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E_1^k, another quantitative variant at cholinesterase locus 1

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SUMMARY Two families segregating for the atypical (E_1^a) allele at cholinesterase locus 1 are described. Unusual results for dibucaine inhibition led to the recognition of a new allele (E_1^k) also segregating in these families. The enzymatic and immunological data are consistent with the hypothesis that E_1^k causes reduction of 'usual' (E_1^u) molecules by about 33%. Whether the reduction of E_1^u caused by E_1^k is caused by retarded synthesis or accelerated degradation of serum cholinesterase remains to be determined.

Several quantitative variants at serum cholinesterase (E.C.3.1.1.8) locus 1 have been described. These result in lowered serum cholinesterase activity and, when interacting with the E_1^a (atypical) allele, give dibucaine inhibitions below those of $E_1^uE_1^a$ heterozygotes. Interaction with E_1^a led to the discovery of the E_1^s (Liddell *et al.*, 1962) and E_1^j (Garry *et al.*, 1976) variants. E_1^s exists in several forms (Rubinstein *et al.*, 1970) and results in 95 to 100% diminution of E_1^u molecules; E_1^j results in about 66% reduction. One family has been described in which several members have greatly increased amounts of apparently normal serum cholinesterase (Neitlich, 1966; Yoshida and Motulsky, 1969). The gene responsible has been named E Cynthiana but it is not yet known if it is active at cholinesterase locus 1 or locus 2.

We present here two families with a quantitative variant at cholinesterase locus 1 which results in about 33% reduction of E_1^u molecules; both of these families were recognised by means of interaction with the E_1^a gene.

Methods

The enzymatic and immunological methods used were the same as given in earlier publications (Garry *et al.*, 1976; Rubinstein *et al.*, 1976; Dietz *et al.*, 1973). (Table II in Garry *et al.* (1976) gives the cholinesterase activities and inhibitions of the known phenotypes as determined in our laboratory.)

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Results

S. FAMILY

The index case (II.1) was discovered by means of prolonged apnoea after administration of succinylcholine. The cholinesterase activities and inhibitions of the sera of the family members are given in the Table. Two individuals (III.2 and III.3) have dibucaine inhibitions in a range not previously found. This family can be explained by assuming segregation for a new allele[1] which must have entered the family through II.2 who is considered to be genotype $E_1^uE_1^k$.

[1]Termed E_1^k in honour of Dr Werner Kalow who clarified the recognition and inheritance of the E_1^a allele by means of dibucaine inhibition.

Table *Enzymatic results on members of S. and J. families*

Family	*Pedigree No.*	*Presumed genotype*	*Cholinesterase activity*	*Inhibition, %*	
				Dibucaine	*Fluoride*
S.	I.1	$E_1^uE_1^a$	5·62	74·3	81·2
	II.1	$E_1^aE_1^a$	1·35	18·5	83·6
	2	$E_1^uE_1^k$	6·74	83·4	77·7
	3	$E_1^uE_1^a$	4·81	76·8	82·5
	4	$E_1^aE_1^a$	1·29	14·8	82·2
	III.1	$E_1^uE_1^u$	8·97	84·5	79·3
	2	$E_1^aE_1^k$	2·21	60·0	81·9
	3	$E_1^aE_1^k$	2·67	65·3	78·2
	4	$E_1^uE_1^u$	8·09	84·4	81·3
	5	$E_1^uE_1^a$	6·91	75·5	79·4
	IV.1	$E_1^uE_1^a$	8·72	74·5	79·8
	2	$E_1^uE_1^k$	9·38	84·6	79·5
	3	$E_1^uE_1^k$	8·60	83·8	82·1
J.	II.1	$E_1^aE_1^k$	2·97	63·1	79·7
	2	$E_1^aE_1^a$	2·10	10·5	80·0
	4	$E_1^uE_1^k$	5·26	85·5	80·4
	5	$E_1^uE_1^a$	6·78	74·2	85·2
	III.1	$E_1^uE_1^a$	5·53	75·8	78·0
	2	$E_1^aE_1^k$	3·28	66·0	79·0
	3	$E_1^uE_1^u$	7·76	82·2	80·3
	4	$E_1^uE_1^u$	8·49	82·7	81·5

Subjects IV.2 and IV.3 must also be $E_1^uE_1^k$. Subjects III.2 and III.3 are assigned genotype $E_1^aE_1^k$. The pedigree and most likely genotypes are given in Fig. 1. Though I.2 may have been $E_1^uE_1^s$ (in which case II.1 and II.4 would be $E_1^aE_1^s$), the fact that II.1, II.3, II.4, III.2, III.3, and III.5 all have at least one E_1^a gene suggests that I.2 was probably $E_1^uE_1^a$ (or, more remotely, $E_1^aE_1^a$ or $E_1^aE_1^s$).

J. FAMILY

The index case (II.2) was also discovered by prolonged apnoea after succinylcholine. The data on family members are given in the Table. Two individuals, II.1 and III.2, have dibucaine inhibitions unlike those found in known phenotypes but similar to those of III.2 and III.3 in the S. family. Assuming the same explanation as that given for the S. family, II.1 and III.2 are assigned genotype $E_1^aE_1^k$. Either I.1 or I.2 must have been $E_1^uE_1^a$; the other could have been $E_1^aE_1^k$ or $E_1^sE_1^k$. Since the E_1^a gene is more frequent than the E_1^s gene, $E_1^aE_1^k$ is more likely; that is why II.2 is assigned $E_1^aE_1^a$ rather than $E_1^aE_1^s$. (Very remotely, I.1 could have been $E_1^aE_1^s$ and I.2 $E_1^aE_1^k$. II.4 would then be $E_1^sE_1^k$. Against this is the cholinesterase activity of 5·26 found in II.4 as $E_1^sE_1^k$ would be expected to give a very much lower value.) II.4 is, therefore, classified as $E_1^uE_1^k$. The most probable genotypes are given along with the pedigree in Fig. 2.

Figure 3 clarifies the identification of $E_1^aE_1^k$ with respect to previously known genotypes in terms of dibucaine and fluoride inhibitions, The $E_1^aE_1^k$ area falls just between those for $E_1^uE_1^a$ and $E_1^aE_1^j$. $E_1^uE_1^k$ cannot be distinguished from $E_1^uE_1^u$, $E_1^uE_1^s$, or $E_1^uE_1^j$ by inhibitions.

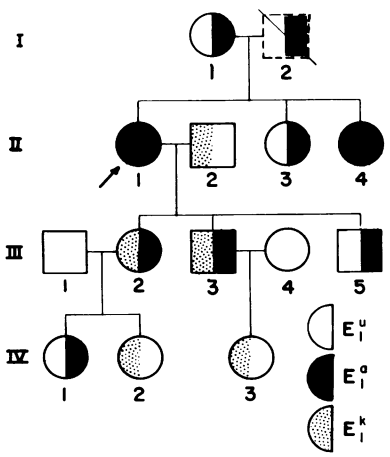

Fig. 1 *Pedigree of the S. family.*

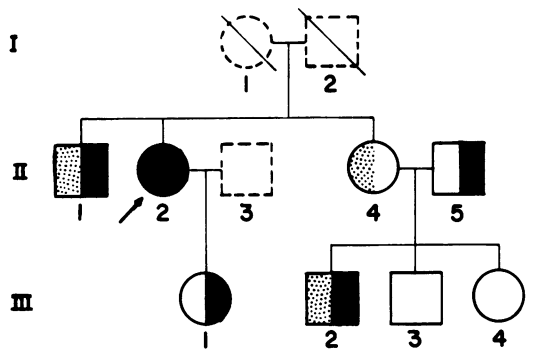

Fig. 2 *Pedigree of the J. family.*

Following the reasoning used for E_1^j (Garry *et al.*, 1976), the E_1^k allele can be explained as resulting in reduced numbers of circulating E_1^u molecules whether because of reduced synthesis or accelerated degradation. Fig. 4 (derived from Fig. 4 of Garry *et al.*, 1976) relates dibucaine inhibitions to mixtures of E_1^u and E_1^a molecules in various proportions. The average dibucaine inhibition of the four $E_1^aE_1^k$ subjects from the two families given above indicates that the ratio of E_1^u to E_1^a molecules in their sera is about 40:60. This corresponds to an approximate 33% reduction of E_1^u molecules caused by the E_1^k allele. E_1^k causes less reduction of E_1^u molecules than E_1^j—33% vs 66%. The average cholinesterase activity of $E_1^aE_1^k$ sera should, therefore, be higher than that of $E_1^aE_1^j$ sera. This is what is observed, 2·78 vs 1.93.

The relative diminution of E_1^u molecules in the sera of the two $E_1^aE_1^k$ subjects (II.1 and III.2) in the J. family was also shown immunologically. Fig. 5 (based on Fig. 4 of Rubinstein *et al.*, 1976) relates the

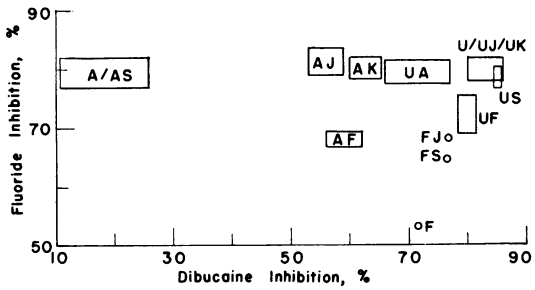

Fig. 3 *Distribution of the known cholinesterase phenotypes. Each variant is judged from its relative inhibition by fluoride and dibucaine in phosphate buffer.*

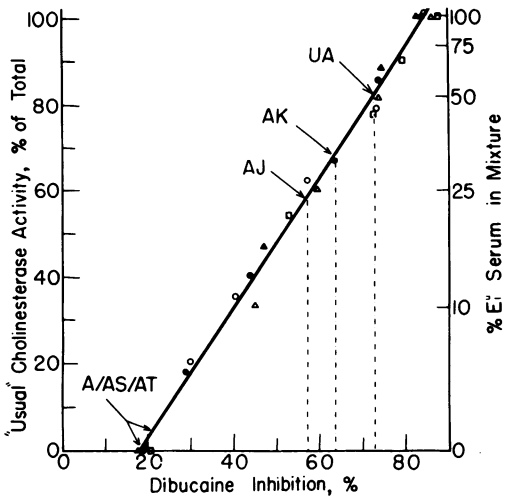

Fig. 4 *Dibucaine inhibition of mixtures of $E_1^uE_1^u$ and $E_1^aE_1^a$ sera. Different symbols represent mixtures of different sera. The left ordinate represents the percentage of total activity resulting from $E_1^uE_1^u$ as estimated from the activities of the unmixed sera. The right ordinate gives the percentage of $E_1^uE_1^u$ serum when starting with $E_1^uE_1^u$ and $E_1^aE_1^a$ sera of average activities. The arrows indicate the mean dibucaine inhibition of the phenotypes listed.*

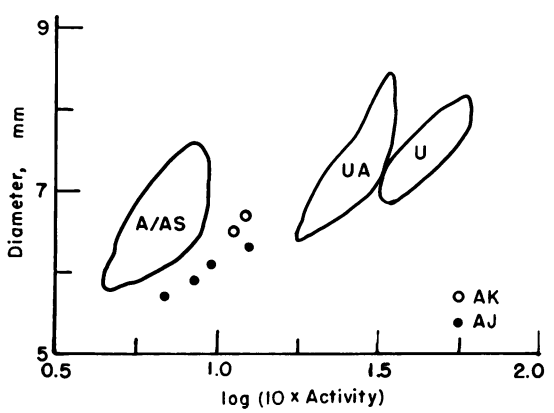

Fig. 5 *Immunodiffusion of serum cholinesterase variants. In every case 5 µl undiluted serum was used. The large circled areas represent at least 20 different sera for each class shown. The solid dots represent the four AJ sera from the pedigree given in Garry et al. (1976) and the two small open circles the two AK sera from the J. family described here.*

cholinesterase activity of sera of various genotypes to immunological reactivity by gel immunodiffusion. The two $E_1^aE_1^k$ sera are found in an area of the plot distinct from $E_1^uE_1^u$, $E_1^uE_1^a$, and $E_1^aE_1^a$ sera and close to that of $E_1^aE_1^j$ sera.

Discussion

The recognition of E_1^k in the two pedigrees given here is based on interaction of E_1^a with the resultant production of dibucaine inhibitions unlike any found hitherto—precisely the way in which E_1^j was also found (Garry *et al.*, 1976). Inspection of Fig. 3 and 4 shows that other quantitative variants may well be discovered in the same way. Dibucaine inhibitions falling between 30 to 50%, if segregating appropriately, would indicate quantitative variants intermediate in their effects between E_1^s and E_1^j. Such variants, if they exist, should account for roughly 75 to 90% reduction of E_1^u molecules.

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