomal dominant transmission (Dubreuil and Roy, 1970; Isaacs et al., 1975; Eng et al., 1978; Byrne et al., 1982). In these family studies, however, some gene carriers were free of clinical symptoms and could be identified only by their altered muscle histology, thus illustrating the variable expression of the disease. Sporadic cases of CCD have also been reported and could be new mutations or, more trivial, be members of families that have been only superficially diagnosed. As a consequence, a reliable estimation of population frequency and mutation rate in CCD is impossible at present.

A number of associated clinical features have been described in patients with central core disease, including kyphoscoliosis, congenital hip dislocation, foot deformities, and joint contractures. These skeletal alterations seem not to be related to the severity of muscle weakness and some of them, like other features, appear to be nonspecific associations.

Episodes of malignant hyperthermia after inhalational anesthesia of CCD patients were first noted in 1973 (Denborough et al., 1973) and have been reported repeatedly since (Eng et al., 1978; Frank et al., 1980; Shuaib et al., 1987; Krivosic-Horber and Krivosic, 1989). There are numerous case reports in the literature concerning the susceptibility to malignant hyperthermia in myopathic patients, including dystrophic, atrophic, metabolic, and myotonic myopathies. However, malignant hyperthermia seems to be associated more frequently with CCD than with any of the other muscle disorders. In a systematic study Shuaib et al. (1987) reached the conclusion that "all patients with central core disease should be considered at risk for malignant hyperthermia unless in vitro contracture tests show that the particular patient is free of the trait."

The locus for the human malignant hyperthermia susceptibility gene (MHS) has recently been localized to the chromosome 19q12–13.2 region by linkage to DNA markers (MacKenzie et al., 1989, 1990; MacLennan et al., 1989; McCarthy et al., 1990; McKenzie...
et al., 1990). Extensive studies on halothane-sensitive porcine strains suffering from similar symptoms after exposure to halothane make the ryanodine receptor (the Ca^{2+}-release channel of the sarcoplasmic reticulum) a likely candidate for the primary biochemical defect in this condition (Rousseau et al., 1987; Mickelson et al., 1988; MacLennan et al., 1990). Moreover, the ryanodine receptor gene (RYR1) belongs to a large conserved syntenic linkage group located on chromosomes 19 in man, 7 in mouse, and 6 in pigs (McCarthy et al., 1990; Cavanna et al., 1990; Davies et al., 1989). The strong association of MHS and CCD prompted us to start a linkage study in CCD families with the same set of DNA markers that map to the pertinent segment of human 19q.

MATERIALS AND METHODS

The patients from the three families shown in Fig. 1 have been examined clinically and/or histopathologically as indicated by the different pedigree symbols. In Family A, individuals A-I-2, A-II-2, and A-III-1 have a history of generalized hypotonia from birth ("floppy infants") and proximal muscle weakness. Symptoms were nonprogressive but persisted throughout life, conferring to the patients physical abilities inferior to those of their peers. In contrast, patient A-II-3 never learned to walk and has been wheelchair-bound since early childhood. Perhaps surprisingly, muscle histology in the two brothers A-II-2 and A-II-3 was similar and showed a predominance of type I fibers and central cores in almost all fibers. The cores lacked phosphorylase and oxidative enzyme activity. The other family members were examined by the same neurologist and found unaffected. In Family B, patient B-I-1 has suffered from muscle weakness since infancy. His motor development was delayed and he now shows proximal muscle hypotrophy and weakness (Gowers' maneuver). In his muscle biopsy, structured central cores were found in all type I fibers (ATPase reaction and light and electron microscopy). The clinical history and muscle performance of his daughters B-II-2 and B-II-4 resembled his own. A muscle biopsy was not done. Their siblings had a normal clinical status. In Family C, the index patient C-II-1 was reported as a floppy infant and achieved the motor milestones with marked delay. Today she is not capable of strong physical exercise but can manage everyday activities. Her muscle biopsy showed an excess of type I fibers, almost all containing central cores devoid of oxidative enzyme activity. Her brother and father have presented a very similar picture of generalized proximal muscle weakness since childhood. They did not undergo a muscle biopsy. The uncle (C-I-1) and the mother (C-I-3) are clinically nonconspicuous.

DNA preparations from peripheral blood leukocytes, Southern blot analysis, and DNA probe labeling procedures were according to standard protocols (Sambrook et al., 1989).
TABLE 1

Lod Scores for Linkage of CCD to Various Chromosome 19 Markers

<table>
<thead>
<tr>
<th>Locus</th>
<th>Probe</th>
<th>Location</th>
<th>Lod scores at θ of 0.00</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>D19S20</td>
<td>pJCZ3.1</td>
<td>19</td>
<td>-∞</td>
<td>-2.35</td>
<td>-1.46</td>
<td>-0.64</td>
<td>-0.25</td>
<td>-0.06</td>
</tr>
<tr>
<td>D19S11</td>
<td>p13-1-25</td>
<td>p13.2-cen</td>
<td>-∞</td>
<td>-0.94</td>
<td>1.07</td>
<td>0.97</td>
<td>0.68</td>
<td>0.30</td>
</tr>
<tr>
<td>D19S13</td>
<td>pHW60</td>
<td>19q12</td>
<td>1.51</td>
<td>1.35</td>
<td>1.19</td>
<td>0.86</td>
<td>0.53</td>
<td>0.22</td>
</tr>
<tr>
<td>D19S28</td>
<td>p5B18</td>
<td>19q13.1</td>
<td>0.80</td>
<td>0.79</td>
<td>0.68</td>
<td>0.45</td>
<td>0.23</td>
<td>0.07</td>
</tr>
<tr>
<td>D19S18</td>
<td>pPM6.7</td>
<td>19q13.1</td>
<td>2.41</td>
<td>2.22</td>
<td>2.01</td>
<td>1.51</td>
<td>0.94</td>
<td>0.57</td>
</tr>
<tr>
<td>D19S8</td>
<td>p17.1</td>
<td>19q13.2</td>
<td>0.43</td>
<td>0.38</td>
<td>0.33</td>
<td>0.22</td>
<td>0.11</td>
<td>0.04</td>
</tr>
<tr>
<td>D19S22</td>
<td>pEFD4.2</td>
<td>19q</td>
<td>1.00</td>
<td>0.87</td>
<td>0.74</td>
<td>0.49</td>
<td>0.25</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Data from Schonk et al. (23) and LeBeau et al. (14).

The polymorphisms used for the linkage studies are defined by the following probes and restriction enzymes, respectively: pJCZ3.1/TaqI (locus D19S20), p13-1-25/BamHI (D19S11), pHW60/TaqI and BglII (D19S13), p5B18/TaqI (D19S28), pPM6.7/EcoRI (D19S18), and pEFD4.2/TaqI (D19S22); further details are given in LeBeau et al. (1989). The physical location of these marker loci was adapted from Schonk et al. (1989).

Two-point lod scores were calculated using the LINKAGE program package (version 5.3) of Lathrop and Lalouel (1984). The likelihood ratios for the location of the CCD locus in relation to chromosome 19q markers were estimated by the EXCLUDE program (Edwards, 1987).

RESULTS

A total of seven chromosome 19 probes detecting eight informative polymorphisms in these families were used for linkage analysis (Fig. 1). Two-point lod scores between the CCD locus and each of the marker loci are given in Table 1. A peak lod score of z = 2.41 at zero recombination fraction (θ = 0.00) was obtained for CCD and the D19S18 locus. Lower but still positive lod scores all peaking at θ = 0.00 were also observed for the other chromosome 19 long-arm markers tested, whereas the two short-arm probes (D19S20 and D19S11) showed recombination to the CCD locus. Although none of the observed pairwise lod score values reaches the significance level (z ≥ 3), the complete "haplotype" of chromosome 19q alleles strongly suggests a linkage of the CCD locus to this chromosome arm. When the chromosomal location was tested with the EXCLUDE program (Edwards, 1987), the probability was 0.9995 for the CCD locus being on chromosome 19. With respect to the marker loci D19S13, D19S28, D19S18, D19S8, and D19S22, which have been physically mapped to 19q intervals (Schonk et al., 1989), the highest likelihood ratio (1.8 × 10⁶:1) for the location of the CCD locus was found in the proximal segment of 19q13.1 (Fig. 2).

DISCUSSION

Taken together, our data suggest linkage of CCD to DNA markers on the proximal long arm of human chromosome 19. A preliminary fine mapping of the CCD gene with respect to physically mapped loci was obtained by a multipoint location analysis (Fig. 2). As
no recombination has been observed between the CCD locus and these 19q markers, the peaking of the likelihood ratios in proximal 19q13.1 is determined largely by the informativity of the individual polymorphisms. Interestingly, this location coincides with the previous mapping of MHS and RYR1 to the same chromosome segment. In fact, the index patients from two families had positive in vitro contracture tests and must thus be considered at risk for malignant hyperthermia (Ellis et al., 1984). The MHS test results, however, were not used for the classification of family members with respect to CCD to avoid a circular argument. The strong association of MHS to CCD suggests a correlation of the underlying molecular defects. If mutations in the RYR1 gene turn out to be the primary defects in human MHS (MacLennan et al., 1990), it shall be interesting to see if CCD is caused by allelic mutations. If not, it remains to be seen which interaction at the structural or functional gene level causes the concurrent observation of malignant hyperthermia and myopathy in CCD patients. The final cloning and characterization of the MHS and CCD genes then will require a more precise subchromosomal localization of the genes by extended linkage studies and physical mapping strategies.

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Note added in proof. Recently, linkage of the CCD locus to 19q13.1, another DNA marker on 19q13, has been demonstrated in a single large pedigree in Australia (Haan et al., 1990, Hum. Genet. 86: 187–190).

REFERENCES


