Identification of the $E_1^f E_1^k$ cholinesterase genotype

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SUMMARY A family segregating for the A, F, and K alleles at cholinesterase locus 1 is described. This work, undertaken after the proband suffered prolonged apnoea after the use of suxamethonium during the delivery of her third child, resulted in the identification of the $E_1^f E_1^k$ genotype in her oldest son.

Since the introduction of the short acting muscle relaxant succinyl dicholine (suxamethonium) in 1951 and the subsequent discovery that certain patients exhibited a greater than usual sensitivity to the drug, interest has been directed towards identifying the genetically controlled variants of serum cholinesterase (acetylcholine acyl hydrolase E.C. 3.1.1.8). Some of these variants have been found to be the biochemical basis for this prolonged relaxant effect. The atypical, dibucaine resistant variant described in 1957 by Kalow and Genest,¹ the fluoride resistant variant identified by Harris and Whittaker² in 1961, and the silent gene reported in 1962 by Liddell *et al*³ were the first cholinesterase variants to be recognised. Taken in pairs, these three variants, together with the usual gene, result in 10 possible cholinesterase genotypes, all of which have been described and identified. In 1976, Garry *et al*⁴ identified the J allele, while two years later Rubinstein *et al*⁵ reported the K allele. These are both quantitative

cholinesterase variants which, using the currently available inhibition techniques, can only be identified when associated with the atypical variant, as in the $E_1^a E_1^j$ and $E_1^a E_1^k$ genotypes.

This report describes a family segregating for the A, F, and K alleles at cholinesterase locus 1, where one member must, on genetic grounds, be of the $E_1^f E_1^k$ genotype, although his inhibitor numbers are indistinguishable from those associated with the $E_1^u E_1^f$ genotype. $E_1^f E_1^k$ also seems the likely genotype in a second member of the family, but confirmation in her case is lacking.

Methods

Serum cholinesterase activities were measured by the benzoylcholine method of Kalow and Lindsay⁶ at 37°C as recommended by King and Morgan.⁷ In this laboratory the range of activities for the $E_1^u E_1^u$ usual homozygote by this method is 1.98 (0.40) U/ml [mean (1 SD), n=126].

Cholinesterase genotypes were identified by the degree of inhibition of benzoylcholine hydrolysis at

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FIGURE Family pedigree.

25°C using dibucaine,¹ fluoride,² and the dimethyl carbamate of (2-hydroxy-5-phenyl-benzyl) trimethylammonium bromide (Roche Ro 02-0683).⁸

All estimations were performed using a Gilford 2600 recording spectrophotometer (Ciba Corning Diagnostics Ltd) incorporating a Rapid Sampler module and Thermostat temperature controller.

Results

The family pedigree is shown in the figure. The proband, II.5, was investigated immediately after the delivery of her third child when she experienced a prolonged period of apnoea after the use of suxamethonium. Her results then, and those obtained on a repeat specimen six weeks post partum, when her serum cholinesterase had returned to its normal level, together with those for the rest of the family are shown in the table. Genotypes were allocated by reference to the inhibitor ranges published by Evans.⁹

Discussion

In genotyping the children of patient II.5 and her husband II.6, it became clear that the genotype of their oldest son, III.3, was of particular interest.

Four possible genotypes can result from the union of an $E_1^a E_1^k$ mother and an $E_1^u E_1^f$ father: $E_1^u E_1^a$, $E_1^a E_1^f$, $E_1^u E_1^k$, and $E_1^f E_1^k$. The inhibitor numbers for the two younger sons, III.4, and III.5, classify them unequivocally as $E_1^a E_1^f$ and $E_1^u E_1^a$ respectively, but those of III.3 are indistinguishable from inhibitor numbers associated with the $E_1^u E_1^f$ genotype. Since, in this situation, clearly neither $E_1^u E_1^f$ (excluded on genetic grounds) nor $E_1^a E_1^k$ (excluded by its inhibitor numbers) is a possible classification, the inevitable conclusion must be that III.3 is heterozygous for the fluoride resistant and Kalow cholinesterase variants $E_1^f E_1^k$. This is a further example of a genotype recently described for the first time by Whittaker *et al.*¹⁰ The fact that the inhibitor numbers of the $E_1^u E_1^f$ and $E_1^f E_1^k$ genotypes are indistinguishable is in marked contrast to the observed differences between the inhibitor numbers associated with the $E_1^u E_1^a$ and $E_1^a E_1^k$ genotypes. Indeed, the characteristic inhibitor numbers of the K variant, when coupled with the atypical dibucaine resistant gene, constitute the only way of identifying it by present techniques.¹¹

Having established the genotype of III.3 as $E_1^f E_1^k$, attention focused on those other members of the family whose inhibitor numbers classified them as $E_1^u E_1^f$. Could any of them be more

TABLE Family cholinesterase results.

Pedigree No	Serum cholinesterase (U/ml at 37°C)	Dibucaine No	Fluoride No	Ro02-0683 No	Presumed genotype §
I.1	1.16	75	48	—	$E_1^u E_1^f$
I.3	1.39	70	45	90	$E_1^u E_1^f$
I.4	1.54	80	57	—	$E_1^u E_1^k$ ($E_1^u E_1^u$)
I.5	0.58	70	46	93	$E_1^f E_1^k$ ($E_1^u E_1^f$)
I.6	1.68	80	57	—	$E_1^u E_1^u$ ($E_1^u E_1^k$) ($E_1^u E_1^s$)
I.7	1.64	81	57	—	$E_1^u E_1^k$
I.8	0.56	62	50	—	$E_1^u E_1^a$
I.9	2.39	82	58	—	$E_1^u E_1^u$
I.10	1.70	73	41	—	$E_1^u E_1^f$
I.11	2.07	66	48	—	$E_1^u E_1^a$
II.1	1.15	79	57	—	$E_1^u E_1^k$ ($E_1^u E_1^s$)
II.2	1.54	77	50	—	$E_1^u E_1^f$
II.3	1.08	81	59	—	$E_1^u E_1^k$
II.5*	{ 0.26† 0.79‡	{ 52 53	{ 45 44	{ — 62 }	$E_1^a E_1^k$
II.6	1.87	76	51	95	$E_1^u E_1^f$
II.8	1.77	76	48	—	$E_1^u E_1^f$
II.9	1.34	75	49	—	$E_1^u E_1^f$
II.10	1.66	80	58	—	$E_1^u E_1^u$
II.11	1.82	66	48	—	$E_1^u E_1^a$
III.1	2.18	82	58	—	$E_1^u E_1^u$
III.2	2.08	81	58	—	$E_1^u E_1^u$
III.3	1.66	72	46	94	$E_1^f E_1^k$
III.4	0.99	47	30	62	$E_1^a E_1^f$
III.5	1.71	61	49	70	$E_1^u E_1^a$

*Proband.

†Results immediately post partum.

‡Results six weeks post partum.

§Possible alternative genotypes shown in brackets.

Pedigree Nos I.2, I.12, I.13, I.14, II.4, II.7, II.12, II.13, III.6, III.7, and III.8 were not tested.

appropriately genotyped $E_1^fE_1^k$? Since the K allele appeared to be confined to the maternal side of the proband's family, $E_1^uE_1^f$ phenotypes on the paternal side were eliminated from further consideration, leaving only subjects I.1, I.3, I.5, and II.2 to be considered. Except in the case of I.5, there was no available evidence to support an $E_1^fE_1^k$ ascription in any of these subjects. I.5, however, had a low cholinesterase activity and inhibitor numbers consistent with a fluoride resistant variant; she had a son, II.1, with a low enzyme activity, but normal inhibition numbers and she comes from a family where the K allele is known to be segregating. An $E_1^fE_1^k$ classification in her case might explain the low enzyme activity of II.1 in terms of an $E_1^uE_1^k$ genotype, though it must be admitted that an $E_1^uE_1^k$ genotype or even $E_1^uE_1^s$ in his father, I.6, cannot be excluded as the basis for the son's low activity. Another detailed cholinesterase screen on the family of I.6 would be needed to provide support for this alternative theory. Since the cholinesterase activity of I.5 is considerably lower than that of her two $E_1^uE_1^f$ sibs, I.1 and I.3, evidence does seem to be weighted towards an $E_1^fE_1^k$ ascription in her case, a conclusion supported also by the data reported by Whittaker *et al*¹⁰ in their family study. Genetic information from a study of the parents of I.5 would be necessary to confirm the genotype. Unfortunately, this information is not available, and so, until new techniques are developed to permit the identification of the Kalow variant other than in combination with the dibucaine resistant gene, her genetic status, unlike that of III.3, lacks this final confirmation.

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