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SHORT REPORT

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Several interacting genes influence the malignant hyperthermia phenotype

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Abstract Malignant hyperthermia (MH), a potentially lethal disorder of skeletal muscle calcium homeostasis, manifests only on exposure to certain anaesthetic drugs. The mode of inheritance appears to be autosomal dominant with both locus and allelic heterogeneity having been reported. Association analysis of eight MH candidate loci in UK families has indicated that several genes influence susceptibility in individual families, rather than MH simply being a major gene defect. In support of this hypothesis, we present data on a replica analysis of an independent sample of European MH families.

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Malignant hyperthermia (MH) has an estimated prevalence of 1 in 10,000 and is only triggered in susceptible individuals following exposure to certain anaesthetic drugs. The ryanodine receptor gene (*RYR1*) on chromosome 19q13.1 encodes a skeletal muscle calcium release channel and accounts for susceptibility in at least 50% of MH families. Other implicated loci include *CACNA1S* (1q32) and *CACNA2D1* (7q11.23-q21.1) and loci on 3q13.1 (MHS4) and 5p (MHS6). *CACNA1S* and *CACNA2D1* encode subunits of the dihydropyridine receptor, a voltage-gated calcium channel, functionally coupled to the ryanodine receptor.

The in vitro contracture test (IVCT) was the first reliable method to assess MH phenotype and involves measuring the contracture response of a muscle specimen to certain agents (European Malignant Hyperthermia Group 1984). Although the pattern of inheritance according to IVCT phenotype appears to be autosomal dominant, some families have been identified where the IVCT phenotype and the susceptibility genotype are discordant (Adeokun et al. 1997; Fagerlund et al. 1997). One explanation is that several independent genes may influence MH susceptibility in an individual family. Using the extended transmission disequilibrium test (ETDT; Sham and Curtis 1995), we investigated the role of candidate loci on six chromosomes in a sample of 77 UK nuclear families. Our analysis indicated that that MH loci on chromosomes 3q, 5p and 7q were involved in families that had previously demonstrated linkage to *RYR1* (19FAM, n=61). In families that showed no evidence for chromosome 19 involvement (OTHER, n=16), an effect from chromosome 1q was detected (Robinson et al. 2000). This was the first demonstration that multiple genes may influence susceptibility to MH in individual families. Our objective has been to test these findings in an independent data set.

We investigated a sample of 131 independent nuclear MH families from seven European MH centres. ETDT analysis was performed for the candidate regions previously studied. All families were analysed together (Table 1). In addition, families were stratified into distinct groups according to the posterior probability of linkage to *RYR1*

Table 1 Results of ETDT analysis for the entire sample set for chromosome 1, 3, 5, 7 and 19 markers (*TDT* transmission disequilibrium test). *Numbers* in *bold* represent results from the current analysis, the other numbers are results obtained from a previous analysis of 77 UK nuclear families (Robinson et al. 2000). Family material was selected and marker genotyping conducted as described in Robinson et al. (2000)

Marker	Candidate locus	Number of transmissions from heterozygous parents	*P* value for χ^2 test for:	
			Genotype-wise TDT	Allele-wise TDT
D19S191		97	0.25	0.07
↑		**71**	**0.0002**	**0.033**
2.7 cM	*RYR1*			
↓				
D19S422		131	0.02	0.002
		157	**0.094**	**0.224**
CACNL1AS	*CACNL1AS*	119	0.001	0.0006
		133	**0.008**	**0.048**
D3S1281	*MHS4*	118	0.003	0.01
		65	**0.121**	**0.065**
D5S674		124	0.34	0.34
↑		**170**	**0.018**	**0.010**
12 cM	*MHS6*			
↓				
D5S418		152	0.07	0.26
		153	**0.625**	**0.972**
D7S634		120	0.01	0.0001
↑		**109**	**0.159**	**0.085**
2–5 cM	*CACNA2D1*			
↓				
D7S849		71	0.02	0.26
		105	**0.006**	**0.009**

as previously described (Robinson et al. 2000). Families with a posterior probability of linkage to RYR1≥0.5 were partitioned into the "19FAM" pedigree set (n=100). Remaining families were grouped into the "OTHER" pedigree set (n=31). Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

ETDT analysis of the entire data set confirmed the role of the *RYR1* region in MH susceptibility with P=0.0002 for D19S191, which lies proximal to *RYR1* (Table 1). For remaining candidate loci, the effects of chromosomes 1 and 7 were confirmed in the entire data set as previously observed. In contrast to the earlier study, a chromosome 3 effect was not detected and chromosome 5 gave significant results in the entire data set. On analysis of the partitioned data set, a chromosome 1 effect was detected in the "19FAM" grouping (genotype-wise TDT P=0.009). Genotype-wise TDT results were also significant for D5S674 (P=0.013) and D7S634 (P=0.043) in "19FAM". In the "OTHER" grouping, only D7S849 was significant (genotype-wise TDT P=0.006; data not shown).

These results support the hypothesis that several genes influence susceptibility to MH and suggest that the phenotype is influenced by a major locus (*RYR1*) and the effect of modifier genes, perhaps by way of a threshold type model (Scriver and Waters 1999; Dipple and McCabe 2000). Following family stratification, results confirm a role for loci on chromosomes 5 and 7 in *RYR1*-linked families, with the influence of chromosomes 1 and 3 being less clear. In families with no evidence of *RYR1* linkage, a chromosome 7 effect was also detected compared with the chromosome 1 effect seen previously (Robinson et al. 2000). As the proportion of families within each grouping is similar to that in the first study, an explanation for these diverse findings and the observed variation in significance levels for the two sample sets is the genetic background of the sample, or the possible consequences of the method used for family stratification.

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