The Silent Gene for Serum Pseudochoolinesterase

A. SZEINBERG, SERENA PIPANO, E. OSTFELD, and L. EVIATAR

From the Departments of Chemical Pathology and Anaesthesia, Tel Aviv University Medical School, Government Hospital Tel Hashomer, Tel Hashomer, Israel

The investigations of human serum pseudochoolinesterase (acetylcholine acylhydrolase) (International Union of Biochemistry, 1961 : 3.1.1.8) suggest the existence of four allelic genes controlling the formation of different enzyme types: gene E₁⁺ for usual esterase, E₁⁺⁺ for atypical (dibucaine resistant) esterase, E₁⁻ for a fluoride-resistant type, and E₁⁻⁻ for a 'silent' gene. The four genes result in the existence of 10 different genotypes (Harris, 1964).

Until now 7 cases (in 6 families) of complete pseudochoolinesterase deficiency, compatible with the homozygous genotype E₁⁺⁻⁻, have been described (Liddell, Lehmann, and Silk, 1962; Doenicke, Gurtner, Kreutzberg, Remes, Spiess, and Steinbereithner, 1963; Hodgkin, Giblett, Levine, Bauer, and Motulsky, 1965; Goedde, Fuss, Ritter, and Baitsch, 1965a; Dietz, Lubrano, and Rubinstein, 1965). During a survey on the frequency of atypical pseudochoolinesterase in various population groups in Israel, a new case of complete absence of the enzyme was detected by us. Family examinations demonstrated a similar absence of enzyme activity in the serum of two children of the propositus (Fig.). The present report describes the detailed findings in the affected family.

Material and Methods

The survey has been conducted on a random sample of blood bank donors and hospital patients not suffering from liver, kidney, or neoplastic diseases. All the sera were examined by the screening paper-spot test of Harris and Robson (1963), modified as follows: filter paper Whatman No. 31 ET was used instead of No. 17, the control tubes contained 0.05 ml. serum diluted in 2 ml. phosphate buffer instead of 4 ml. as originally described. All the samples, which in the screening test suggested the presence of atypical enzyme or showed low enzymatic activity, were examined for definitive classification by spectrophotometric procedures for pseudochoolinesterase activity and the dibucaine number (Kalow and Genest, 1957), fluoride number (Harris and Whittaker, 1961), and inhibition by RO2-0683 (Liddell, Lehmann, and Davies, 1963).

All the determinations were carried out in Beckman DU spectrophotometer at 240 mμ at 26° C. The enzyme activity was expressed in arbitrary units defined by Kalow and Lindsay (1955) as micromoles of acetylcholine hydrolysed by 1.0 ml. serum in one hour at 37° C. The normal range of activity obtained upon examination of 108 adult healthy blood donors was 130-378 units, mean ± S.D. = 247.0 ± 50.7 units.

Results

During the survey 2347 subjects were examined and 91 cases of the intermediate phenotype (E₁⁺⁻⁻) and 4 of the atypical phenotype (most probably E₁⁺⁻⁻⁻ genotype) were found. Subdivision of the materials according to the ethnic origin of the subjects suggested the existence of significant differences in the frequency of the E₁⁺⁻⁻ gene between some Jewish groups. A detailed presentation of these results has been reported (Szeinberg, Pipano, and Ostfeld, 1966).

Among the subjects examined during the survey, one healthy blood bank donor (45-year-old Jewish male, born in Morocco) did not demonstrate any serum pseudochoolinesterase activity in the screening test. No activity was also demonstrated by the spectrophotometric assay using 1/200, 1/100, or 1/40 dilutions of the serum. A second sample obtained two weeks later gave similar results. Of the 90 children of the propositus, 6 were examined, and 2 sons (III.6 and III.7) did not demonstrate any pseudochoolinesterase activity; 2 other sons, 2 daughters (III.2, III.3, III.4, and III.5), and the wife of the propositus (II.1) had enzyme activities of 169, 163, 175, 130, and 140 units with normal inhibition characteristics (Table). There was no consanguinity between the propositus and his wife.

The absence of pseudochoolinesterase activity in the 2 sons suggested that, either their mother was also a carrier of the silent gene, or that we had encountered a family with an unusual mutation, namely a dominant inheritance of the silent gene.
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In view of those two alternative possibilities, additional members of the family were examined. The father of the propositus (I.1) had 108 units and the mother (I.2) 99 units enzyme activity with normal inhibition characteristics. These results negated the probability of a dominant transmission of the silent gene and suggested that both parents were of the heterozygous EₜₛE₄ₛ genotype, as the activity of their sera was below the normal limit. Among 5 sibs of the propositus, 3 (II.3, 4, 5) had low enzyme activity (90, 72, 78 units) compatible with genotype EₜₛE₄ₛ, and 2 (II.7, 10) normal activity (237 and 173 units) compatible with genotype EₜₛEₜₛ.

According to this analysis the most probable explanation of the absence of enzyme activity in the two members of the third generation was an inheritance of recessive silent genes both from the propositus and his wife. The fact that the wife had enzyme activity of 140 units, i.e. above the lower normal limit, did not preclude the possibility of a heterozygous EₜₛE₄ₛ genotype. It has been conclusively demonstrated in other families reported so far that persons of this genotype may have enzyme activities within the normal range (Liddell et al., 1962; Simpson and Kalow, 1964; Hodgkin et al., 1965; Szeinberg, Pipano, and Ostfeld, 1965; Dietz et al., 1965). The most probable genetic make-up of the investigated family is presented in the figure.

Lehmann and Liddell (1964) demonstrated a slight inhibition of normal pseudocholinesterase

### TABLE

RESULTS OF THE FAMILY INVESTIGATION

<table>
<thead>
<tr>
<th>Individual</th>
<th>Sex and Age (yr.)</th>
<th>Pseudocholinesterase Level (Units/ml.)</th>
<th>Dibucaine Number</th>
<th>Fluoride Number</th>
<th>R02-0683 Number</th>
<th>Presumed Genotype</th>
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<tr>
<td>I.1</td>
<td>M 72</td>
<td>108</td>
<td>78</td>
<td>54</td>
<td>96</td>
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<tr>
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<td>-</td>
<td>-</td>
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<tr>
<td>I.3</td>
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<td>90</td>
<td>81</td>
<td>56</td>
<td>98</td>
<td>EₜₛE₄ₛ</td>
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<tr>
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<td>72</td>
<td>81</td>
<td>59</td>
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<td>EₜₛE₄ₛ</td>
</tr>
<tr>
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<td>55</td>
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<td>95</td>
<td>EₜₛE₄ₛ</td>
</tr>
<tr>
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<td>-</td>
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</table>

Normal range 130-378
Normal mean ±SD 247.0±50.7 73.8±49.0 54.7±77 92.1±100
activity by serum from the patient with complete pseudocholinesterase deficiency. No such inhibition could be demonstrated in the cases reported by Hodgkin et al. (1965). Sera of our 3 subjects with complete absence of activity were investigated for inhibitory activity in the following experiment: equal volumes of normal serum and anenzymic serum were mixed. The mixture was tested immediately for pseudocholinesterase activity; in addition, one part of the mixture was incubated at 37°C for 30 minutes and 1 hour, another aliquot was diluted 1:100 with the phosphate buffer used for the pseudocholinesterase activity estimation and also incubated for 30 minutes and 1 hour. None of the mixtures demonstrated any evidence for the presence of inhibitor in the anenzymic sera.

**Discussion**

Starch gel electrophoresis of serum of the 2 cases of Hodgkin et al. (1965) showed absence of the 4 isozyme bands associated with normal pseudocholinesterase activity. Immunological studies indicated that these subjects did not produce any antigenically similar protein detectable by the methods used. On the other hand, Goede, Gehring, and Hofmann (1965b) who studied 2 cases, which did not show any pseudocholinesterase activity in the spectrophotometric assays, demonstrated a slight esterase activity (2–3% of normal) by a micromanometric assay. The activity was located at the C4 isozyme band after starch gel electrophoresis of serum. In addition, immunological investigations demonstrated the presence of a protein antigenically similar to the usual pseudocholinesterase protein. Further investigations are required in order to establish whether the divergent results obtained by the two groups of investigators were due to differences in techniques, or to the existence of different mutants of the so-called 'silent gene'.

All the presently available pedigree data suggest that the silent gene is an allele of the normal esterase and its two allelic mutants (E1a and E1b). However, an alternative hypothesis has been also discussed by Simpson and Kalow (1964), namely that the gene determining absence of pseudocholinesterase is an independent, non-allelic gene which suppresses the action of E1b or E1a genes. The presence of a gene suppressing the action of E1a was not considered to be likely, as it would predict too high frequency for individuals without pseudocholinesterase activity. The hypothesis of a suppressor of E1a gene was excluded on the grounds that no segregation of E1a gene was observed among relatives of persons with the hypothetical genotype E1aE1aSS (s represents the suppressor gene and S its non-suppressor allele). This conclusion (with a chance probability of p = 0.02) was based on results seen in 14 offspring in a critical type of mating. Hodgkin et al. (1965) suggested that more offspring from the critical matings were required to disprove this removed hypothesis.

The material available for such an analysis has now been enlarged. The critical matings analysed by the previous investigators were between normal (E1aE1bSS) and silent gene heterozygotes (E1aE1bSs). 3 children of such a mating were present in a family described previously by us (subjects III.3, 4, 5, Szeinberg et al. 1965) and 3 children in the family described by Dietz et al. (1965) (subjects III.22, 23, 24 in family G). All were phenotypically normal. Thus the number of phenotype U offspring in this type of mating is now 20, and the probability of this being a chance occurrence is (3/4)20 ≈ 0.003.

In the family described here, additional types of mating provide material for testing the suppressor gene theory. Both parents of the propositus are obligatory heterozygotes (E1aE1bSs) and their mating should result in 10/16 phenotype U, and 2/16 phenotype I (intermediate dibucaine resistant), 3/16 phenotype A (typical dibucaine resistant), and 1/16 homozygous silent gene children (on the assumption (Simpson and Kalow, 1964) that one suppressor gene does not completely suppress the action of two E1a genes). Five sibs of the propositus were tested and all were phenotypically normal. In the family of Hodgkin et al. (1965) two sibs of the propositus, and in the family 'C' of Dietz et al. (1965) one sister of the propositus also showed normal dibucaine resistance. The probability of not finding cases with increased dibucaine resistance among these 8 offspring is (11/16)8 ≈ 0.049.

The mating between the propositus of our family (homozygote silent E1aE1aSS) and his wife (heterozygous silent E1aE1bSs) should produce 1/4 dibucaine resistant E1aE1aSs (phenotype A) offspring. Among the 6 examined children of this couple none with the atypical enzyme was found, the probability of chance being (3/4)6 ≈ 0.178.

The combined data from all the critical matings exclude the hypothesis that the silent gene is independent of E1 locus and suppresses the action of E1a gene with the probability of (3/4)20 × (11/16)8 × (3/4)6, i.e. about 0.000003.

Hodgkin et al. (1965) suggested that the heterozygotes E1aE1b produced on the average about two-thirds of the enzyme activity of normal homozygotes. This conclusion was based on results seen in 5 obligatory heterozygotes studied by them, a family studied by Simpson and Kalow (1964), and a personal
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communication of Harris (Hodgkin et al., 1965). The individual variations were, however, very large (range of 28–113% of mean normal activity in the material of Simpson and Kalow (1964), and 31–83% in that of Hodgkin et al. (1965)), and obviously additional information would be valuable in this respect. There were 10 obligatory heterozygotes $E_1^cE_1^s$ in the families described by Dietz et al. (1965): the mean enzyme activity in the males was 75% (range 68–83%) and among the females 78% (63–99%) of mean normal activity. The two obligatory heterozygotes in the family of Case 10 described by Goedde et al. (1965a) had 59% and 39% of mean normal activity. In the families described by us there were 10 obligatory heterozygotes (II.1, 4, 7, in Szeinberg et al. (1965)); and I.1, 2; II.1: III.2, 3, 4, 5 in the present communication). The mean activity in these 10 subjects was 53% of mean normal and the range was 40–71%. In view of these results it seems that the collection of more data is indicated in order to decide whether persons with only one $E_1^c$ gene produce significantly more than one-half of normal enzyme activity. It should be remembered that this type of investigation is further complicated by the influence of age, sex, and environmental factors on the serum pseudocholinesterase activity, as demonstrated by Simpson and Kalow (1965).

Summary

A Jewish family from Morocco with a complete absence of serum pseudocholinesterase activity in father and two sons was discovered during a population screening in Israel. The family data were compatible with the hypothesis that these cases were homozygous for the 'silent' gene, allelic to the 'usual' pseudocholinesterase gene.

Genetic considerations from this and previously reported families exclude an alternative hypothesis, that the 'silent' gene is independent of the $E_1$ locus and suppresses the action of the $E_1^s$ gene, with a probability of about 0.00003.

Serum with complete absence of pseudocholinesterase activity failed to inhibit normal pseudocholinesterase activity.

Heterozygotes for the 'usual' and 'silent' genes in the present family and another one previously described by the authors had a mean of 53% of normal enzyme activity (range 40–71%).

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References


