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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. © 1991 Academic Press, Inc.

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 (Dubowitz 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

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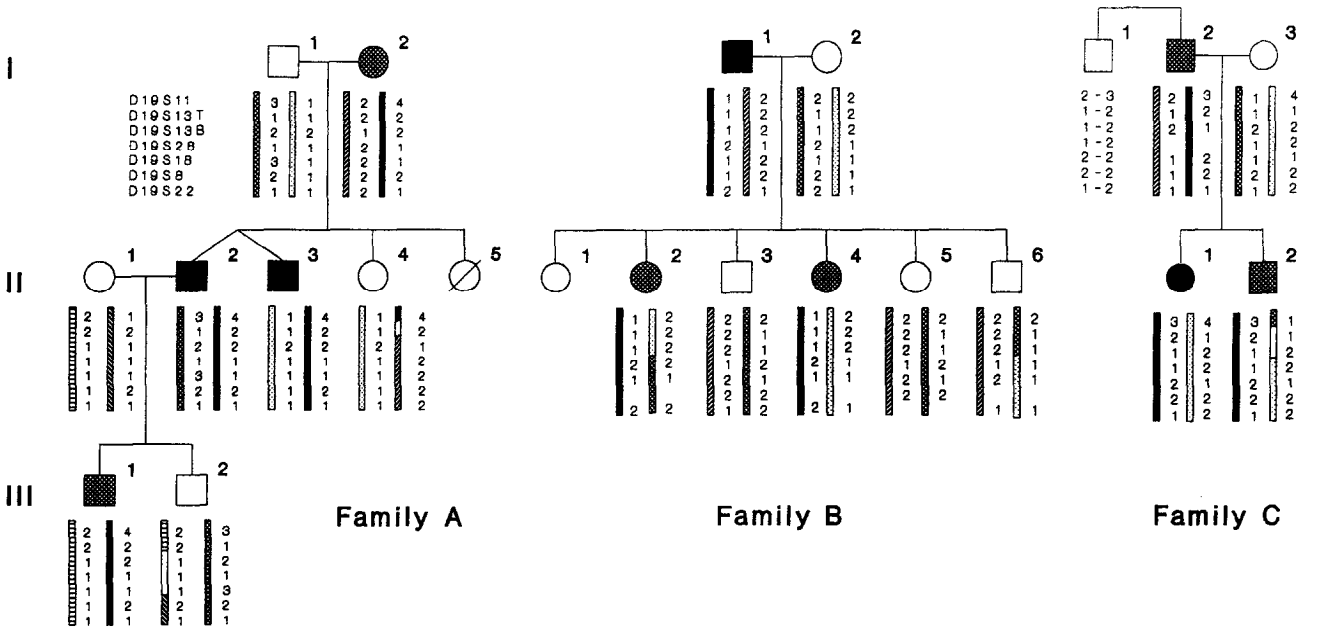

FIG. 1. Segregation of chromosome 19 DNA markers in three CCD families. Pedigree symbols: *black,* affection status ascertained by clinical examination and muscle histology; *cross-hatched,* affection confirmed by clinical examination only; *white,* clinically normal. The most probable DNA marker haplotypes are drawn below each individual. The chromosomes carrying the CCD mutation are given in black.

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

Locus	Probe	Physical location[a]	Lod scores at θ of					
			0.00	0.05	0.10	0.20	0.30	0.40
D19S20	pJCZ3.1	19	$-\infty$	−2.35	−1.46	−0.64	−0.25	−0.06
D19S11	p13-1-25	19p13.2–cen	$-\infty$	0.94	1.07	0.97	0.68	0.30
D19S13	pHW60	19q12	1.51	1.35	1.19	0.86	0.53	0.22
D19S28	p5B18	19q13.1	0.90	0.79	0.68	0.45	0.23	0.07
D19S18	pPM6.7	19q13.1	2.41	2.22	2.01	1.51	0.94	0.37
D19S8	p17.1	19q13.2	0.43	0.38	0.33	0.22	0.11	0.04
D19S22	pEFD4.2	19q	1.00	0.87	0.74	0.49	0.25	0.07

[a] 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/*Taq*I (locus D19S20), p13-1-25/*Bam*HI (D19S11), pHW60/*Taq*I and *Bgl*II (D19S13), p5B18/*Taq*I (D19S28), pPM6.7/*Eco*RI (D19S18), p17.1/*Taq*I (D19S8), and pEDF4.2/*Taq*I (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 ($\theta = 0.00$) was obtained for CCD and the D19S18 locus. Lower but still positive lod scores all peaking at $\theta = 0.00$ 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 ($z \geq 3$), 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 (1.8×10^6:1) 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

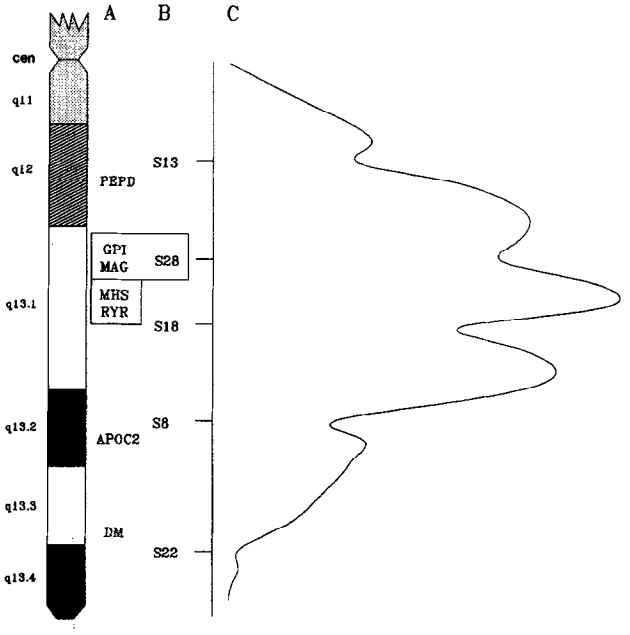

FIG. 2. Likelihoods for the location of the CCD locus on chromosome 19q. (A) Approximate physical location of known genes and (B) polymorphic DNA segments (the order of loci within boxes remains to be determined). (C) Likelihood ratios for the various positions of the CCD locus with respect to known markers (arbitrary units).

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 D19S9, 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

1. Byrne, E., Blumbergs, P. C., and Hallpike, J. F. (1982). Central core disease: Study of a family with five affected generations. *J. Neurol. Sci.* **53:** 77–83.
2. Cavanna, J. S., Greenfield, A. J., Johnson, K. J., Marks, A. R., Nadal-Ginard, B., and Brown, S. D. M. (1990). Establishment of the mouse chromosome 7 region with homology to the myotonic dystrophy region of human chromosome 19q. *Genomics* **7:** 12–18.
3. Davies, W., Harbitz, I., Fries, R., Stranzinger, G., and Hauge, J. G. (1988). Porcine malignant hyperthermia carrier detection and chromosomal assignment using a linked probe. *Anim. Genet.* **19:** 203–12.
4. Denborough, M. A., Dennett, X., and Anderson, R. M. (1973). Central core disease and malignant hyperpyrexia. *Br. Med. J.* **1:** 272–276.
5. Dubowitz, V., and Roy, S. (1970). Central core disease of muscle: Clinical, histochemical and electron microscopic studies of an affected mother and child. *Brain* **93:** 133–146.
6. Edwards, J. H. (1987). Exclusion mapping. *J. Med. Genet.* **24:** 539–543.
7. Ellis, F. R., Halsall, P. J., Ording, H., *et al.* (European Malignant Hyperpyrexia Group) (1984). A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. *Br. J. Anaesth.* **56:** 1267–1269.
8. Eng, G. D., Epstein, B. S., Engel, W. K., McKay, D. W., and McKay, R. (1978). Malignant hyperthermia and central core disease in a child with congenital dislocating hips: Case presentation and review. *Arch. Neurol.* **35:** 189–197.
9. Frank, J. P., Harati, Y., Butler, I. J., Nelson, T. E., and Scott, C. I. (1980). Central core disease and malignant hyperthermia. *Ann. Neurol.* **7:** 11–17.
10. Greenfield, J. G., Cornman, T., and Shy, G. M. (1958). The prognostic value of the muscle biopsy in the "floppy infant." *Brain* **81:** 461–470.
11. Isaacs, H., Heffron, J. J. A., and Badenhorst, M. (1975). Central core disease: A correlated genetic, physiochemical, ultramicroscopic, and biochemical study. *J. Neurol. Neurosurg. Psychiatry* **38:** 1177–1186.
12. Krivosic-Horber, R., and Krivosic, I. (1989). Central core disease associated with malignant hyperthermia sensitivity. *Presse Med.* **18:** 828–831.
13. Lathrop, G. M., and Lalouel, J. M. (1984). Easy calculations of lod scores and genetic risks on small computers. *Am. J. Hum. Genet.* **36:** 460–465.
14. LeBeau, M. M., Ryan, D., Jr., and Pericak-Vance, M. A. (1989). Report of the committee on the genetic constitution of chromosomes 18 and 19: Human Gene Mapping 10. *Cytogenet. Cell Genet.* **51:** 338–357.
15. MacKenzie, A. E., Korneluk, R. G., Duff, C., Worton, R. G., and MacLennan, D. H. (1989). Localization of the human ryanodine receptor gene with respect to an established human chromosome 19 linkage group. *Am. J. Hum. Genet.* **45**(Suppl.): A149. [Abstract]
16. MacKenzie, A. E., Korneluk, R. G., Zorzato, F., Fuji, J., Phillips, M., Iles, D., Wieringa, B., Leblond, S., Bailly, J., Willard, H. F., Duff, C., Worton, R. G., and MacLennan, D. H. (1990). The human ryanodine receptor gene: Its mapping to 19q13.1, placement in a chromosome 19 linkage group, and exclusion as the gene causing myotonic dystrophy. *Am. J. Hum. Genet.* **46:** 1082–1089.
17. MacLennan, D. H., Zorzato, F., Fuji, J., Otsu, K., Phillips, M., Lai, F. A., Meissner, G., Green, N. M., Willard, H. F., Britt, B. A., Worton, R. G., and Korneluk, R. G. (1989). Cloning and localization of the human calcium release channel (ryanodine receptor) gene to the proximal long arm (cen-q13.2) of human chromosome 19. *Am. J. Hum. Genet.* **45**(Suppl.): A205. [Abstract]
18. MacLennan, D. H., Duff, C., Zorzato, F., Fuji, J., Phillips, M., Korneluk, R. G., Frodis, W., Britt, A., and Worton, R. G. (1990). Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature* **343:** 559–561.
19. McCarthy, T. V., Healy, J. M., Heffron, J. J., Lehane, M., Deufel, T., Lehmann-Horn, F., Farrall, M., and Johnson, K. (1990). Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12–13.2. *Nature* **343:** 562–564.
20. Mickelson, J. R., Gallant, E. M., Litterer, L. A., Johnson, K. M., Rempel, W. E., and Louis, C. F. (1988). Abnor-

mal sarcoplasmic reticulum ryanodine receptor in malignant hyperthermia. *J. Biol. Chem.* **263:** 9310–9315.

21. ROUSSEAU, E. C., SMITH, J. S., AND MEISSNER, G. (1987). Ryanodine modifies conductance and gating behaviour of single calcium release channels. *Am. J. Physiol.* **253:** C364–C368.
22. SAMBROOK, J., FRITSCH, E. F., AND MANIATIS, T. (1989). "Molecular cloning: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
23. SCHONK, D., COERWINKEL-DRIESSEN, M., VAN DALEN, I., OERLEMANS, F., SMEETS, B., SCHEPENS, J., HULSEBOS, T., COCKBURN, D., BOYD, Y., DAVIS, M., RETTIG, W., SHAW, D., ROSES, A. D., ROPERS, H. H., AND WIERINGA, B. (1989). Definition of subchromosomal intervals around the myotonic dystrophy gene region at 19q. *Genomics* **4:** 384–396.
24. SHUAIB, A., PAASUKE, R. T., AND BROWNELL, K. W. (1987). Central core disease. Clinical features in 13 patients. *Medicine (Baltimore)* **66:** 389–396.
25. SHY, G. M., AND MAGEE, K. R. (1956). A new congential nonprogressive myopathy. *Brain* **79:** 610–619.