Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12–13.2

Tommie V. McCarthy*, J. M. Sandra Healy*, James J. A. Heffron*, Mary Lehane†, Thomas Deufel‡, Frank Lehmann-Horn§, Martin Farrall‖¶ & Keith Johnson‖¶

* Department of Biochemistry, University College, Cork, Ireland
† Department of Anaesthetics, University College, Cork, Ireland
‡ Dr Von Haunersches Children's Hospital, University of Munich, Lindwurmstrasse 4, D-8000 Munich 2, FRG
§ Department of Neurology, Technical University of Munich, Mohlstrasse 28, D-8000 Munich 80, FRG
‖ Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, Norfolk Place, London W2 1PG, UK
¶ Present addresses: MRC Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ, UK (M.F.), and Department of Anatomy, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, UK (K.J.)

MALIGNANT hyperthermia (MH) is an inherited human skeletal muscle disorder and is one of the main causes of death due to anaesthesia[1]. The reported incidence of MH varies from 1 in 12,000 in children to 1 in 40,000 in adults[2,3]. MH is triggered in susceptible people by all commonly used inhalational anaesthetics; it is characterized by a profoundly accelerated muscle metabolism, contractures, hyperthermia and tachycardia[1,4,5]. Susceptibility to MH (MHS) is predicted by contracture tests on muscle tissue obtained by biopsy[6,7]. An almost identical disorder known as porcine MH exists in pigs[8,9]. The genetics of the porcine syndrome have been extensively studied[10]; the locus controlling expression of porcine MH is genetically linked to the glucose phosphate isomerase locus (*GPI*)[11]. In man, *GPI* has been mapped to the q12–13.2 region of chromosome 19 (refs 10–12). We have now investigated genetic linkage in several extended Irish pedigrees in which MHS is segregating as an autosomal dominant trait. Here we show linkage between MHS and DNA markers from the *GPI* region of human chromosome 19 with a maximum log likelihood ratio (lod score) of 5.65 at the *CYP2A* locus. These results indicate that human and porcine MH are most probably due to mutations in homologous genes, and also provide a potentially accurate and noninvasive method of diagnosis for MHS.

A standardized test (based on contractures induced in muscle *in vitro* by caffeine and halothane) for the investigation of MH susceptibility in individuals has been established and validated by the European MH group[7,15]. This test allows the following diagnoses to be made: MH-susceptible (MHS), MH-negative (MHN), and MH-equivocal (MHE).

The locus controlling expression of porcine MH is very tightly linked to the *GPI*[11], alpha 1-B glycoprotein (*GA1B*)[16] and H blood group antigen loci[17]. This linkage group, spanning about 12 centimorgans (cM; a centimorgan is equivalent to 1% recombination between two loci and corresponds to $\sim 1 \times 10^6$ base pairs of DNA), is highly conserved in humans and maps to chromosome 19q12–13.2 (ref. 16). This region of chromosome 19 contains the myotonic dystrophy (*DM*) locus and several well-defined polymorphic loci including an apolipoprotein gene cluster (*APOC1*, *APOC2* and *APOE*), *BCL3*, *CYP2A* and

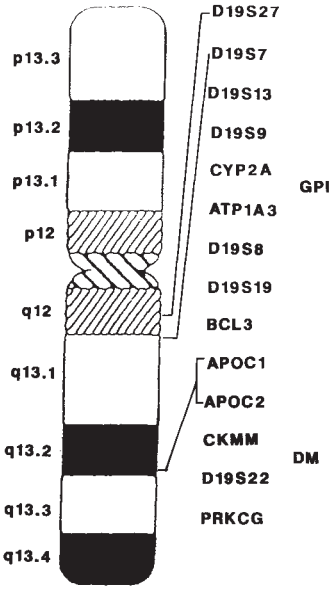

FIG. 1 Linkage map of 14 markers on chromosome 19q. Data are from a multipoint analysis of the CEPH pedigrees[19] with the location of *GPI* from somatic cell hybrid data[18] and the position of the *DM* locus distal to *CKMM* from analysis of recombinant family members[26–28].

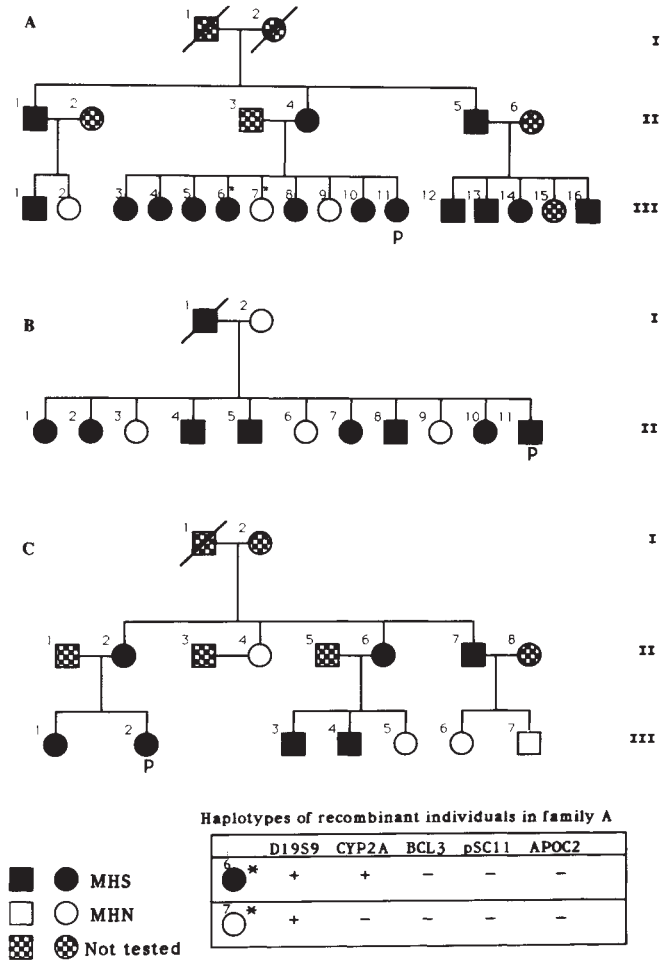

FIG. 2 Pedigrees of three Irish MHS families. Individuals were diagnosed as MHS or MHN by the standardized European contracture test[7]. There were no MHE individuals in these families. In each pedigree the proband (P) suffered a clinical episode of MH during anaesthesia and recovered. DNA was isolated from peripheral blood of all individuals for genotyping with polymorphic DNA markers. The alleles of the markers *D19S9*, *CYP2A*, *BCL3*, *pSC11* and *APOC2* were combined into haplotypes for all members of family A. The haplotypes of the two recombinant individuals III-6 and III-7 in family A are shown at the bottom and are denoted by an asterisk in the pedigree. +, Affected individual's genotype; −, unaffected individual's genotype.

TABLE 1 Lod score for MHS versus *D19S9, CYP2A* and *APOC2** markers

Equal male and female recombination fractions

	Recombination frequency (θ)									
	0.00	0.001	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.40
D19S9 vs MH	2.07	2.10	2.30	2.50	2.42	2.23	1.98	1.68	1.35	0.59
CYP2A vs MH	0.74	0.74	0.72	0.64	0.55	0.45	0.36	0.27	0.18	0.05
APO* vs MH	2.60	2.68	3.07	3.59	3.56	3.31	2.95	2.45	2.00	0.84

Female θ varying only (male $\theta = 0.5$)

	Recombination frequency									
	0.00	0.001	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.40
D19S9 vs MH	0.21	0.25	0.47	0.80	0.88	0.85	0.77	0.65	0.51	0.19
CYP2A vs MH	0.08	0.08	0.08	0.07	0.05	0.04	0.03	0.02	0.01	0.00
APO* vs MH	0.70	0.73	0.95	1.26	1.31	1.25	1.15	1.00	0.84	0.45

Male θ varying only (female $\theta = 0.5$)

	Recombination frequency									
	0.00	0.001	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.40
D19S9 vs MH	1.54	1.54	1.51	1.39	1.24	1.08	0.91	0.73	0.55	0.19
CYP2A vs MH	0.37	0.37	0.36	0.32	0.26	0.21	0.16	0.12	0.08	0.02
APO* vs MH	0.17	0.22	0.68	1.35	1.53	1.50	1.36	1.18	0.92	0.37

Lod scores were calculated with the LINKAGE program[21] for combined sexes ($\Theta_{male} = \Theta_{female}$), male recombination alone (Θ_{male}, $\Theta_{female} = 0.5$) and female recombination alone ($\Theta_{male} = 0.5$, Θ_{female}). The frequency of the *MHS* mutant allele was kept at 0.001; lod scores calculated with MHS frequencies of 0.0001 or 0.01 did not appreciably vary. Two 'liability' classes were defined for MHS: class 1 (patients who had undergone an MH crisis) defined with complete penetrance; and class 2 (MHS, unaffected and unknown individuals) with reduced penetrance (0.99) and a 1% phenocopy rate. The latter liability class allows for potential inaccuracies in diagnosis with the muscle biopsy test. Three probes (*APOC2, pSC11* and *BCL3*) have been physically mapped to a 300-kilobase interval[25] and lod scores for these three markers were combined into a single haplotype (APO*).

D19S9[18]. The order of 14 markers spanning 63 cM on 19q is known (Fig. 1) from both physical and genetic mapping studies prompted by research to isolate the *DM* gene[18,19]. Because of the phenotypic similarity of human and porcine MH, it seemed likely that the human MH locus would map to 19q. We tested this possibility by investigating linkage between MHS and markers that span 19q12–13.2 in three large Irish families in which MHS is segregating as an autosomal dominant trait (Fig. 2). We typed the markers *D19S9, BCL3, pSC11* and *APOC2* in these families and calculated the two-point lod scores (Table 1).

The maximum two-point lod score generated for these markers was 3.67 at recombination fraction $\theta = 0.073$ for the haplotype of markers *APOC2, pSC11* and *BCL3* (Table 1). The genetic distance between *D19S9* and *APOC2* has been estimated to be 20 cM from linkage analysis in the CEPH panel of families[19,20]

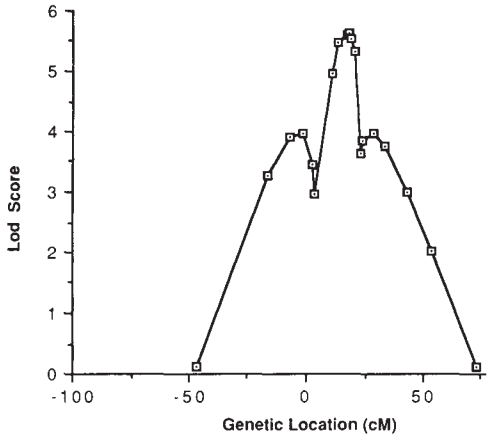

FIG. 3 Location map summarizing location scores ($\log_{10}$ likelihood difference) calculated for *MHS* at various map positions in a fixed marker map. Joint likelihoods were calculated for the six loci simultaneously. Here the support for linkage of MHS is calculated at various map locations in a fixed map of polymorphic markers. The most likely location for *MHS* is at *CYP2A* with a lod score of 5.65. On the *x*-axis *APOC2* is arbitrarily set at 0 cM, *D19S9* is at +20 cM, and *CYP2A* at +15 cM, based on linkage maps published for this region[19,20].

and no sex difference in recombination is seen across this interval[20]. Several polymorphic loci map into this interval (Fig. 1), and one of these, *CYP2A*, was informative in family A (Fig. 2). Multipoint joint likelihood calculations were computed with the LINKAGE computer program[21], and are shown as a location map (Fig. 3). The maximum lod score in the multipoint analysis using *CYP2A* was 5.65 at $\theta = 0$. The multipoint analysis indicates that *D19S9* and *APOC2* flank the *MHS* locus.

Inspection of the haplotypes in family A shows that markers proximal to *BCL3* segregate with MHS in affected individual III-6. By contrast, the allele of the *D19S9* marker that usually segregates with MHS, segregates with *MHN* in individual III-7 (Fig. 2). Taken together, these two recombination events confirm the mapping of *MHS* and *CYP2A* into the interval between *D19S9* and *APOC2*. In somatic cell hybrids, *CYP2A* and *GPI* cosegregate[18], which, with the data presented here, strongly indicates that human MH and porcine MH are homologous disorders.

Although the defect responsible for MHS is not known, biochemical evidence indicates that the skeletal muscle ryanodine receptor is abnormal in pigs affected with MH[22]. The human ryanodine receptor gene has recently been localized to the cen-q13.2 region of chromosome 19 (ref. 23), and in the accompanying letter MacLennan *et al.*[24] show that polymorphic markers for this gene cosegregate with the human MH susceptibility trait. These data, taken together with the linkage studies presented here, indicate that the ryanodine receptor gene is a highly likely candidate for the *MHS* gene. □

Received 14 July; accepted 19 December 1989.

1. Britt, B. A. in *Malignant Hyperthermia* (ed. Britt, B. A.) 325–367 (Martinus-Nijhoff, Amsterdam, 1987).
2. Britt, B. A. & Kalow, W. *Can. Anaesth. Soc. J.* **17,** 293 (1970).
3. Snow, J. C. *Archs Opthal., N.Y.* **84,** 407 (1970).
4. Berman, M. C., Harrison, G. G., Bull, A. B. & Kench, J. E. *Nature* **225,** 653–655 (1970).
5. Ellis, F. R. & Heffron, J. J. A. in *Recent Advances in Anaesthesia and Analgesia* (eds Atkinson, R. S. & Adams, A. P.) 173–207 (Churchill-Livingstone, New York, 1985).
6. Rosenberg, H. & Reed, S. *Anesth. Analg.* **62,** 415–420 (1983).
7. European Malignant Hyperpyrexia Group *Br. J. Anaesth.* **56,** 1267–1271 (1984).
8. Hall, L. W., Woolf, N., Bradley, J. W. P. & Jolly, D. W. *Br. med. J.* **2,** 1305 (1966).
9. Harrison, G. G. *et al. Br. med. J.* **3,** 594–595 (1968).
10. Archibald, A. L. & Imlah, P. *Anim. Blood Grps biochem. Genet.* **16,** 253–263 (1985).
11. Andresen, E. & Jensen, P. *Nord. Vet. Med.* **29,** 502–504 (1977).
12. Ingram, P. H. *et al. Biochem. Genet.* **15,** 455–476 (1977).
13. Brook, J. D. *et al. Hum. Genet.* **68,** 282–285 (1984).
14. Lusis, A. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 3929–3933 (1986).

15. Ording, H. in *Malignant Hyperthermia* (ed. Britt, B. A.) 267–277 (Martinus-Nijhoff, Amsterdam 1987).
16. Eiberg, H. *et al. Cytogenet. Cell Genet.* **51,** 994 (1989).
17. Rasmusen, B. A. & Christian, L. L. *Science* **191,** 947–948 (1976).
18. Schonk, D. *et al. Genomics* **4,** 384–396 (1989).
19. Harley, H. *et al. Cytogenet. Cell Genet.* **51,** 1011 (1989).
20. Nakamura, Y. *et al. Genomics* **3,** 67–71 (1988).
21. Lathrop, G. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **81,** 3443–3446 (1984).
22. Mickelson, J. R. *et al. J. biol. Chem.* **263,** 9310–9315 (1988).
23. MacLennan, D. H. *et al. Am. J. hum. Genet. Suppl.* **45,** abstract 803 (1989).
24. MacLennan, D. H. *et al. Nature* **343,** 559–561 (1990).
25. Shaw, D. J. *et al. Hum. Genet.* **83,** 71–74 (1989).
26. Brunner, H. G. *et al. Genomics* **5,** 589–595 (1989).
27. Johnson, K. *et al. Genomics* **5,** 746–752 (1989).
28. Korneluk, R. G. *et al. Genomics* **5,** 596–604 (1989).

ACKNOWLEDGEMENTS. We thank the Irish Health Research Board for partially funding this work, EMBO for supporting S.J.M.H. on a short term fellowship, Drs Michael Morgan and Pelin Faik at Guy's Hospital for typing *GPI* in the families, Dr Bill Davies of the Norwegian College of Veterinary Medicine, Oslo for providing the pig *GPI* probe and Professor Bob Williamson of St Mary's Hospital for discussion and critical reading of this manuscript.

Vasoactive intestinal peptide regulates mitosis, differentiation and survival of cultured sympathetic neuroblasts

David W. Pincus*, Emanuel M. DiCicco-Bloom*† & Ira B. Black*

Departments of * Neurology and † Pediatrics, Division of Developmental Neurology, Cornell University Medical College, 515 East 71st Street, New York, New York 10021, USA

ALTHOUGH acute, millisecond-to-millisecond actions of neurotransmitters are well documented, diverse longer-term effects have been discovered only recently[1–5]. Emerging evidence indicates that these signals regulate a variety of neuronal processes, from phenotypic expression to neurite outgrowth[6–19]. Here we show that a single putative transmitter, vasoactive intestinal peptide, can exert multiple, long-term effects simultaneously: it stimulates mitosis, promotes neurite outgrowth and enhances survival of sympathetic neuron precursors in culture. As the peptide seems to be a normal presynaptic transmitter in the sympathetic system[19–25], synaptic transmission may exert hitherto unexpected effects.

To study early neuronal ontogeny, we developed a fully defined, dissociated cell culture system composed of an enriched population of sympathetic precursors or neuroblasts[26]. Embryonic neuroblasts, derived from superior cervical ganglia from rats of 15.5 days' gestation, were identified immunocytochemically by detection of neurofilament and the catecholamine biosynthetic enzyme, tyrosine hydroxylase[26]. Earlier work indicated that these cells enter the mitotic cycle in culture[26,27]. We investigated the effect of vasoactive intestinal peptide (VIP) using this system, because in the adult rat the peptide is localized to presynaptic fibres that innervate these ganglion neurons[19,22–24]. VIP elicits a striking twofold increase in [^{3}H]thymidine incorporation per culture, indicating that the peptide increases neuroblast DNA synthesis (Fig. 1*a*). This increase in incorporation is comparable to that stimulated by the insulin growth factors in this system[26] (Fig. 1*b*). Moreover, increased synthesis reflects a larger number of blasts entering mitosis, not simply an enhanced incorporation by a pre-existent mitotic population: VIP more than doubles the percentage of tyrosine hydroxylase-positive cells incorporating thymidine into nuclei (Fig. 1*c*). Consequently, VIP increases the mitotic population *in vitro*. Finally, the effects of VIP on mitosis are highly specific as secretin and glucagon, two closely related peptides, do not alter DNA synthesis (data not shown).

In addition to regulating mitosis, VIP markedly increases the number and complexity of neurites. The peptide evokes a sixfold rise in the number of process-bearing cells 10 h after plating (Figs 2*a*, *b* and 3*a*). The elevation is not due to selective survival of a process-bearing subpopulation, because VIP does not alter the overall cell number at this time. Further, the neuritogenic effects are sustained, because VIP also markedly increases the percentage of neuroblasts with long processes at 24 h (Figs 2*c*, *d* and 3*b*). Our observations suggest that VIP simultaneously promotes differentiation and mitosis in the same cultures.

TABLE 1 VIP content of embryonic day-15.5 ganglion

Tissue	VIP (pg per μg protein)
SCG	1.12 ± 0.059 (3.07 ± 0.093 pg per ganglion)
Liver	0
Adult ileum	0.62 ± 0.117

Ganglia were dissected, pooled (10–20 per sample) and frozen at −70 °C. Adult ileum[49] and embryonic liver were examined as positive and negative assay controls, respectively. Tissues were extracted in 0.01 M HCl and radioimmunoassayed for VIP using a commercial kit (Peninsula). The lowest quantity of VIP detectable by this method was 1 pg. The antibody exhibited <0.001% cross-reactivity with other neuropeptides including VIP 1–12, peptide histidine-isoleucine or substance P at 10,000-fold molar excess. Values are corrected for recovery (80%), expressed as pg per μg acid-extractable protein and represent the mean ±s.e.m. of at least 4 samples.

During ontogeny, both precursor proliferation and cell survival contribute to the formation of stable neuronal populations[28,29]. To investigate whether VIP influences neuroblast survival, we determined the cell numbers after a longer period in culture, namely 4 days. In fact, VIP exposure results in >300% increase in the number of cells per culture compared with the control (Fig. 3*c*). Virtually the entire surviving population expressed tyrosine hydroxylase, suggesting that VIP exerts effects on cells of the sympathetic lineage (data not shown). Moreover, even in the presence of the mitotic inhibitor aphidicolin (at 5 μg ml^{-1})[30], which inhibits [^{3}H]thymidine incorporation by 80%, VIP treatment still increases cell numbers over the controls (control: 893 ± 174 cells per dish; VIP: 7,750 ± 638 cells per dish; differ at $P < 0.001$ by Student's *t*-test). Thus, in addition to influencing mitosis and differentiation, VIP seems to exert a trophic effect on sympathetic neuroblasts.

We characterized the specific nature of this effect on survival by testing other neuropeptides closely related to VIP. Secretin, peptide histidine-isoleucine and growth hormone-releasing factor, which are known to alter transmitter traits in the superior cervical ganglion (SCG)[19,22,23], have no trophic effects in culture; only VIP enhances neuronal survival (data not shown).

As VIP has a function in the mature sympathetic nervous system, we looked for VIP in embryonic day 15.5 SCG by radioimmunoassay of ganglia extracts (Table 1) and found significant amounts were present (2.3–3.0 pg per ganglion). This suggests that the transmitter is physiologically important during development.

The multiple effects of VIP on mitosis, process elaboration and cell survival could be mediated by direct action on neuroblasts or by indirect action through non-neuronal cells. In fact, non-neuronal cells made up less than 1.5% of the cells plated initially. Moreover, after four days of incubation, non-neuronal numbers did not increase in control medium or in the presence of VIP. Thus, the peptide did not promote division or survival of the non-neuronal population. The tiny number of non-neuronal cells and the rarity of non-neuron–neuron contacts indicates that VIP probably elicits neuroblast mitosis, differentiation and survival in culture directly.

The VIP concentrations necessary to exert these effects on sympathetic neuroblasts are higher than other reported K_d values for VIP binding in the nervous system, which range between 1 and 70 nM (refs 31, 32), but a low-affinity VIP receptor with an apparent K_d of 285 nM has been reported[33]. This K_d value is comparable to the half-maximal VIP concentration