A family segregating for E₁^j and E₁^k at cholinesterase locus 1

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SUMMARY A family segregating for the A, J, and K alleles at cholinesterase locus 1 is described. Several further examples of the AJ and AK phenotypes occur in this family, and one member of the family, by genetic analysis, is phenotype JK. In relation to possible succinylcholine apnoea, phenotypes AJ, AK, and JK should all be considered vulnerable.

Several quantitative variants at serum cholinesterase locus 1 (E.C.3.1.1.8) have been described. The most frequently encountered is E₁^s which gives rise to a 97 to 100% reduction in enzyme activity.¹ Other, and rarer, alleles are E₁^j,^{2 3} which is associated with a 65 to 70% reduction, and E₁^k,⁴ with which activities are reduced by only about 33%. The latter alleles were discovered in families segregating for the atypical (E₁^a) allele by finding sera with anomalous dibucaine inhibition values. In every case, family studies confirmed the inherited nature of the abnormality. E₁^k has been described in two families and E₁^j identified in two families and possibly in a third.⁵

We describe here a family in which the data are best explained by postulating segregation for E₁^a, E₁^j, and E₁^k. One family member is consistent with genotype E₁^jE₁^k.

Methods

The sera from members of the M family were studied in two laboratories. The methods used in laboratory A, based upon propionylthiocholine, have been described elsewhere,^{6 7} and table 1 gives values for selected phenotypes relevant to the M family. The significance of the various inhibition values in assigning phenotypes is readily grasped by reference to fig 3 in Rubinstein *et al.*⁴ Patients with cholinesterase phenotypes susceptible to succinylcholine usually have serum cholinesterase activities of less than 4.0 U/ml by this method.⁹ Repeatability of results on the same serum at different times has

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TABLE 1 Laboratory A: results for selected serum cholinesterase phenotypes

Nomenclature		Activity*	Inhibition %±1 SD	
Phenotype	Genotype	U±1 SD	Dibucaine	Fluoride
U	E ₁ ^u E ₁ ^u	8.44±1.78	83.6±1.3	79.7±1.2
UA	E ₁ ^u E ₁ ^a	5.84±1.76	72.7±3.1	80.0±1.6
A	E ₁ ^a E ₁ ^a	1.90±0.61	19.9±2.7	84.0±1.8
AS	E ₁ ^a E ₁ ^s	1.30±0.37	20.7±4.1	82.3±3.4
SS†	E ₁ ^s E ₁ ^s	0.03±0.01	5.3±4.3	35.7±6.1
ST†	E ₁ ^s E ₁ ^t	0.18±0.05	67.7±4.3	67.7±1.7
US	E ₁ ^u E ₁ ^s	4.61±0.57	84.4±0.8	79.3±1.4
AJ (5)	E ₁ ^a E ₁ ^j	1.33-2.52	53.4-59.9	79.1-83.8
AK (4)	E ₁ ^a E ₁ ^k	2.21-3.28	60.0-66.0	78.2-81.9
UJ (3)	E ₁ ^u E ₁ ^j	4.53-4.91	81.2-85.4	77.9-80.5
UK (4)	E ₁ ^u E ₁ ^k	5.26-9.38	83.4-85.5	77.7-82.1

* Activity is expressed in U/min per ml at 37°C with propionylthiocholine as substrate.

† SS and ST correspond to S₁ and S₂ in our previous paper.¹ For the reason for a change in nomenclature, see table 1 of Scott.⁸ In the bottom half of the table, because of the small number of cases, ranges rather than SD are given. The number in parentheses after the phenotypes gives the number of cases previously encountered.^{2 4} Values for other phenotypes may be found in the same publications.

previously been studied (see table 3 in Dietz *et al.*⁹) and found to be excellent. The coefficient of variation (CV) for five samples assayed several times was between 3.3 and 6.8%, whereas the CV of the dibucaine and fluoride inhibitions on three of the specimens exceeded 1.5% in only one case which was 3%. Further data to the same effect are found for the usual phenotype in table 2 which shows a CV of enzymatic activity as high as 9%, but the CV of the inhibitions did not exceed 3%. Similar but less extensive comparisons for unusual phenotypes, again

TABLE 2 Interday assays of cholinesterase

Sample	N	Activity		Inhibition			
		U/ml $\pm$ SD	CV (%)	Dibucaine % $\pm$ SD	CV (%)	Fluoride % $\pm$ SD	CV (%)
1	13	6.71 $\pm$ 0.60	8.9	84.3 $\pm$ 1.6	1.9	80.0 $\pm$ 2.4	3.0
1a	5	7.23 $\pm$ 0.30	4.1	83.5 $\pm$ 1.0	1.2	79.5 $\pm$ 0.6	0.8
2	9	9.38 $\pm$ 0.69	7.3	83.7 $\pm$ 1.4	1.7	79.2 $\pm$ 1.3	1.6
2a	6	10.85 $\pm$ 0.34	3.1	83.8 $\pm$ 1.8	2.1	79.8 $\pm$ 0.4	0.5

Samples 1 and 1a and 2 and 2a from two subjects. The assays were repeated N times in a period of 8 to 13 months.

TABLE 3 Assay of unusual phenotypes of cholinesterase at two different times

Sample	Date obtained	Phenotype	Activity U/min/ml	Inhibition (%)	
				Dibucaine	Fluoride
A	9/79	AA/AS	1.64/2.27*	18.4/19.4	84.3/81.2
B	3/79	UF	8.27/8.19	79.2/81.7	73.0/75.6
C	3/79	AF	2.52/2.69	63.3/57.8	69.2/62.5
D	11/78	A/AS	1.62/1.71	18.6/21.6	81.4/82.8
E	12/77	UF	8.38/8.84	80.2/77.5	73.3/69.7
F	12/77	UA	7.93/6.97	75.1/74.1	80.5/78.9
G	12/77	UF	7.60/8.09	80.7/79.5	74.7/75.1
H	12/77	AF	4.71/4.50	61.8/55.0	60.7/67.2

*Assayed at different times. Day sample obtained and 1/80.

TABLE 4 Repeated cholinesterase assays on a single person

Date	Activity		Inhibition (%)	
	U/min/ml	Dibucaine	Fluoride	
11/73	13.16	82.7	77.9	
7/74	9.44	83.8	80.5	
9/74	11.81	82.2	78.2	
5/76	8.12	83.7	78.1	
7/76	9.20	82.6	81.0	
1/78	9.69	82.9	78.5	
Mean $\pm$ SD	10.2 $\pm$ 1.87	83.0 $\pm$ 0.6	79.0 $\pm$ 1.4	
CV (%)	18.3	0.8	1.7	

showing low variation for the inhibitions, are given in table 3. Concerning repeatability of results on sera drawn from the same subject at different times, in addition to the two comparisons in table 2, table 4 gives results on a subject with the usual phenotype who had six samples drawn over a 5-year period. As expected, although there is moderate variation in the enzymatic activity, the inhibitions show little variation. None of the variance tabulated in tables 2 to 4 or in previous publications would change the assignment of phenotype. This is true even in subjects whose serum cholinesterase activity is depressed by disease; the inhibitions are not altered.

TABLE 5 Laboratory B: results for selected cholinesterase phenotypes

Phenotype	Genotype	Activity (U/ml $\pm$ 1 SD)	Inhibition (% $\pm$ 1 SD)		
			Dibucaine	Fluoride	Ro-02-0683
U	$E_1^u E_1^u$	4.56 $\pm$ 1.21	81.0 $\pm$ 2.8	61.5 $\pm$ 3.7	96 $\pm$ 1.4
UA	$E_1^u E_1^a$	2.99 $\pm$ 0.90	61.6 $\pm$ 4.2	48.5 $\pm$ 4.46	70.5 $\pm$ 4.7
A	$E_1^a E_1^a$	0.96 $\pm$ 0.36	19.0 $\pm$ 4.0	23.6 $\pm$ 2.6	12.9 $\pm$ 4.3
UF	$E_1^u E_1^f$	3.90 $\pm$ 0.58	74.7 $\pm$ 1.6	48.5 $\pm$ 2.2	95.5 $\pm$ 1.3
AF	$E_1^a E_1^f$	0.94 $\pm$ 0.45	47.8 $\pm$ 3.6	34.3 $\pm$ 4.9	65.0 $\pm$ 3.0
US	$E_1^u E_1^s$	2.39 $\pm$ 0.74	81.5 $\pm$ 2.4	61.3 $\pm$ 4.2	96.9 $\pm$ 1.0
FS	$E_1^f E_1^s$ (3)	1.63-1.68	62-65	39-43	93-94
FF	$E_1^f E_1^f$ (1)	1.3	64	38	95
AK	$E_1^a E_1^k$ (6)	1.64-2.27	47-57	42-48	55-66
UK	$E_1^u E_1^k$ (4)	3.65-5.25	79-84	58-62	95-96

In laboratory B enzyme activities were measured using the method of Dietz *et al.*,⁷ modified according to Evans and Wroe.¹⁰ Using this method, enzyme activities of less than 1.68 mmol/l are associated with succinylcholine sensitivity. Inhibitor numbers in this laboratory are based upon benzoylcholine hydrolysis using dibucaine,¹¹ fluoride,¹² and Hoffman La Roche Ro-2-0683.¹³ Enzyme activities and inhibitor values of relevant phenotypes are shown in table 5.

Medical history

Patient III.4 (figure) was a 15-year-old boy weighing 59 kg. Before bilateral antral washout he was given 60 mg succinylcholine after which he suffered apnoea lasting 20 minutes.

FIGURE Pedigree of the M family. The arrow identifies the proband.

TABLE 6 Enzymatic results on members of M family

Pedigree No	Presumed phenotype	Laboratory A			Laboratory B			
		Cholinesterase activity (U)	Inhibition (%)		Cholinesterase activity (U)	Inhibition (%)		Ro-02-0683
			Dibucaine	Fluoride		Dibucaine	Fluoride	
I.1	UJ	9.16	82.2	78.0	5.2	83	68	97
I.2	AK	4.02	70.3	81.3	2.6	55	43	64
II.1	UA	6.79	74.7	80.1	3.6	67	43	75
II.2	JK	3.09	83.1	79.8	1.5	80	60	95
II.3	UA	5.63	77.7	82.2	3.2	66	50	77
III.1	AK	1.36	64.4	81.4	0.8	51	46	54
III.2	UJ/UK	3.44	82.1	80.8	2.0	83	65	98
III.3	UK/UJ	5.49	82.0	80.4	3.3	82	59	97
III.4*	AJ/AK	1.77	61.4	87.6	1.1	41	38	45
III.5	AK	2.96	69.5	80.1	1.7	56	47	62
III.6	AJ	1.90	55.4	79.8	1.1	41	39	48

*Proband.

Examination of his cholinesterase status gave findings which were not easy to interpret and accordingly investigations were widened to include as many members as possible of his close family. During the course of these studies an unconjugated hyperbilirubinaemia was observed in some members of the family including the proband. This was mild in III.4 and III.5 but severe in III.3. It was not associated with any other abnormality of liver function. To verify that bilirubin does not alter the cholinesterase assay, bilirubin was added to several sera of different phenotypes at a concentration of 20 mg/100 ml. Neither the assay nor the dibucaine or fluoride inhibitions were affected significantly.

Results and discussion

Results of enzyme activities and inhibitor numbers on the proband and members of his immediate family as obtained in the two laboratories are shown in table 6. The genetic explanation that best accounts for the biochemical results in this pedigree is given in the figure. For subjects III.2, III.3, and III.4 there is legitimate uncertainty in the ascription; the preferred interpretation is given first in the table and the alternative ascription second.

On genetic grounds II.2 is heterozygous for both E_1^j and E_1^k although there is nothing in the analytical results which proves this ascription. As it is a quantitative variant of E_1^u , the dibucaine and fluoride inhibition values are located in the same area in fig 3 of Rubinstein *et al*⁴ as those of the usual phenotype. In addition the pedigree provides several more examples of $E_1^aE_1^k$ and $E_1^aE_1^j$.

Based on the cholinesterase activities in both laboratories for AJ subjects and the JK subject II.2 in this pedigree, and the values in each laboratory below which succinylcholine sensitivity is frequent,

AJ and JK subjects must be considered as vulnerable phenotypes. The situation is not so clear with AK subjects. Using the cutoff value of laboratory A, AK subjects would be vulnerable; by the criterion of laboratory B they might not be. However, as a precaution we feel AK subjects should be added to the list of susceptible phenotypes until more data are available.

The routine measurement of serum cholinesterase activity as a predictor of susceptibility to succinylcholine apnoea will occasionally reveal sera with activities in the vulnerable range unassociated with acquired causes of cholinesterase deficiency. An example might be II.2 in the present pedigree. Although family studies might indicate the inherited nature of the abnormality, unfortunately at present this can only be clarified biochemically if the family is also segregating for E_1^a . For this reason, subjects with phenotypes UJ, UK, JK, JS, and KS are probably overlooked.

Assuming that the hyperbilirubinaemia in subjects III.3, III.4, and III.5 is inherited, the certain ascription of II.1 and the fact that III.3 cannot have the E_1^a gene indicate that the locus for hyperbilirubinaemia in this family cannot be on the same chromosome as that containing cholinesterase locus 1.

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