# Clinical Workshop

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## A Pharmacogenetic Study of a Family Exhibiting Atypical and "Silent" Genes for Plasma Cholinesterase

EDWARD A. ERNST, M.D.,* AND J. CRISPIN SMITH, PH.D.†

Two sisters were encountered, each having experienced prolonged apnea following a therapeutic dose of succinyldicholine (SDC). Congenital absence of typical plasma cholinesterase (pseudocholinesterase, acylcholine acylhydrolase, EC 3.1.1.8) (PChE) was suspected.

Polymorphism is exhibited by PChE and the type is genetically determined by two allelic, co-dominant autosomal genes.[1] The symbols used for genes stimulating the production of various types include $E_u$ for typical,[2] $E_a$ for atypical[3] and $E_f$ for the extremely rare, fluoride-inhibition resistant gene,[4] not encountered in this study. A "silent" gene, $E_s$, has been proposed[5] which holds a place on the chromosome, but fails to stimulate the production of any of these types. As regards the typical, atypical and "silent" genes, the possible genotypes and corresponding phenotypes are as follows:

| *Genotype* | *Phenotype* |
|---|---|
| homozygote $E_uE_u$ | typical plasma cholinesterase only |
| homozygote $E_aE_a$ | atypical plasma cholinesterase only |
| homozygote $E_sE_s$ | neither cholinesterase |
| heterozygote $E_uE_s$ | typical plasma cholinesterase only |
| heterozygote $E_aE_s$ | atypical plasma cholinesterase only |
| heterozygote $E_uE_a$ | typical and atypical cholinesterase (genes are co-dominant) |

Typical PChE has been established as the enzyme responsible for the rapid intravascular destruction of SDC.[6] It is present in more than 99 per cent of the population.[2] However, atypical PChE hydrolyzes SDC very slowly at concentrations used pharmacologically.[7] When this drug is given to the rare person with a gene complement of $E_aE_a$, $E_aE_s$, or $E_sE_s$, the rate of destruction is greatly reduced and paralytic apnea is prolonged. Plasma cholinesterase studies were done on the aforementioned sisters and on their 51-member family of four generations to determine their phenotypes and their deduced genotypes.

### METHODS

Members of the family lived in different states, requiring three locations for plasma collection. This was done by the same person to standardize the procedure and to insure enzymatic preservation. Venous blood was drawn, heparinized, centrifuged, and the resultant plasma decanted and individually labeled. The plasma was immediately frozen and maintained in a frozen state until enzymatic studies were done.

Acetylcholine was used in this study as a substrate to reveal the amount of enzymatic activity present by a null-point potentiometric, titrimetric procedure using the *p*H Stat (Radiometer, Copenhagen) and the results were reported in micromoles of substrate hydro-

* Resident, Department of Anesthesia, Scott Research Laboratory, Fairview General Hospital, Cleveland, Ohio. Present address: College of Medicine, University of Florida, Gainesville, Florida 32601.

† Chief, Anesthesiology Research Laboratory, Montefiore Hospital and Medical Center, New York, New York.

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TABLE 1. O-DN and ACh Data Grouped According to Possible and Deduced Genotypes

| Group | Patient* | O-DN Number | ACh Rate | Possible Genotypes | Deduced Genotypes |
|---|---|---|---|---|---|
| A | 10 | 0 | 19 | $E_sE_s$, $E_aE_a$, $E_aE_s$ | $E_aE_s$ |
| | 12 | 0 | 10 | | |
| B | 28 | 91 | 184 | $E_uE_a$, $E_uE_s$ | $E_uE_s$ |
| | 29 | 100 | 63 | | |
| | 30 | 50 | 69 | | |
| | 22 | 57 | 126 | | |
| | 36 | 65 | 133 | | |
| | 19 | 65 | 121 | | |
| | 18 | 66 | 221 | | |
| C | 21 | 66 | 139 | $E_uE_a$ | $E_uE_a$ |
| | 27 | 66 | 178 | | |
| | 5 | 67 | 121 | | |
| | 20 | 67 | 115 | | |
| | 7 | 68 | 95 | | |
| | 25 | 71 | 75 | | |
| | 17 | 86 | 329 | | |
| | 6 | 87 | 310 | | |
| | 52 | 90 | 314 | | |
| | 44 | 88 | 279 | | |
| | 16 | 89 | 269 | | |
| | 40 | 91 | 246 | | |
| | 11 | 91 | 245 | | |
| | 38 | 87 | 226 | | |
| | 48 | 90 | 233 | | |
| | 35 | 92 | 234 | | |
| | 39 | 87 | 218 | | |
| D | 46 | 91 | 225 | $E_uE_u$, $E_uE_s$ | $E_uE_u$ |
| | 45 | 91 | 223 | | |
| | 47 | 90 | 216 | | |
| | 49 | 91 | 216 | | |
| | 34 | 93 | 213 | | |
| | 24 | 94 | 213 | | |
| | 8 | 90 | 203 | | |
| | 41 | 91 | 205 | | |
| | 23 | 95 | 212 | | |
| | 26 | 96 | 210 | | |
| | 50 | 93 | 186 | | |
| | 51 | 93 | 175 | | |
| | 42 | 94 | 170 | | |
| | 4 | 94 | 165 | | |
| | 43 | 93 | 160 | | |
| | 32 | 94 | 154 | | |
| | 37 | 91 | 145 | | |
| E | 13 | 98 | 146 | $E_uE_u$, $E_uE_s$ | $E_uE$? |
| | 15 | 98 | 145 | | |
| | 33 | 95 | 121 | | |
| | 14 | 95 | 104 | | |
| | 9 | 95 | 94 | | |
| | 31 | 98 | 73 | | |

* Refers to number used on genealogy chart.

lyzed/ml. plasma/hr. (ACh test). The final substrate concentration was $2 \times 10^{-2}$ $M$ and the assay was carried out at 30° C. with 0.1 $N$ sodium hydroxide as titrant.

The type of enzyme was revealed by a dibucaine inhibition test. Normal and atypical PChE are differentially inhibited by this substance. Typical PChE hydrolyzes most substrates rapidly but is inhibited 80 per cent or more by dibucaine. Atypical PChE is inhibited very little by dibucaine (20% or less). A plasma containing both typical and atypical PChE has an intermediate inhibition by dibucaine of about 60 per cent. In this study, the percentage of inhibition of the PChE activity by dibucaine, using o-nitrophenyl butyrate as a substrate was determined on all plasma samples and was reported as percentage of inhibition (O-DN test). The assays were carried out at 30° C. using a Technicon Auto-Analyser. The blank contained $10^{-5}$ $M$ physostigmine to inhibit all cholinesterase activity.

## RESULTS AND DISCUSSION

The O-DN and ACh data are presented in table 1. Members of the family are grouped according to their possible and deduced genotypes.

The two members of group A are the propositus (no. 10) and her sister (no. 12). They have O-DN numbers of zero, revealing no inhibition of their enzymes by dibucaine and demonstrating, therefore, an absence of typical PChE. Of their three possible genotypes, they cannot be $E_sE_s$ because they hydrolyzed a small amount of acetylcholine. They cannot be $E_aE_a$ because each produced a daughter (no. 28 and no. 29) with a high O-DN number (above 94). If they were homozygotes $E_aE_a$, each would have had to

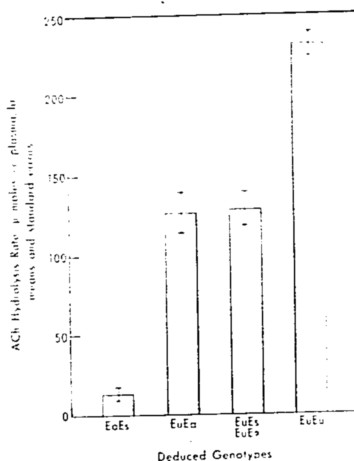


FIG. 1. Acetylcholine hydrolysis rates (μmoles/ml. plasma/hour) of various genotypes (means and standard errors).

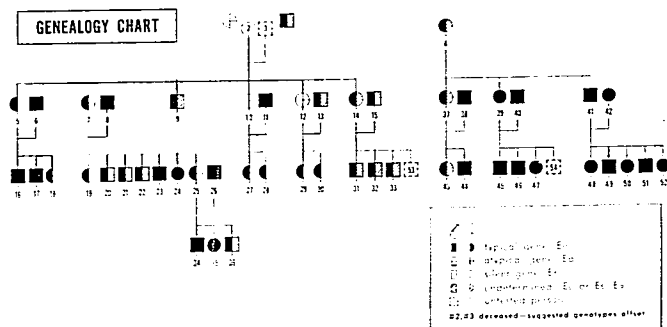


FIG. 2. Genealogy chart of the family. Propositus is number 10.

contribute an $E_a$ gene to her daughter causing the daughter to produce atypical PChE and lowering her O-DN number to between 50 and 70. Therefore, they had to contribute a "silent" gene and have $E_aE_s$ genotypes.

Members of group B are the daughters. Their high O-DN numbers indicate that they produce only typical PChE and have no atypical enzyme. Of their two possible genotypes, they cannot be $E_uE_u$ because their mothers had no typical gene to contribute. Therefore, they have $E_uE_s$ genotypes.

Members of group C are conventional heterozygotes $E_uE_a$ producing both typical and atypical plasma cholinesterase and having intermediate O-DN numbers and ACh hydrolysis rates. They are easily identified by their O-DN numbers (50–71).

The large amount of inhibition as indicated by the high O-DN numbers in group D, reveals that there is only typical PChE present. The high ACh hydrolysis rates indicate an abundance of this enzyme. It is deduced, therefore, that members of group D have two genes stimulating its production and have $E_uE_u$ genotypes.

Finally, there are ten persons (group E) whose genotype cannot be definitely established. They have high O-DN numbers and so produce only typical enzyme. Their genotype could be $E_uE_u$ or $E_uE_s$ and they have temporarily been assigned $E_uE$?. It is tempting to include them in group B with the established $E_uE_s$ daughters. There are several reasons to suggest this grouping. (1) The "silent" gene has been found in four members of the family and it is possible that it occurs in others. (2) The low ACh rates in group E could result from only one $E_u$ gene stimulating typical PChE production. The ACh rates of the $E_uE_s$ genotype bracket those of group E. (3) Most members of group E have an interfamilial relationship that would be compatible with an $E_uE_s$ genotype. On the other hand there is reason to include them with the $E_uE_u$ genotype. Their low ACh rates could be due to a decreased production of typical PChE in an $E_uE_u$ individual. This enzyme is produced in the liver and is reduced in liver disease, sever burns, tuberculosis, heart failure, chronic anemia, etc.[8] The incidence of the "silent" gene in the general population is reported to be very low.[2] There are two genetically unrelated spouses in group E and the possibility of them both having a "silent" gene is unlikely. Concerted efforts have been made to develop an objective laboratory test to differentiate the "silent" gene. None have been successful. Identification of this gene is still made only when a discrepancy is found between the phenotypes and genotypes in families which have atypical genes.[9,10] Therefore, definitive genotypes of the members of group E cannot be established at the present time.

Regardless of their genotypes, they have the same ACh hydrolysis rate and functional ca-

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pacity as the $E_uE_s$ person. A bar graph (fig. 1) demonstrates the ability of various genotypes to hydrolyze acetylcholine. $E_uE?$ is included with $E_uE_s$ because of their similarities. The practical point that this chart makes is that heterozygotes $E_uE_s$ or $E_uE_a$ have a lowered functional capacity to hydrolyze choline esters as compared with $E_uE_u$ homozygotes. The "silent" gene heterozygote is frequently classified with the typical homozygote; they both produce only the typical enzyme, and give a similar inhibitor test result (high O-DN number). This is erroneous because the "silent" gene heterozygote, is neither functionally nor genetically the same, with half the functional activity and lesser reserves. Genetically this type has the possibility of contributing a "silent" gene to his progeny. The small amount of hydrolysis occurring in an individual with no typical PChE can be seen in the $E_aE_s$ bar.

The relationship between members of the family is revealed in the genealogy chart (fig. 2) constructed from the data in table 1. The fraternal aunt (no. 4) and her descendants are included. Neither of the deceased parents could have been a homozygote. Three genes are necessary to explain the compliment of their six children; typical, atypical and "silent." These genes can be supplied only by heterozygous combinations of $E_aE_s$-$E_uE_s$, $E_aE_s$-$E_uE_a$, or $E_uE_a$-$E_uE_s$. If the mother (no. 2) were $E_aE_s$ and the father (no. 3) $E_uE_s$, the possibility of the fraternal aunt (no. 4) being $E_uE_s$ would be supported. Against this possibility is that their combination produced no $E_sE_s$ homozygote. Six progeny, however, are not enough to justify a conclusion.

Excluding the genetically unrelated spouses, the atypical genes occurred in 50 per cent of the primary family. At least four members had "silent" genes. Total absence of typical genes occurred in 7.7 per cent as compared to 0.04 per cent in the general population. The Chi square test revealed no correlation between the occurrence of these genes and sex.

## Conclusion and Summary

The physician using succinylcholine or other esters such as procaine which are destroyed by enzymatic hydrolysis is interested, in the final analysis, in knowing the ability of his patient to inactivate these agents. An ACh test will demonstrate the functional capacity regardless of the genotype and, if it is low, an inhibitor test will reveal whether the defect is acquired, perhaps remediable, or congenital and without the means for correction. Therefore, these tests are of value before operation in any patient suspected of having a low functional plasma cholinesterase level. Heterozygotes $E_uE_a$ or $E_uE_s$ would probably not have trouble with the usual SDC exposure, but their reserves could be further reduced by superimposed hepatic or hematologic disease. Exposure to commercial anticholinesterase such as insecticides [11] and ecothiopate iodide (Phospholine) [12, 13] ophthalmic drops provide an additional hazard.

Routine pre-anesthetic enzymatic studies are impractical in most hospitals. The patient with atypical PChE usually denies knowledge of previous trouble involving members of his family, and the anesthesiologist first encounters this problem during the anesthetic. If the problem is recognized and the patient simply given adequate ventilatory support, no harm is done. Confirmatory studies should be done later and his genetic relatives studied, and the knowledge used for any future anesthetic experience.

This study supports the following: concept of a "silent" gene for plasma cholinesterase; the occurrence of atypical and "silent" genes in 49 members of one family; the reduced functional capacity of the "silent" gene heterozygote; and the value of enzymatic studies on the genetic relatives of patients exhibiting no typical plasma cholinesterase.

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# Pulmonary Surfactant: Determinations from Lung Extracts of Patients Receiving Diethyl Ether or Halothane

R. N. MILLER, M.D.,* AND P. A. THOMAS, LTC, MC †

Beecher [1] first suggested that the decreased lung volume observed in postoperative patients were the result of diffuse partial alveolar hypoventilation. Subsequently Bendixen *et al.*[2] proposed that progressive atelectasis might occur in patients with controlled ventilation under general anesthesia unless periodic hyperventilation was practiced. The possible contribution of inhalation anesthetics to depletion, deactivation or destruction of the surface active alveolar material to aggravate a state of progressive atelectasis has not been studied in the clinical setting. In a series of patients undergoing thoracic surgical procedures in this clinic, the surface tension of lung extracts prepared from specimens was normal, suggesting that the anesthetic and technique of administration did not adversely affect pulmonary surfactant.[3] The present investigation was undertaken to substantiate this impression.

* Assistant Professor of Anesthesiology, Washington University School of Medicine, Division of Anesthesiology, Barnes Hospital, St. Louis, Missouri 63110.
† Chief, Department of Surgery and Thoracic Surgery Service, Valley Forge General Hospital, Visiting Assistant Professor of Surgery, Temple University School of Medicine, Philadelphia.
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Address reprint requests to P. A. Thomas, LTC, MC, Department of Surgery, Valley Forge General Hospital, Phoenixville, Pennsylvania 19460.

## PROCEDURE

Ten patients with pulmonary disease necessitating operation were selected for this study. Operation was done for removal of tuberculous residua, neoplasm or diagnosis of neoplasm (table 1). Eight were men, 2 women, whose ages varied from 19 to 50 years. They were in good health except for the pulmonary disease.

Each patient received meperidine 75 mg. and scopolamine 0.2 mg. one hour prior to induction of anesthesia.

Thiopentothal and succinylcholine were administered intravenously for tracheal intubation. Ventilation was controlled manually and anesthesia supplemented by a mixture of 70

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