MALIGNANT HYPERTHERMIA AND THE FLUORIDE-RESISTANT GENE

M. WHITTAKER AND J. J. BRITTEN

SUMMARY

One hundred and six individuals from 33 families with a history of malignant hyperthermia have been investigated for plasma cholinesterase variants. An increased frequency of the fluoride-resistant gene has been found. Although an adequate explanation for our results is elusive, some hypotheses are discussed.

Many investigations have been carried out in the pathophysiology and biochemistry of malignant hyperthermia (MH), but in spite of the extensive clinical research an understanding of the syndrome in humans remains obscure. The rare and sporadic nature of the hyperthermic response during anaesthesia is alien to the systematic collection of physiological and biochemical data from patients. However, the increasing awareness of the syndrome by anaesthetists and the recognition that rapid and vigorous therapy is essential for a patient's survival has reduced the fatalities in susceptible individuals.

Possible predictive tests for MH during anaesthesia have to date proved to be disappointing. An increased creatine phosphokinase (CPK) activity (Isaacs, Frere and Mitchell, 1973) is by no means a universal finding in susceptible individuals and, indeed, there are other causes of an increased enzymic activity, for example i.m. injections, physical activity, trauma and alcoholism. The report of Zsigmond and others (1972) of excessive amounts of brain-type CPK isoenzyme in the serum of 21 of 23 MH-susceptible individuals has not been confirmed by others (Moore, Watson and Summery, 1976; Hassan, Meltzer and Cho, 1978).

The principal identification of individuals susceptible to MH is by pharmacological assessment of muscle biopsy, but Halsall, Cain and Ellis (1979) have reported that the diagnosis of insusceptibility to MH must be based on results obtained from multiple samples. It is not surprising that much research has been focused on the analysis of blood samples in attempts to develop a reliable predictive test for individuals susceptible to the syndrome.

The unexpected results of Whittaker, Spencer and Searle (1977), reporting a high frequency of the fluoride-resistant gene for plasma cholinesterase not only in patients who had survived MH but in additional families who had lost at least one blood relation as a consequence of the syndrome, are impossible to interpret. Ellis, Cain and others (1978) have indicated that the fluoride-resistant gene has an increased frequency in relatives of patients who had experienced MH. We report our plasma cholinesterase studies of blood samples received from patients and relatives with a personal or family history of MH.

MATERIALS AND METHODS

Ten-millilitre blood samples, heparinized or clotted, were obtained by venepuncture from individuals with personal or family history of MH. Blood samples were sent by first class post to Exeter, where they were examined immediately or the plasma was kept frozen until required.

Plasma cholinesterase activity was measured by the hydrolysis of benzoylcholine in phosphate buffer 0.07 mol litre⁻¹, pH 7.4 at 26 °C (Kalow and Lindsay, 1955). Dibucaine and fluoride numbers were determined by the method of Kalow and Genest (1957) or Harris and Whittaker (1961). Additionally, bromide numbers (Dickson, 1978), chloride numbers (Whittaker, 1968a) and pancuronium numbers (Whittaker and Britten, 1980) were measured for some samples.

RESULTS

One hundred and six blood samples from 33 families were received from anaesthetists practising in Britain. The segregation of the cholin-
TABLE I. Segregation of cholinesterase genotypes in families with history of MH

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of families (total = 33)</th>
<th>Number of individuals (total = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_1^uE_1^u$</td>
<td>13</td>
<td>43</td>
</tr>
<tr>
<td>$E_1^uE_1^f$</td>
<td>15</td>
<td>59</td>
</tr>
<tr>
<td>$E_1^uE_1^*$</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>$E_1^fE_1^f$</td>
<td>15</td>
<td>59</td>
</tr>
<tr>
<td>$E_1^fE_1^*$</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>$E_1^<em>E_1^</em>$</td>
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<td>59</td>
</tr>
<tr>
<td>$E_1^<em>E_1^</em>$</td>
<td>15</td>
<td>59</td>
</tr>
</tbody>
</table>

Frequency of genes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MH families</th>
<th>Normal population (British)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_1^u$</td>
<td>0.7783</td>
<td>0.9775</td>
</tr>
<tr>
<td>$E_1^f$</td>
<td>0.2028</td>
<td>0.0025</td>
</tr>
<tr>
<td>$E_1^*$</td>
<td>0.0188</td>
<td>0.0200</td>
</tr>
</tbody>
</table>

DISCUSSION

It appears from our results that there is a high probability that a rare plasma cholinesterase gene occurs in families reported to be susceptible to MH. Supportive evidence for such an hypothesis has been reported by Ellis, Cain and others (1978) from their investigations of relatives of individuals who have experienced MH. The relatives were classified as sensitive or non-sensitive from in vitro pharmacological investigations of muscle biopsy samples (Ellis, Harriman et al., 1978). Additionally, dibucaine and fluoride numbers were measured on blood samples from all patients and their findings are summarized in table III. Contradictory evidence arises from the findings of the Danish Malignant Hyperthermia Investigation Unit which investigated 26 patients from 13 different families in Denmark (Ørding, Hanel and Viby-Mogensen, 1981). Eighteen patients in this survey were classified as MH-susceptible by in vitro muscle biopsy tests according to Ellis, Harriman and others (1978). Plasma cholinesterase phenotypes were characterized by measurement of dibucaine, fluoride, chloride, scoline and urea numbers as described by Viby-Mogensen and Hanel (1977). On this basis only one individual had a rare gene ($E_1^*$), although this could be interpreted from the figures quoted as the $E_1^f$ gene. It is probable that family studies justify the $E_1^*$ gene, although no supportive family data are given to resolve this ambiguity. Nevertheless, a real difference exists between the British and Danish findings for which no obvious explanation is apparent. The frequency of the $E_1^uE_1^f$ phenotype in the two healthy populations is appreciably the same—0.5% in England (Whittaker, 1968b) and 0.25% in Denmark (Hanel, Viby-Mogensen and Schaffalitzky de Muckadell, 1978) and an isolated "pocket" or ethnic contribution to the English sample can be discounted since the samples were received from many regions in Britain.

Our findings, even setting aside the contradictory evidence of the Danish survey, are embarrassing. It is widely accepted that MH has a dominant inheritance (Britt, Locher and Kalow, 1969) although there appears to be variable expressivity and incomplete penetrance. The plasma cholinesterase variants, however, show a recessive inheritance and by no means all individuals with the fluoride-resistant gene are susceptible to MH. This is borne out by considering the esterase genes found in these families is presented in table I. Inhibition parameters of seven propositi who have survived MH are shown table II.

TABLE II. Propositii reported to have survived malignant hyperthermia. BzCh = enzymic activity (benzoylcholine $\mu$nol min$^{-1}$ ml$^{-1}$ x 100); DN = dibucaine number; FN = fluoride number; BrN = bromide number; ClN = chloride number; PN = pancuronium number. *Genotype confirmed by family studies. Frequency of $E_1^u$, gene = 0.0714; Frequency of $E_1^f$, gene = 0.4286

<table>
<thead>
<tr>
<th>Name</th>
<th>BzCh</th>
<th>DN</th>
<th>FN</th>
<th>BrN</th>
<th>ClN</th>
<th>PN</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. B.</td>
<td>99</td>
<td>72</td>
<td>46</td>
<td>45</td>
<td>22</td>
<td>87</td>
<td>$E_1^uE_1^f$</td>
</tr>
<tr>
<td>L. W.</td>
<td>86</td>
<td>63</td>
<td>47</td>
<td>43</td>
<td>20</td>
<td>67</td>
<td>$E_1^uE_1^*$</td>
</tr>
<tr>
<td>D. S.</td>
<td>100</td>
<td>80</td>
<td>53</td>
<td>43</td>
<td>17</td>
<td>92</td>
<td>$E_1^fE_1^*$</td>
</tr>
<tr>
<td>M. L.</td>
<td>99</td>
<td>79</td>
<td>53</td>
<td>34</td>
<td>9</td>
<td>89</td>
<td>$E_1^fE_1^*$</td>
</tr>
<tr>
<td>L. O'B.</td>
<td>106</td>
<td>80</td>
<td>52</td>
<td>29</td>
<td>16</td>
<td>91</td>
<td>$E_1^fE_1^*$</td>
</tr>
<tr>
<td>J. M.</td>
<td>106</td>
<td>78</td>
<td>53</td>
<td>27</td>
<td>6</td>
<td>87</td>
<td>$E_1^uE_1^*$</td>
</tr>
<tr>
<td>L. P.</td>
<td>124</td>
<td>81</td>
<td>51</td>
<td>41</td>
<td>17</td>
<td>87</td>
<td>$E_1^uE_1^*$</td>
</tr>
</tbody>
</table>

Range for $E_1^uE_1^u$: 80-120 78-83 56-67 33-46 13-21 38-92
Range for $E_1^uE_1^f$: 69-104 74-81 45-54 36-46 18-25 83-92
Range for $E_1^uE_1^*$: 61-92 57-68 44-53 41-49 24-30 58-69
frequency of the $E_{1s}E_{1f}$ phenotype, which occurs at a frequency of 1:200 of the population, whereas it is estimated that the occurrence of MH is 1 in 14000. It is possible that we are finding a new variant which is masquerading as a fluoride-resistant phenotype. Evidence for such an hypothesis is meagre and much more work is required to justify this concept. We have tried many structurally different inhibitors in attempts to resolve this possibility, with no success. It is sobering to reflect that the Danes, like us, use benzoylcholine as substrate in phosphate buffer pH 7.4. However, our ranges of inhibition numbers for characterizing the various genotypes do differ slightly.

There is much evidence in the literature that MH is a complex syndrome and Denborough (1977) has defined two predisposing myopathies. One is dominantly inherited and usually subclinical. The other myopathy occurs in young boys and is associated with a number of striking physical features. The second myopathy is inherited as a recessive characteristic, but several of our patients were young girls and Denborough's findings do not help to resolve our problem.

The inheritance of porcine MH is at present uncertain (Hall, Lucke and Lister, 1980). It now seems probable that porcine MH is inherited as an autosomal recessive characteristic (Andresen and Jensen, 1977; Eikelenboom et al., 1978); further investigations of the porcine syndrome may resolve our problem.

On a different tack, our difficulties might be resolved by deeper consideration of the actual clinical syndrome which our patients or their relatives experienced. It is now customary to terminate anaesthesia to abort an attack of MH whenever jaw stiffness lasting more than 2 min after suxamethonium is observed (Relton et al., 1972; Corballo, 1975). Stovner, Innes and Holen (1976) reported jaw stiffness in four of 10 cases of MH; in three of these four cases the anaesthetist ignored the clinical sign and the patient died with fully developed signs of MH. Anaesthesia was terminated in the fourth case of jaw stiffness and the patient recovered. Melvoll, Stovner and Whittaker (1980) have reported suxamethonium-induced jaw stiffness and myalgia associated with atypical plasma cholinesterase. There is a reasonable doubt whether the jaw stiffness is indeed indicative of MH, although termination of anaesthesia following jaw stiffness is not in doubt. So we must query whether all cases referred to us as MH or "family history" are indeed bona fide.

The ready interpretation as MH, by some anaesthetists, for a patient's observation that "one of my relatives died under anaesthesia" might account for a few of our cases, but by no means all. Jago and Payne (1976) have discussed the problems of diagnosing MH in patients undergoing surgery for conditions inherently associated with fever and advocate the careful exclusion of other possible causes of some of the clinical characteristics associated with MH.

The conflicting results of the Danish and British workers demand further investigation. We are convinced that a fluoride-resistant gene is not a predictive test for MH, but the possible correlation between the rare plasma cholinesterase variants and MH is certainly a research problem which can only be unmasked with the co-operation of practising anaesthetists.

ACKNOWLEDGEMENTS

Financial support from the Medical Research Council is gratefully acknowledged. The co-operation of many anaesthetists who have sent blood samples from propositi and relatives with a family history of MH has been invaluable.

REFERENCES


L'HYPERTERMIE MALIGNE ET LE GENE RESISTANT AU FLUORURE

RESUME

Cinq six individus appartenant a 33 familles ayant des antécédents d’hyperthermie maligne ont été soumis à des recherches dans le but de trouver des variantes de la cholinesterase du plasma. On a constaté une augmentation de la fréquence du gène résistant au fluorure. Bien qu’une explication adéquate sur ces résultats soit assez aléatoire, on traite, dans cet article, de certaines des hypothèses.

BÖSARTIGE HYPERTERMIE UND DAS FLUORIDRESISTENTE GEN

ZUSAMMENFASSUNG


HIPERTERMIA MALIGNE Y EL GENE RESISTENTE AL FLUORURO

SUMARIO

Se han investigado ciento seis (106) individuos procedentes de 33 familias con un historial de hipertermia maligna, en lo relativo a variables de colinesterasas del plasma. Se encontró un aumento de la frecuencia del gen resistente al fluoruro. Aunque una explicación adecuada de nuestros resultados es elusiva, se discuten algunas hipótesis.