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STUDIES ON FORMAL GENETICS OF THE PSEUDO-CHOLINESTERASE* POLYMORPHISM; AN ATYPICAL SEGREGATION IN A FAMILY†

H. W. GOEDDE, W. FUSS,‡ D. GEHRING and H. BAITSCH

Institute of Anthropology, Section of Biochemical Genetics, Albertstr. 11, Freiburg i. Br., Germany

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Abstract—A family is reported, in which 'silent' and 'dibucaine-resistant' pseudo-cholinesterase phenotypes are found simultaneously. The various phenotypes were differentiated by the estimation of the enzyme activity and the rate of inhibition by dibucaine and sodium fluoride.

IN RECENT publications it has been shown, that very likely a more complicated model of formal genetics has to be assumed to account for the pseudo-cholinesterase polymorphism than the primarily suggested 2-allele-model.[6, 10–14] Lehmann[12] supposes at least 4 alleles belonging to one gene locus (Table 1). However not all the phenotypes have been exactly identified that were to be expected as a consequence of this suggestion (population genetics[1, 3, 6, 10]). A family is described, in which we have found some different pseudo-cholinesterase phenotypes simultaneously. This family indicates the existence of a 'silent gene'.

METHODS

The activity of pseudo-cholinesterase was determined by the spectrophotometric method standardized by Kalow.[7] The decrease of extinction indicating the hydrolysis of benzoylcholine as substrate was estimated at 240 mμ with a "Zeiss PMQ II" spectrophotometer. The concentration of the substrate was 5×10^{-5} M benzoylcholine;[7] the concentration of the inhibitors: 10^{-5} M dibucaine[7] resp. 5×10^{-5} M sodium fluoride.[4] Solvent was 0·66 M phosphate buffer of pH 7·4 at 26°. The absorption cells had a light path of 10 mm. After incubation for 1 min, the decrease of absorbance was followed for 3 min.

RESULTS AND DISCUSSION

The subject (II_{45}) reacted upon an injection of succinyldicholinchloride, a widely used muscle relaxant in surgery, with an abnormally prolonged period of

* Acylcholine-acylhydrolase, E C 3.1.1.8.

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muscular paralysis and apnoea (about 7–8 hr). The vast majority of individuals that have been found to be excessively sensitive to succinyldicholinchloride, possess an atypical variant of the enzyme pseudo-(serum)-cholinesterase.[2, 7-9] With the usual dose (0·5–1 mg/kg body weight), this atypical enzyme protein has practically no affinity to the substrate succinyldicholinchloride.

TABLE 1. PHENOTYPES AND GENOTYPES OF PSEUDOCHOLINESTERASE-POLYMORPHISM

Alleles*	Ch^N	Ch^D	Ch^F	Ch^S
		Phenotypes		
Genotypes	Formale	Values		
		Activity[7]	Dibucaine-numbers†	Fluoride-numbers†
Homozygote				
Ch^N/Ch^N	Ch (NN)	238 (178–330)‡	80 (71–85)[3, 8, 9]	61 (≥ 55)[3, 4, 5]
Ch^D/Ch^D	Ch (DD)	95	22 (< 23)[8, 9]	23 (20–27)[4, 5]
Ch^F/Ch^F	Ch (FF)		66[15]	35[15]
Ch^S/Ch^S	Ch (SS)	0	0	0
Heterozygote				
Ch^N/Ch^D	Ch (ND)	142 (138–164)‡	62 (43–70)[3, 8, 9]	48 (40–55)[3, 4, 5]
Ch^N/Ch^F	Ch (NF)	156	74[15]	52[15]
Ch^N/Ch^S	Ch (NS)	133 (81–167)‡	80	60
Ch^D/Ch^F	Ch (DF)		49[15]	35[15]
Ch^D/Ch^S	Ch (DS)	45	21	24
Ch^F/Ch^S	Ch (FS)			

* Ch^N = normal allele; Ch^D = dibucaine-resistant allele; Ch^F = fluoride-resistant allele; Ch^S = 'silent gene' allele.

† Mean values, taken from literature and our own experiments.

‡ The present paper.

In these cases the relaxant effect is not neutralized by enzyme activity but only by hydrolysis and renal elimination. The activity tests[7-9] and the estimation of the inhibitor constants[4, 5, 9] first suggested that the subject (II_{45}) would be a homozygote for the abnormal allele Ch^D ('dibucaine-resistant') of the normal autosomal gene Ch^N, which controls the synthesis of the pseudo-cholinesterase (Fig. 1). Because one daughter of the subject showed quite normal inhibitor constants, we pursued

large investigations on the family of the subject in order to elucidate the Mendelian genetics of this atypical segregation. Following results were obtained:

The subject (II_{45}) is married to a woman (II_{46}) who shows a normal enzyme activity and inhibitor constants in accordance to the genotype Ch^N/Ch^N. They have two daughters (III_{72} and III_{74}), who genetically are of special interest; both would have been heterozygotes Ch^N/Ch^D in consequence of the hypothesis that II_{45} would be homozygous (Ch^D/Ch^D). Regarding the values of the inhibitor constants (dibucaine- and fluoride-numbers), one daughter (III_{72}) would have been homozygous (Ch^N/Ch^N) for the allele Ch^N, the other one (III_{74}) a heterozygote (Ch^N/Ch^D). The statement of III_{72} suggested that the subject (II_{45}) is not homozygous for the allele Ch^D but heterozygous for the alleles Ch^D and Ch^S ('silent gene'). Liddell *et al.*[13] and Woolf *et al.*[14] first pointed out the existence of such a 'silent gene'; it can be discovered by a low pseudo-cholinesterase-level ('dosis effect'), which is specially obvious in cases of heterozygotes for the 'silent gene' and the normal allele Ch^N, where normal inhibitor constants are found.[14]

If an individual shows atypical inhibitor constants as our propositus (II_{45}), it is still difficult to conclude the presence of the 'silent gene', because the measured enzyme activity is reduced too much. Therefore, the homozygous phenotype (Ch^D/Ch^D) cannot easily be differentiated from that of heterzygotes with Ch^D/Ch^S. Heterozygous individuals of the genotype Ch^N/Ch^S have a similarly reduced enzyme activity as the heterozygotes with Ch^N/Ch^D, but they differ by their inhibitor constants (dibucaine- and fluoride-numbers;[10] Table 1).

On the assumption that there are the two atypical alleles Ch^D and Ch^S besides of the normal allele Ch^N in the investigated family, the atypical statements of the pedigree can be interpreted easily (Fig. 1 and Table 2). We concluded the existence of the 'silent gene' from the fact, that 4 of 20 examined members have an obviously reduced enzyme activity in the family of the father of II_{45}; two (II_{37} and II_{42}) of seven brothers and sisters of the subject show a reduced enzyme activity and according to this normal dibucaine- and fluoride-numbers. One sister (II_{38}) shows the same genetic statement as the subject (Ch^D/Ch^S). The daughter III_{72} of the subject has an enzyme activity of 42 units ($\Delta E/3$ min/ml serum (26°)) (normal values 65–105 units) and quite normal dibucaine- and fluoride-numbers. In consequence to these results, this daughter is heterozygous for the alleles Ch^N and Ch^S.

The estimated enzyme activities and inhibitor constants of the subject (II_{45}) and his brothers and sisters (II_{37}, II_{38}, II_{39}, II_{40}, II_{42}, II_{43}) make it possible to determine the genetic constitutions of the not tested parents of the subject (I_9 and I_{10}): In accordance to these results one parent has been heterozygous for the 'silent gene' (Ch^N/Ch^S), the other one heterozygous for the atypical 'dibucaine-resistant' allele (Ch^N/Ch^D). This conclusion is founded on the enlarged investigation of the whole family (Fig. 1 and Table 2):

The atypical allele Ch^D has repeatedly been detected in the family of the mother of II_{45}. The members II_{47}, II_{50}, II_{52} and III_{77} are heterozygotes for this allele (Ch^N/Ch^D). Reduced enzyme activities in coincidence with normal inhibitor constants pointing to the 'silent gene' (Ch^S) have only been found in the family of the father of II_{45} (II_{23}, II_{25}, II_{31}, III_{61}). The low enzyme activities of III_{65}, III_{66} and III_{67} cannot be taken as arguments because the mother (II_{33}) shows normal values of

TABLE 2. INHIBITOR CONSTANTS AND ACTIVITIES OF TESTED SERA

II	19	20	21	23	25	30	31	32	33	37	38	39	40	42	43	44	45	46	47	50	52	53	54	57
Activity per ml serum	108	114	97	40	40	71	57	39	87	38	43	83	68	33	52	89	20	77	54	52	53	91	74	123
DN	80	81·5	78·9	78·3	80·7	80	78·1	78·7	78	80	21	82	81·5	81·8	60	79·8	21·1	84	64·1	62·5	65·4	81	77·1	80
FN	59	58·5	57·7	60·9	60	58·7	62·3	60	57·5	60	23·2	59	60	60·6	48	59·6	26·3	64·2	48·4	48·5	48·5	58·7	60·5	60·7
Ch	NN	NN	NN	NS	NS	NN	NS	NS	NN	NS	DS	NN	NN	NS	DN	NN	DS	NN	DN	DN	DN	NN	NN	NN
III	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	81	82
Activity per ml serum	121	99	82	54	80	85	78	64	52	40	69	108	69	104	42	97	63	90	85	57·5	83	98	71·5	107
DN	80·8	78·9	78·6	79	81·8	79·8	78·9	77·9	80	79·1	80	80·8	81	84·6	81·6	79·4	64·3	79·2	79·2	62·5	82	84·7	74·6	81·3
FN	56·3	56·9	60	59·4	58	58·7	58·9	55·6	59·1	58·1	60	56·8	60·6	58·8	59·2	57·7	48·4	60	60	47·5	58·8	60	58	62·5
Ch	NN	NN	NN	NS	NN	NN	NN	NS	NS	NS	NN	NN	NN	NN	NS	NN	DN	NN	NN	DN	NN	NN	NN	NN

enzyme activity and inhibitor constants. Evidently she is not carrying the 'silent gene', whereas the father of these three children has a reduced enzyme activity and normal inhibitor constants and consequently possesses the 'silent gene'. He did not appear to be a consanguineous relative to the subject (II_{45}).

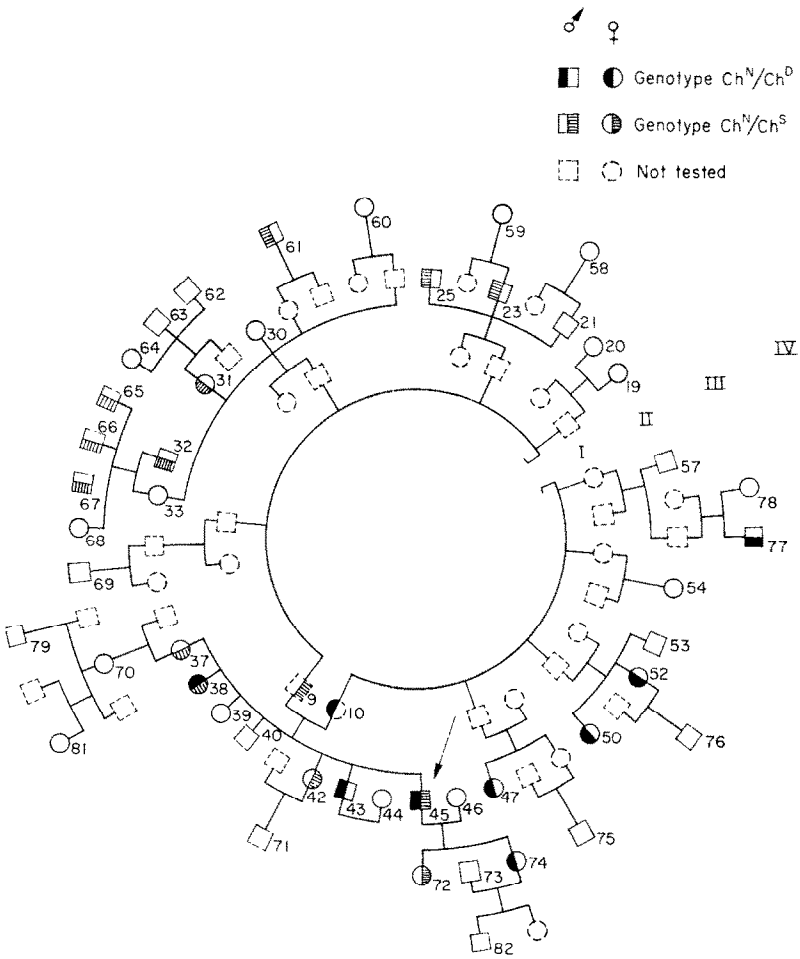

FIG. 1. Pedigree of the investigated family.

Figure 2 shows that the tested individuals can be divided in four groups:
(1) normal phenotype (Ch^N/Ch^N);
(2) heterozygous phenotype for the 'silent gene' (Ch^N/Ch^S);
(3) heterozygous phenotype for the 'dibucaine-resistant' allele (Ch^N/Ch^D); and
(4) the two single points that are representing the propositus (II_{45}) and his sister (II_{38}), both of them heterozygous for the 'dibucaine-resistant' allele and the 'silent gene' (Ch^D/Ch^S).

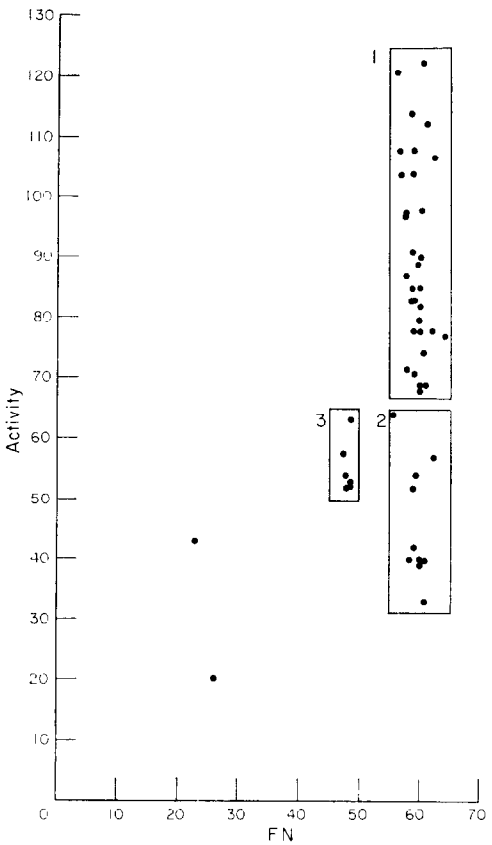

FIG. 2. Distributions of enzyme activities and fluoride numbers in the 50 individuals studied.

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