SHORT COMMUNICATION

Multipoint Mapping of the Central Core Disease Locus

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A linkage analysis with 12 DNA markers from proximal 19q was performed in eight families with central
core disease (CCO). Two-point analysis gave a peak lod score of Z = 4.95 at θ = 0.00 for the anonymous marker
D19S190 and of Z = 2.53 at θ = 0.00 for the ryanodine receptor (RYR1) candidate gene. Multipoint linkage
data place the CCO locus at 19q13.1, flanked proximally by D19S190/D19S28 and distally by D19S47. This map location includes the RYR1 gene. The results of the linkage study present no evidence for genetic het-
erogeneity of CCO. © 1993 Academic Press, Inc.

Central core disease (CCO, MIM 171000), first reported by Shy and Magee (18), is a nonprogressive con-
genital myopathy that is inherited as an autosomal dominant trait. The name “central core disease” was coined
because of the characteristic “cores” that appear upon histochemical staining of transverse sections of skeletal
muscle biopsies. Ultrastructural analysis has demonstrated that the normal fibrillar architecture is disrupted
within the cores and that organelles such as the sarcoplas 
plasmic reticulum or mitochondria are missing. Therefore,
various histochemical stains show a lack of oxidative
enzymes and phosphorylase activities. The presence
of cores in a significant proportion of type I skeletal
muscle fibers is required for a definite diagnosis of CCO.
The clinical diagnosis in infancy is usually based on con-
genital hypotonia and delay in motor development.
Later, a static diffuse weakness of proximal muscle
groups predominates, individually ranging from very
mild to severe but usually not or only gradually progressive. A severe and life-threatening complication often
associated with CCO is a potentially fatal malignant hyperthermic reaction to general anesthetics (1, 9).

A disorder clinically and biochemically comparable to

human malignant hyperthermia (MHS) is the so-called
stress syndrome in pigs. A cysteine for arginine ex-
change of amino acid 615 in the ryanodine receptor, the
calcium release channel of the sarcoplasmic reticulum, is
considered the genetic defect in this trait (3, 17). The
respective gene (RYR1) is part of a conserved linkage
group localized on human chromosome 19q13.1 (13).
Linkage between RYR1 and MHS in man was reported
(14, 15) and, recently, the porcine RYR1 mutation has
also been identified in several human MHS families (4,
6; Y. Feist, B. Rübsam, E. J. Hartung, and C. R. Müller,
unpublished results). However, linkage to the RYR1
gene has been excluded in other MHS families (2, 11),
and a second locus on chromosome 17 has been sug-
gested (12). Thus, MHS in man is clearly a genetically
heterogeneous disease. The strong association between
CCO and MHS prompted the first linkage studies with
chromosome 19q markers in CCO families. Indepen-

FIG. 1. Pedigrees of the eight CCO families. Pedigree symbols:
black, affection status ascertained by clinical examination and muscle
histology; cross-hatched, affection confirmed by clinical examination
only; white, examined clinically normal.

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205

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dently two groups mapped the CCO gene to the proximal long arm of chromosome 19 (5, 8). As this assignment coincides with the localization of the RYR1 and the MHS loci on 19q12-19q13.2, the RYR1 gene also became a likely candidate gene for CCO. Mutations of the RYR1 gene in CCO patients, however, have not been reported to date. Here, we present a refined genetic linkage map for CCO.

The eight unrelated CCO pedigrees studied were depicted in Fig. 1. Clinical details of some of the families (families 1, 2, and 3) were previously published in (8). All family members were examined by expert neurologists, and the diagnosis of CCO was established histologically in at least one patient from each family while his/her relatives were classified afterward on clinical and genetic grounds. The complementing diagnostic approaches (clinical, genetic, and/or histopathological) are indicated by different pedigree symbols in Fig. 1.

Twelve genetic markers were typed in leukocyte DNA as described (8; see Table 1). Typing of the CA-repeat markers D19S199, D19S190 (7), D19S47, and APOC2 (19) followed the original protocols. The most likely order of chromosome 19 markers was adapted from (7, 11). Linkage calculations were made using the LINKAGE program package (10; version 5.3). Both histologically and clinically examined individuals were classified as affected, and penetrance of CCO was set to 0.95.

Two-point lod scores between the CCO locus and each of the marker loci are summarized in Table 1. The highest two-point lod score of $Z = 4.95$ at zero recombination fraction ($\theta = 0.00$) was observed for D19S190. A positive though not significant lod score was also found for the RYR1 candidate gene. A single phase-known recombination was observed between CCO and D19S49 in family CCO8. Multipoint lod score analysis yielded a peak lod score for CCO in the interval flanked by D19S199/D19S28 and D19S47, a segment that includes the RYR1 gene (Fig. 2). This interval has a significantly higher likelihood than the flanking regions D19S49/D19S191/D19S28 and D19S47-APOC2. As D19S191 and D19S28 have been located on the same cosmide contig, less than 50 kb apart (D. E. Iles, unpublished data), these two markers were considered very closely linked ($\theta < 0.01$).

**TABLE 1**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Probe</th>
<th>Informative families</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>D19S49</td>
<td>CA repeat</td>
<td>1,8</td>
<td>-0.57</td>
<td>-0.96</td>
<td>-0.22</td>
<td>-0.56</td>
<td>-0.01</td>
</tr>
<tr>
<td>D19S28</td>
<td>pBR322</td>
<td>2,3</td>
<td>0.99</td>
<td>0.79</td>
<td>0.68</td>
<td>0.45</td>
<td>0.23</td>
</tr>
<tr>
<td>D19S191</td>
<td>CA repeat</td>
<td>2,4-9</td>
<td>3.11</td>
<td>2.79</td>
<td>2.45</td>
<td>1.76</td>
<td>1.05</td>
</tr>
<tr>
<td>RYR1</td>
<td>HRR1-5</td>
<td>2-6</td>
<td>2.53</td>
<td>2.42</td>
<td>2.53</td>
<td>1.70</td>
<td>1.66</td>
</tr>
<tr>
<td>D19S18</td>
<td>pPMI.7</td>
<td>1,2,3,5,6</td>
<td>4.13</td>
<td>3.72</td>
<td>3.20</td>
<td>2.34</td>
<td>1.36</td>
</tr>
<tr>
<td>D19S190</td>
<td>CA repeat</td>
<td>2-9</td>
<td>4.95</td>
<td>4.53</td>
<td>4.06</td>
<td>2.99</td>
<td>1.81</td>
</tr>
<tr>
<td>D19S47</td>
<td>CA repeat</td>
<td>1-9</td>
<td>0.79</td>
<td>0.47</td>
<td>0.11</td>
<td>0.02</td>
<td>0.77</td>
</tr>
<tr>
<td>APO C2</td>
<td>CA repeat</td>
<td>1,2,3,8</td>
<td>2.31</td>
<td>2.10</td>
<td>1.80</td>
<td>1.26</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*For the RYR1 locus a haplotype was constructed from five RFLPs of the HRR subclones (14).*

The same applies for the marker pair D19S190 and D19S18. The multipoint curve largely reflects the informativity of markers. The small minimum at the RYR1 locus was due to an inferred crossover event in family CC03 and must have occurred between proximal and distal RFLPs within the RYR1 gene. As CC03 is a two-generation pedigree it was impossible to pinpoint this crossover to one of the children.

Our data are in full agreement with the close linkage of CCO to the RYR1 gene as found by Mulley *et al.* (16). Therefore, RYR1 remains a candidate gene for CCO. The strong association of MHS to CCO suggests a common link between the underlying molecular defects. Our results further support the hypothesis of CCO and subforms of MHS being allelic mutations of the same gene. Formally, however, they might as well be encoded by separate, closely linked genes. Our data also predict that CCO maps close to the RYR1 locus, or indeed could cosegregate with defects of this gene. So far, linkage studies including a total of nine unrelated families of Caucasian origin give no indication for genetic heterogeneity in CCO. Until now a definite diagnosis of CCO has been dependent on the invasive method of muscle biopsy. Indirect genotype analysis may now offer a noninvasive diagnostic test for some CCO families.

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**REFERENCES**


