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Population and pharmacogenetic studies on serum cholinesterase (pseudocholinesterase) on a population of central Italy (Rome)

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Genetica. — *Population and pharmacogenetic studies on serum cholinesterase (pseudocholinesterase) on a population of central Italy (Rome).* Nota (*) di GIOVANNI CERMELE [1], CARLO SANTOLAMAZZA [2], MARISA PULVIRENTI [1], VINCENZO BLANDAMURA [1], ELENA SANPIETRI [3], MICHELE LEVI [4], LAURA E. PACIFICI [1] e GUIDO MODIANO [5], presentata dal Socio G. MONTALENTI.

ABSTRACT. — Serum cholinesterase or pseudocholinesterase (symbol: E, for Esterase), being able to hydrolyse succinyldicholine (SCC), a substance commonly used in the first stage of general anesthesia to induce muscle relaxation, is responsible for the normally short duration (3'-4') of the myorelaxation induced by SCC. The high sensitivity to SCC of subjects with on E activity displays itself as a more or less prolonged apnea following SCC administration that may cause serious clinical problems. The main cause of the low E activity of some subjects is the occurrence of a relatively common allele, the E_1^a, which has associated a low E activity so that the heterozygotes for E_1^a and the normal allele have a moderately prolonged apnea following SCC administration and the $E_1^a E_1^a$ homozygotes' apnea lasts even for hours. Thus the estimate of the frequency of the E_1^a allele is relevant from the clinical, besides of the anthropological, point of view.

In the present paper an E_1^a gene frequency estimate $= 0.011 \pm 0.004$ was arrived at by studying 403 subjects of the Rome population. This estimate was improved by pooling it with another one obtained with another random sample from the same population and became $= 0.011 \pm 0.002$.

Besides and independently from these population studies, the relationship between the E activity and the duration of the SCC-induced muscle relaxation–described by Kalow and Genest long ago (1957)–was reexamined by a different approach. A highly significant negative correlation ($r = -0.37$; $P < 0.001$) was found between these two variables showing that the E activity is one of the major factors upon which depends the SCC-induced myorelaxation time even in the range of the normal values of activity of this enzyme.

KEY WORDS: Population genetics of Central Italy; Pharmacogenetics; Serum cholinesterase.

RIASSUNTO. — *Studi popolazionistici e farmacogenetici sulla colinesterasi sierica (pseudocolinesterasi) in una popolazione dell'Italia centrale (Roma).* La colinesterasi sierica

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o pseudocolinesterasi (simbolo: E, per esterasi), essendo capace di idrolizzare la succinildicolina (SCC), sostanza comunemente usata nel primo stadio della anestesia generale per indurre rilasciamento muscolare, è responsabile della normalmente breve durata (3'-4') del miorilassamento indotto dalla SCC. Soggetti con bassa attività di questo enzima presentano una sensibilità alla SCC maggiore di quella normale che si traduce in un prolungamento più o meno accentuato della apnea indotta dall'SCC che, per il fatto di essere talora del tutto imprevisto, può causare dei problemi clinici gravi. La causa principale della bassa attività E di alcuni soggetti è l'esistenza di un allele, l'E_1^a, a cui è appunto associata una bassa attività di questo enzima, per cui gli eterozigoti per questo allele e quello normale presentano, dopo trattamento con SCC, una apnea moderatamente prolungata, mentre gli omozigoti $E_1^a E_1^a$ rimangono in apnea anche per ore. Da questo deriva l'interesse clinico, oltre che antropologico, di stimare la frequenza di questo allele.

Nel presente lavoro si è determinata la frequenza dell'allele E_1^a in un campione casuale di 403 pazienti della popolazione di Roma e si è giunti alla stima di 0.011 ± 0.004 che, combinata con quella ottenuta su un altro campione della stessa popolazione, è diventata 0.011 ± 0.0027.

Si è inoltre ripreso in esame, ma con un diverso approccio, la relazione già descritta da Kalow e Genest (1957) tra l'attività E e la durata del miorilassamento indotto da SCC. Si è trovata una correlazione negativa ($r = -0.37$) tra queste due variabili, altamente significativa dal punto di vista statistico, che mostra che, anche nell'ambito dei valori normali di attività della pseudocolinesterasi, l'attività di questo enzima è uno dei fattori principali da cui dipende la durata del miorilassamento (e quindi dell'apnea) indotto dall'SCC.

Pseudocholinesterase (abbr. E, for Esterase) is a cholinesterase which differs from the " true " cholinesterase, or acetylcholinesterase, mainly because it hydrolyzes aspecifically the esters of choline (instead of acetylcholine only); it is found predominantly in the serum (hence the name of serum cholinesterase) instead of in specialized cell organules; and for exhibiting an usual hyperbolic function of the activity *vs* substrate concentration instead of the bell-shaped function shown by the " true " cholinesterase (for a general presentation see, for example: Brown *et al.*, 1981; Cermele and Modiano, 1980; Giblett, 1969; Goedde and Agarwal, 1978; Silver, 1974).

The genetics of this enzyme has been exhaustively studied since when, in the fifties (Evans *et al.*, 1952; Bourne *et al.*, 1952; Kalow, 1956; Kalow and Gunn, 1957 and 1959), it had been realized that its activity was the major cause of the short duration of the apnea induced by Succinyldicholine (SCC), a drug commonly administered to patients during general anesthesia to induce muscle relaxation, and that relatively common " low activity " alleles exist and are associated with prolonged SCC–induced apnea (even hours instead of a few minutes in the homozygotes or in the " compound " heterozygotes).

At present two autosomal loci, E_1, and E_2 (or ChE_1 and ChE_2, according to the nomenclature recommended by Shows *et al.* in Human Genetic Mapping 7, Cytogenetics and Cell Genetics *37*, 340-393, 1984) have been identified for pseudocholinesterase.

E_1 controls its activity and kinetic properties. Quite a number of E_1 alleles have been identified (E_1^u, E_1^a, E_1^s, E_1^f, E_1^i and E_1^k) and more or less exhaustively

described in about twenty five years (see reviews quoted above and for the last two alleles see also: Evans *et al.*, 1980; Whittaker and Britten, 1985).

The E_2 locus is responsible for the electrophoretic polymorphism of this enzyme: two alleles, E_2^- and E_2^+, are known. Individuals with no E_2^+ have only four, C_1, C_2, C_3 and C_4 (C for cholinesterase) electrophoretic isozymes while those with the E_2^+ allele display also a fifth electrophoretic band, C_5 (see, for example, Giblett, 1969; Harris, 1980).

The biochemistry, genetic and clinical relevance of pseudocholinesterase had been dealt with in many articles and books (some of them have been quoted above). Population genetic data are listed by Steegmüller, 1975 and Tills *et al.*, 1983.

The present work had been carried out with two purposes:

(*a*) to gather some information on the frequency of the E_1^a allele (which, among the Caucasians, stands out as the by far most important one from the pharmacogenetic point of view) in Italy. In fact to the best of our knowledge

A list of abbreviations.

E = serum cholinesterase or pseudocholinesterase (E for Esterase).

E_1 and E_2 = the two genes that control the enzyme activity and kinetic properties, and the electrophoretic behaviour, respectively, of E.

DN or Dibucaine Number indicates the percent inhibition of the E activity by Dibucaine.

E_1^u, E_1^a, E_1^s and E_1^f are alleles of the E_1 gene, where u = *u*sual; a = *a*typical (low DN); s = *s*ilent; f = *f*luoride resistant.

only one paper (Lucarini *et al.*, 1976), was published so far on the genetic of this enzyme in the Italians, a population very carefully studied for most of the other markers (see Piazza *et al.*, 1982; Modiano *et al.*, 1986).

(*b*) to study the problem of the relationship, among subjects with a *normal* E_1 phenotype, between E activity and duration of the SCC–induced muscle relaxation, by a new approach. This relationship had been in fact deeply investigated a long time ago (Kalow and Gunn, 1957) on patients treated with several doses of SCC and it was shown that a linear relationship exists between the log of the SCC dose (on the abscissa) and that of the duration of the apnea induced by this drug (on the ordinates) and that the intercept of this linear function with the ordinates, *but not its slope*, is correlated with the E activity of the patient (the higher the activity the higher the intercept). The present approach to study the relationship between E activity and sensitivity to SCC had been, in a way, complementary to the Kalow and Gunn's approach since it consisted in studying with a *much less accurate* method (every subject was only treated with a *single* SCC dose so neither the slope nor the intercept of the linear function could be estimated and it was only measured the duration of the muscle relaxation induced by that dose) *many more patients*.

The sample

It consisted of about three hundred and fifty random patients (those affected by a liver disease were however excluded) of both sexes of the 6th Clinica Chirurgica of the University of Rome " La Sapienza " and of random blood donors of the Rome district.

The E activity and the SCC-induced myorelaxation time were measured in 324 of them. In the remaining 79 only the Dibucaine Number was determined.

Materials and Methods

Pseudocholinesterase (E) activity and Dibucaine Number (DN) assays.

Blood samples were kept at room temperature for about two hours following the bleeding; sera obtained by centrifugation were assayed after storage from one day to a month at — 80 °C (the mean E activity of 62 sera re-examined after about one year of further storage was only slightly and non significantly decreased).

The assays were performed in duplicate exactly as described by Modiano *et al.*, 1986, namely according to Kalow and Genest, 1957, except that the final substrate (benzoylcholine) concentration was 10^{-4} M instead of $0.5 \cdot 10^{-4}$ M, so that the linearity between the true E activity and the observed one was maintained through a longer period of time (see Modiano *et al.*, for details).

In our hands the best discriminant value of DN for identifying the carriers of one E_1^a allele turned out to be 65 in both series of data (the present one and that of Modiano *et al.*, 1986) (see also fig. 1) and also on the basis of family studies carried out on 8 large informative families (data not shown).

Duration of the SCC-induced muscle relaxation.

The patients were pre-anesthesized intravenously with atropine sulphate (0.008 mg/Kg).

Induction obtained with sodium thiopentale (5 mg/Kg), was immediately followed by an intravenous administration of SCC (1.0 mg/Kg).

The duration of the SCC-induced muscle relaxation (= sensitivity to SCC) is expressed as the time (in min') elapsed since the end of the intravenous injection of SCC and the moment (approximated at the nearest half-min') when the first contractions of selected muscles (*musculi orbiculares oculi* and muscles of the tongue) became appreciable following suitable stimulation.

Results and Discussion

Estimate of the E_1^a gene frequency.

Out of 403 subjects 9 exhibited a DN lower than 65 (see fig. 1) and were thus classified as carriers of at least one E_1^a allele. Since homozygotes for this

allele could be excluded (even the single patient with a DN = 47 certainly carried an E_1^u allele since his muscle relaxation time was 19.5 min' whereas $E_1^aE_1^a$ and $E_1^aE_1^s$ subjects exhibit myorelaxation times of hours) the estimate of the E_1^a gene frequency in the present sample is $\frac{9}{806} = 0.011 \pm 0.004$, which is in practice identical to that (0.012 ± 0.004) obtained on the different sample from the same population studied by our group (Modiano *et al.*, 1986) also by electrophoretic techniques. Thus the two estimates could be pooled arriving at the improved estimate of 0.011 ± 0.0027 for the population of " Rome " with a SE equal to 25% rather than 36% of the estimate.

This figure compares well with the only one so far published for Italy (0.014 ± 0.006: Lucarini *et al.*, 1976) besides being within the Caucasian range (see Steegmüller, 1975; Tills *et al.*, 1983).

The expected risk of a prolonged apnea associated with homozygosity for E_1^a is then $1.21 \cdot 10^{-4}$. In the present series none $E_1^aE_1^a$ homozygote was found (exp. $\simeq 0.05$) whereas 1 was encountered in the other series (exp. $\simeq 0.05$): altogether 1 *vs* ~ 0.1 expected. A further figure corresponding to the expected pooled frequency of the $E_1^aE_1^s$ and $E_1^aE_1^s$ subjects should be added to $1.21 \cdot 10^{-4}$. This figure is however unknown because reliable estimates for the E_1^s gene frequency are not available for Caucasians. The only information we have is

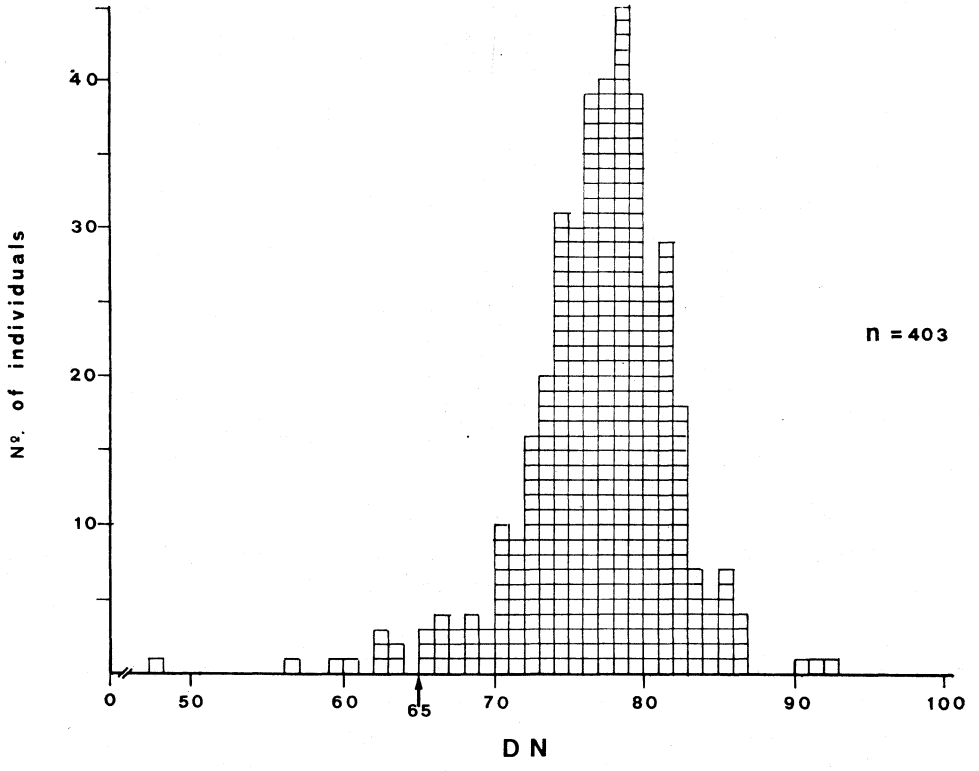

Fig. 1. - Distribution of the Dibucaine Number (DN) in a random sample of 403 subjects of the Rome district.

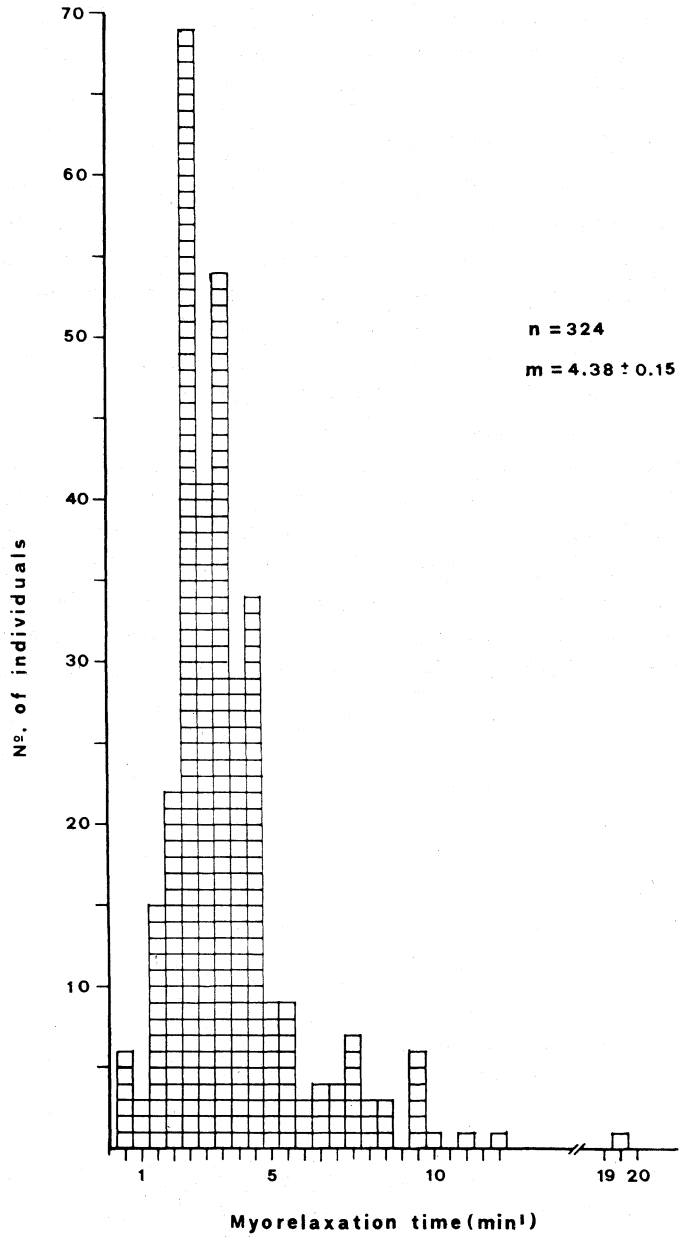

Fig. 2. - Distribution of the SCC-induced myorelaxation time in a random sample of 324 patients of the Rome district.

that this allele is certainly uncommon in this major race since cases of very prolonged SCC-induced apnea are only exceptionally encountered.

The average DN in the subsample of 9 $E_1^a E_1^u$ heterozygotes was 59.8 $\pm$ $\pm$ 1.73 *vs* the mean value of 77.1 $\pm$ 0.25 found in the subsample of 394 individuals with the usual phenotype.

Search for a relationship between E activity and sensitivity to SCC.

324 subjects were studied for both these aspects.

A mean SCC-induced myorelaxation time of 4.38 min' with a SD = 2.80 and a SE = 0.15 was found (see fig. 2).

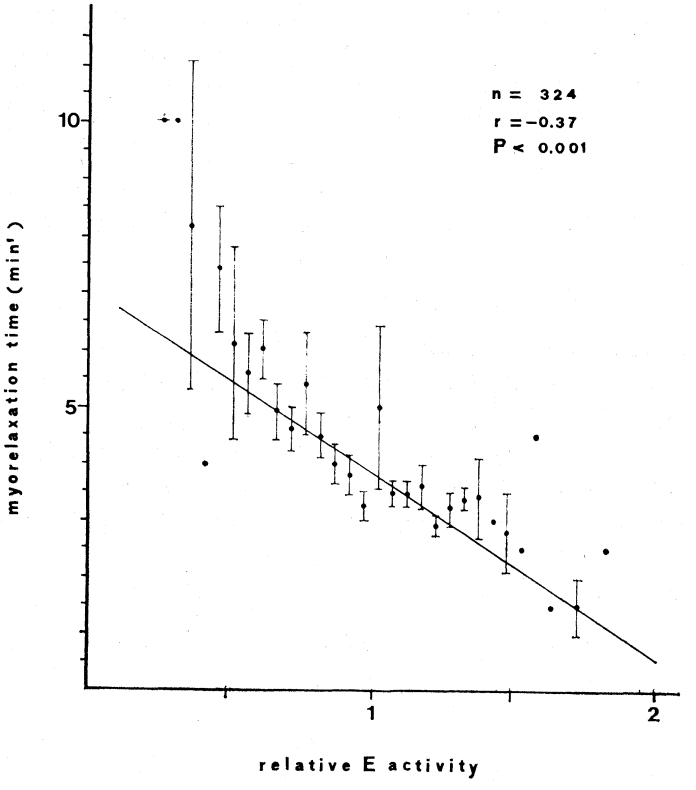

Fig. 3. – Negative correlation between E activity and mean duration of the SCC-induced myorelaxation. For each class of E activity the mean myorelaxation time of the individuals of that class is indicated along with its SE. Single dots refer to classes with a single individual (see text for further explanations).

A highly significant ($P < 0.001$), though not very large, negative correlation ($r = -0.37$) was found in this subsample of 324 subjects between their E activity and SCC-induced myorelaxation time thus confirming the findings obtained by Kalow and Gunn, 1957, by a different approach (see above). The relatively modest value of r calls for other factors, besides of the E activity, affecting the sensitivity to SCC [variations due to: technical imprecision both in determining the E activity and the SCC-induced myorelaxation time; SCC dosages (the body weight is a relatively crude term of reference); and other causes]. In any case the E activity stands out among the major causes of the individual variation of the SCC-sensitivity in the normal range since it alone accounts for approximately 15% ($0.37^2 = 0.14$) of its whole variance besides, of course, being the by far most relevant cause of this variation in the range

of the very high sensitivity to SCC (that is, very prolonged SCC-induced apneas, say in the order of more than ten minutes).

A convenient way of showing the inverse relationship: sensitivity of SCC *vs* E activity consists in subdividing the data into discrete classes of E activity and then in annulling the within-class variation of the myorelaxation time (by definition not associated with E activity variations) by plotting in a graph of these two variables a single dot for each class of E activity calculated as the arithmetical mean of the myorelaxation times of that class (see fig. 3).

The slope of the inverse function shown in fig. 3 is such that one can predict, within a range of E activity around its mean m (in the order of magnitude of $m \pm 0.5\,m$), that is within the normal range and with a standard dose of SCC as indicated above, a time in min' of the SCC-induced muscle relaxation, with this function:

$$\text{min' of SCC-induced myorelaxation time} = 4.38 - (11 \cdot \Delta E)$$

where ΔE indicates the variation of E in terms of its mean m and the *minus* sign is due to the inverse relationship between E and sensitivity to SCC [for example, the time of SCC-induced myorelaxation predicted for an individual with an E activity $= 0.5\,m$ is: $4.38 + (11 \cdot 0.5) = 9.9$ min']. However, as indicated by the relatively low value of r, the prediction power of this function is not very high, that is the predictions are quite approximate.

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