SHORT COMMUNICATION

Multipoint Mapping of the Central Core Disease Locus

S. SCHWEMMLE,*,[1] K. WOLFF,*,[2] L. M. PALMUCCI,† T. GRIMM,* F. LEHMANN-HORN,‡ CH. HÜBNER,§ E. HAUSER,¶ D. E. ILES,‖ D. H. MACLENNAN,⊥ AND C. R. MÜLLER*,[3]

*Institut für Humangenetik der Universität, Biozentrum, W-8700 Würzburg, Germany; †Istituto di clinica delle malattie del sistema nervoso dell' Università di Torino, Italy; ‡Abt. Angewandte Physiologie der Universität, Ulm, Germany; §Universitätskinderklinik Eppendorf, Hamburg, Germany; ¶Universitätskinderklinik Wien, Austria; ‖Department of Cell Biology and Histology, University Hospital Nijmegen, The Netherlands; and ⊥Banting and Best Department of Medical Research, University of Toronto, Canada

Received December 22, 1992; revised April 16, 1993

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 $\theta = 0.00$ for the anonymous marker D19S190 and of $Z = 2.53$ at $\theta = 0.00$ for the ryanodine receptor (RYR1) candidate gene. Multipoint linkage data place the CCO locus at 19q13.1, flanked proximally by D19S191/D19S28 and distally by D19S47. This map location includes the RYR1 gene. The results of the linkage study present no evidence for genetic heterogeneity of CCO.

Central core disease (CCO, MIM 117000), first reported by Shy and Magee (18), is a nonprogressive congenital 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 sarcoplasmic reticulum or mitochondria are missing. Therefore, various histochemical stains show a lack of oxidative enzyme 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 congenital 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 exchange 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 suggested (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-

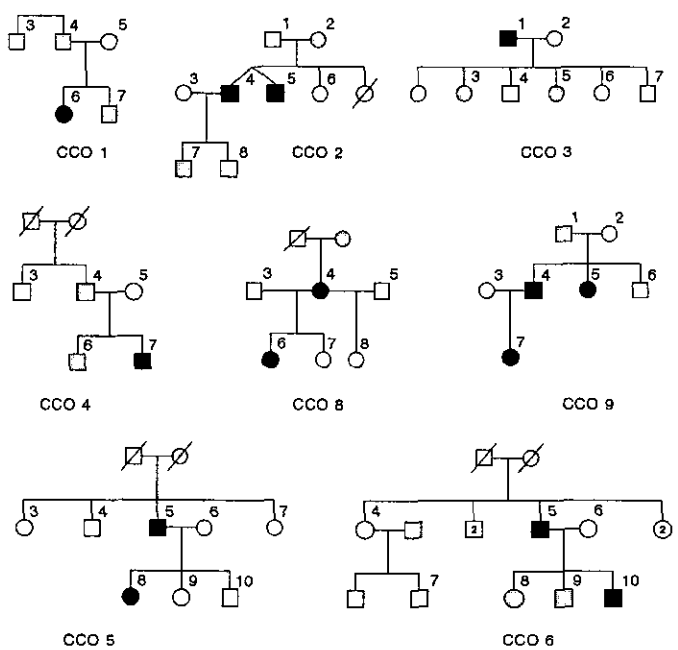

FIG. 1. Pedigrees of the eight CCO families. Pedigree symbols: black, affection status ascertained by clinical examination and muscle histology; cross-hatched, affection confirmed by clinical examination only; white, examined clinically normal.

[1] Present address: Abt. Klinische Genetik, Universität Ulm, Germany.

[2] Present address: Abt. Neuropädiatrie, Universitätskinderklinik, Heidelberg, Germany.

[3] To whom correspondence should be addressed. Telephone: (+)49-931-888-4063. Fax: (+)49-931-888-4069. E-mail: un87 cm@genius.embnet.dkfz-heidelberg.de.

GENOMICS 17, 205–207 (1993)
0888-7543/93 $5.00

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 are 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 D19S191, 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 location analysis yielded a peak location score for CCO in the interval flanked by D19S191/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 cosmid contig, less than 50 kb apart (D. E. Iles, unpublished data), these two markers were considered very closely linked ($\theta < 0.01$).

TABLE 1

Two-Point Lod Scores for CCO vs Chromosome 19q Markers

Locus	Probe	Informative families	Recombination fraction θ 0.00	0.05	0.10	0.20	0.30
D19S49	CA repeat	1, 8	$-\infty$	−0.46	−0.22	−0.06	−0.01
D19S28	p5B18	2, 3	0.90	0.79	0.68	0.45	0.23
D19S191	CA repeat	2, 4–9	3.11	2.79	2.45	1.76	1.05
RYR1[a]	HRR1-5	2–6	2.53	2.42	2.23	1.70	1.06
D19S18	pPM6.7	1, 2, 3, 5, 6	4.13	3.72	3.29	2.34	1.36
D19S190	CA repeat	2–9	4.95	4.53	4.06	2.99	1.81
D19S47	CA repeat	1–9	0.79	4.47	4.11	3.02	1.77
APO C2	CA repeat	1, 2, 3, 8	2.31	2.10	1.80	1.26	0.71

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

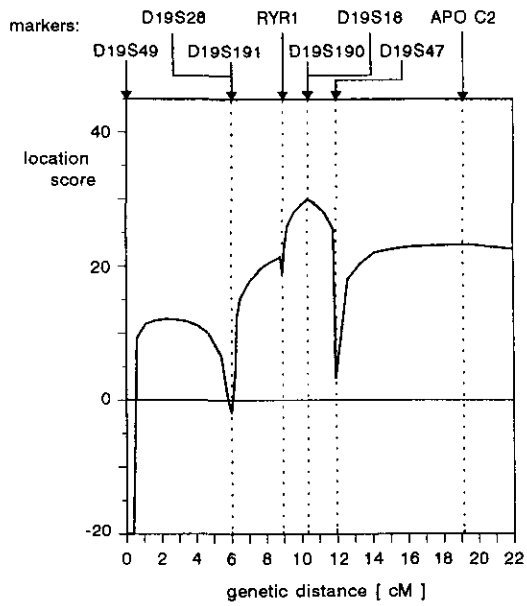

FIG. 2. Multipoint likelihoods for the location of the CCO locus on chromosome 19q13.1–q13.2.

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 CCO3 and must have occurred between proximal and distal RFLPs within the RYR1 gene. As CCO3 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.

ACKNOWLEDGMENTS

This work was supported by grants from the Deutsche Forschungsgemeinschaft (S.S. and C.R.M.), Deutsche Gesellschaft Bekämpfung der Muskelkrankheiten (F.L.-H. and T.G.), Association Française contre le Myopathies (C.R.M.), and the Canadian Genetic Diseases Network of Centers of Excellence (D.H.M.).

REFERENCES

1. Denborough, M. A., Dennett, X., and Anderson, R. M. (1973). Central core disease and malignant hyperpyrexia. *Brit. Med. J.* **1**: 272–276.

2. Deufel, T., Golla, A., Iles, D., Meindl, A., Meitinger, T., Schindelhauser, D., DeVries, A., Pongratz, D., MacLennan, D. H., Johnson, K. J., and Lehmann-Horn, F. (1992). Evidence for genetic heterogeneity of malignant hyperthermia susceptibility. *Am. J. Hum. Genet.* **50:** 1151–1161.
3. Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J., and MacLennan, D. H. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* **253:** 448–451.
4. Gillard, E. F., Otsu, K., Fujii, J., Khanna, V. K., De Leon, S., Derdemezi, J., Britt, B. A., Duff, C. L., Worton, R. G., and MacLennan, D. H. (1991). A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. *Genomics* **11:** 751–755.
5. Haan, E. A., Freemantle, C. J., McCure, J. A., Friend, K. L., and Mulley, J. C. (1990). Assignment of the gene for central core disease to chromosome 19. *Hum. Genet.* **86:** 187–190.
6. Hogan, K., Couch, F., Powers, P. A., and Gregg, R. G. (1992). A cysteine for arginine substitution (R614C) in the human skeletal muscle calcium release channel consegregates with malignant hyperthermia. *Anesth. Analg.* **75:** 441–448.
7. Iles, D. E., Segers, B., Heytens, L., Sengers, R. C. A., and Wieringa, B. (1992). High-resolution physical mapping of four microsatellite repeat markers near the RYR1 locus on chromosome 19q13.1 and apparent exclusion of the MHS locus from this region in two malignant hyperthermia susceptible families. *Genomics* **14:** 749–754.
8. Kausch, K., Lehmann-Horn, F., Janka, M., Wieringa, B., Grimm, T., and Müller, C. R. (1991). Evidence for linkage of the central core disease locus to the proximal long arm of human chromosome 19. *Genomics* **10:** 765–769.
9. Krivosic-Horber, R., and Krivosic, I. (1989). Central core disease associated with malignant hyperthermia sensitivity. *Presse Med.* **18:** 828–831.
10. 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.
11. Levitt, R. C., Nouri, N., Jedlicka, A. E., McKusick, V. A., Marks, A. R., Shutack, J. G., Fletcher, J. E., Rosenberg, H., and Meyers, D. A. (1991). Evidence for genetic heterogeneity in malignant hyperthermia susceptibility. *Genomics* **11:** 543–547.
12. Levitt, R. C., Olckers, A., Meyers, S., Fletcher, J. E., Rosenberg, H., and Meyers, D. A. (1992). Evidence for the localization of a malignant hyperthermia susceptibility locus (MHS2) to human chromosome 17q. *Genomics* **14:** 562–566.
13. 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.
14. MacLennan, D. H., Duff, C., Zorzato, F., Fujii, J., Phillips, M., Korneluk, R. G., Frodis, W., Britt, B. A., and Worton, R. G. (1990). Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature* **343:** 559–561.
15. McCarthy, T. V., Healy, J. M. S., Heffron, J. J. A., Lehane, M., Deufel, T., Lehmann-Horn, F., Farrall, M. *et al.* (1990). Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12–13.2. *Nature* **343:** 562–564.
16. Mulley, J. C., Kozman, H. M., Phillips, H. A., Gedeon, A. K., McCure, J. A., Iles, D. E., Gregg, R. G., Hogan, K., Couch, F. J., MacLennan, D. H., and Haan, E. A. (1993). Refined genetic localization for central core disease. *Am. J. Hum. Genet.* **52:** 398–405.
17. Otsu, K., Khanna, V. K., Archibald, A. L., and MacLennan, D. H. (1991). Cosegregation of porcine malignant hyperthermia and a probable causal mutation in the skeletal muscle ryanodine receptor gene in backcross families. *Genomics* **11:** 744–750.
18. Shy, G. M., and Magee, K. R. (1956). A new congenital nonprogressive myopathy. *Brain* **79:** 610–619.
19. Weber, J. L., and May, P. E. (1989). Abundant class of human DNA polymorphisms which can be typed using the PCR reaction. *Am. J. Hum. Genet.* **44:** 388–396.