Pseudocholinesterase variation in southern African populations

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Summary

Genetic variation at both the El and E2 loci of pseudocholinesterase was studied in 7 southern African populations. In the Ashkenazim and Afrikaners, El locus variation was observed and the frequency of the El' or 'atypical' allele was found to be 0.017 in the Ashkenazim and 0.016 in the Afrikaans-speaking population, similar to those observed in other caucasoid populations. The El', or 'silent' allele, was found to occur in the Afrikaans-speaking population at a frequency 2 - 3 times greater than in other Caucasians, giving an increased risk of succinylcholine sensitivity. The rarity of the El' in the Negro and Khoisan populations was confirmed. All 7 populations showed variation at the E2 locus with high frequencies of the E2' allele in the Caucasians and San, while Negroes showed relatively low frequencies. Coloureds showed intermediate frequencies, consistent with their historical origins.

Pseudocholinesterase (EC 3.1.1.8) is an enzyme produced in the liver, and found in high concentrations in the plasma. Its biosynthesis is under the genetic control of at least two autosomal loci called El and E2 and both are polymorphic in at least some populations. Although the biological functions of the enzyme are still not known, the polymorphism is of clinical interest, since succinylcholine (scoline), a muscle relaxant used in anaesthesia, causes prolonged apnoea in individuals with certain of the El variant phenotypes.

Although the El' (or 'usual') allele is the most common in all populations studied, the distribution of the other El alleles varies considerably among different groups of people. The El' or 'atypical' allele has been shown to be extremely rare or absent among Pygmies, East-Asian populations (Japanese, Koreans, Taiwanese and Filipinos), Eskimos, Negroes, Ice-landers and some South American tribes. It occurs at low but polymorphic frequencies in most European populations, and at high frequencies in Iraqis and Jews. Although a 'fluoride resistant' allele is rare in most caucasoid populations but it occurs at high frequencies among certain groups of Alaskan Eskimos.

The E2 locus is a further source of genetically determined pseudocholinesterase variation but, to date, no clinical significance has been ascribed to this variation. The frequencies of E2 locus alleles are more variable than those at the El locus and do not show as clear a geographical or racial variation. Similar frequencies of the E2' allele responsible for the most commonly encountered variant, the C5 band, have been detected in many populations of different origins, though higher than average frequencies have been found among Caucasians (± 10% E2'/E2 heterozygotes) while the E2' allele is relatively rare among Negro and Asiatic Indian populations (0.3 - 2.9% heterozygotes).

Although a great deal is known about the pseudocholinesterase polymorphisms, data on southern African populations are relatively sparse and this report attempts to rectify this.

Subjects and methods

Seven southern African populations are represented: Ashkenazi Jewish, Afrikaner, Cape Coloured, Tswana (a Nwato group and a mixed group from Taung), Zulu and San (Bushmen). The number of individuals tested are shown in Tables I and II and the areas of origin of the populations are shown on Fig. 1.

### Table I. Observed El locus phenotypes and calculated El' allele frequencies in the 7 populations studied

<table>
<thead>
<tr>
<th>Population</th>
<th>No. studied</th>
<th>I phenotype</th>
<th>El' allele frequency ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi</td>
<td>461</td>
<td>16</td>
<td>3.5</td>
</tr>
<tr>
<td>Afrikaner</td>
<td>255</td>
<td>8</td>
<td>3.1</td>
</tr>
<tr>
<td>Coloured</td>
<td>61</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Nwato</td>
<td>110</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Taung</td>
<td>213</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Zulu</td>
<td>68</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>San (!Kung)</td>
<td>86</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Table II. Observed E2 locus phenotypes and calculated E2' allele frequencies in the 7 populations studied

<table>
<thead>
<tr>
<th>Population</th>
<th>No. studied</th>
<th>C5' phenotype</th>
<th>E2' allele frequency ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi</td>
<td>461</td>
<td>37</td>
<td>7.1</td>
</tr>
<tr>
<td>Afrikaner</td>
<td>255</td>
<td>27</td>
<td>10.6</td>
</tr>
<tr>
<td>Coloured</td>
<td>61</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Nwato</td>
<td>110</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>Taung</td>
<td>213</td>
<td>7</td>
<td>3.3</td>
</tr>
<tr>
<td>Zulu</td>
<td>67</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>San (!Kung)</td>
<td>86</td>
<td>9</td>
<td>10.5</td>
</tr>
</tbody>
</table>
Results

E1 locus

The Ashkenazi and Afrikaner populations contained individuals of the U ("usual") and I ("intermediate") phenotypes (Table I). The results obtained on the Ashkenazim were pooled with those of Lane et al.13 to give a total Ashkenazi sample of 461. Sixteen E1V/E1V heterozygotes were observed among them, while in the Afrikaners there were 8 out of 244, corresponding to E1V allele frequencies of 0.017 ± 0.004 and 0.016 ± 0.006 in the two populations, respectively. No detectable variation was observed in the other 5 populations. Out of 538 coloured, Negro and San individuals studied, not one possessed the E1V allele. The differences between this combined sample and both the Ashkenazi and Afrikaner samples (χ2 values = 24,149 and 13,821 respectively; P < 0.001) are highly significant.

No conclusive evidence for the presence of either the E1' or the E1 allele was found in any of the 7 populations studied. Although no individual possessing the E1' allele was detected in the Afrikaner sample surveyed, we have estimated the frequency of this allele using the data obtained in our laboratory from routine investigations of succinylcholine-sensitive Afrikaners tested in the laboratory over a 9-year period, 4 were found to be E1'/E1' homozygotes. The other 54 were either E1'/E1' heterozygotes or E1'/E1' homozygotes, but these two genotypes could not be distinguished without a family study. The estimation of the frequency of the E1' allele was based on the 'atypical': 'silent' phenotype ratio of 54:4. It was assumed that individuals possessing the E1' and E1' alleles are distributed according to Hardy-Weinberg expectation as q2:2qr: r2, where q is the frequency of E1' and r the frequency of E1'. Thus, q2 + 2qr + r2 = 54:4.

The value of q has been estimated from the number of E1'/E1' heterozygotes to be 0.016 ± 0.006. Substitution of this value into the above expression yielded a value for r, the E1' frequency, of 0.006.

E2 locus

Individuals with both well-defined phenotypes, C5' and C5 (presumably corresponding to the E2'/E2' and E2'/E2' genotypes, respectively), were found in all populations, but no other electrophoretic variants were observed. The detailed results are presented in Table II.

Discussion

The pseudocholinesterase E1 locus determines both quantitative and qualitative characteristics of the serum enzyme. Although 7 populations were screened, variation at the E1 locus was only observed in the 2 caucasoid populations (Ashkenazim and Afrikaners). In the Ashkenazim the estimated E1' frequency of 0.017 ± 0.004, is identical to the frequency observed by Szeinberg et al.3 in a sample of 4196 Ashkenazi Jews in Israel. With this allele frequency, one would expect approximately 1/33000 South African Ashkenazim to be homozygous E1'/E1' and therefore at risk for succinylcholine apnoea. If the E1' frequency in South African Ashkenazim were also similar to that in Israeli Ashkenazim, namely 0.004, one would have expected approximately 3 E1'/E1' heterozygotes to have been found in a sample of approximately 500. It is conceivable that none was found, simply as a result of sampling error.

The pseudocholinesterase results obtained on the Ashkenazi sample are consistent with the conclusion of Lane et al.13 based on data from many gene markers that the South African Ashkenazim are part of a fairly homogeneous world Ashkenazi population. Although the results presented here support the view that the Ashkenazim have remained a well-defined group, they do not preclude an affinity with other Caucasians, too. The E1' allele frequency has been shown to be in the range of 0.015 - 0.030 in many caucasoid populations,1 and the frequency observed in the Ashkenazim is well within this range.

One of the reasons for studying pseudocholinesterase variation in the Afrikaner population was the suggestion from empirical data gathered in our laboratory that there was an increased frequency of scoline-sensitive individuals in this population. The frequency of the E1' allele in the Afrikaner population was estimated to be 0.016 ± 0.006, well within the range of 0.015 - 0.030 quoted for Caucasians.1 It seems, therefore, that an increased frequency of the 'atypical' allele is not the reason for the apparently higher incidence of scoline sensitivity in the Afrikaners. In contrast with the E1' allele frequency, however, the estimated frequency of the E1V allele (0.006) is 2 - 3 times higher than that quoted for Caucasians (0,0017 - 0.0028).14,15 At this frequency, one would expect 1/30000 Afrikaners to be scoline-sensitive due to homozygosity for the E1' allele, which is rather high compared with the 1/100000 figure quoted for other Caucasians.16 Although there may be some error in the E1' frequency estimate, the findings of this study concur with those of two previous studies,17,18 which suggested, although no estimate of the E1' allele was made, that the frequency of the E1' allele was unusually high in South African whites. The proportion of 12 'atypical': 3 'silent' phenotype individuals observed by Panell et al.,15

Fig. 1. Map of southern Africa showing the areas of origin of the various populations.

Blood samples were collected into 10 ml plain Vacutainers by venepuncture. Samples were allowed to clot after which the serum was separated and stored at −20°C, or in liquid nitrogen at −170°C until tested.

Variation at the E1 locus was detected using spectrophotometry, in which enzyme activities were determined by the method of Kalow and Lindsay9 and dibucaine numbers and fluoride numbers by the methods of Kalow and Genest4 and Harris and Whitaker,10 respectively. The modifications suggested by Zsigmond et al.11 for measuring fluoride numbers were introduced.

Variation at the E2 locus was detected electrophoretically using one-dimensional starch-gel electrophoresis.12

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would in fact give a higher frequency of the E1' allele than that calculated here.

An accurate estimate of the E1' allele frequency is extremely difficult to obtain, because of its rarity and the difficulty in identifying E1'/E1' heterozygotes. Estimates must therefore be based on E1'/E1' homozygote frequencies, which are very low, and therefore subject to significant sampling error.

From the frequencies observed in this study, one would expect approximately 1/2066 Afrikaners to be sensitive to scoline. This figure includes the 3 genotypes - E1'/E1', E1'/E1' and E1'/E1'. The corresponding figure in non-Afrikaner-speaking South African Caucasians would be expected to be approximately 1/3090, assuming that their E1' allele frequency is similar to that observed in other Caucasian populations. This figure suggests a 1.5 times higher incidence of scoline-sensitive individuals among Afrikaners than among non-Afrikaner South African Caucasians.

By taking into account the higher E1' allele frequency in Afrikaners, and the fact that they represent approximately 60% of white South Africans, we are able to estimate that the proportion of scoline-sensitive individuals among Afrikaners as compared to non-Afrikaner South African Caucasians would be expected to be approximately 2.2:1. In other words, one would expect to encounter about twice as many Afrikaner as non-Afrikaner scoline-sensitive individuals in the South African white population. The relatively high frequency of the E1' allele in Afrikaners could be due to their origins from a small number of founders.

In contrast with the caucasoid populations, no variation at the E1 locus was found in the Cape Coloured, Negro and Khoisan populations. A number of other studies have also suggested that the E1' allele, if it occurs at all, exists at very low frequencies in the Negro populations of southern Africa. The E1' allele was not found in 326 south-eastern Bantu-speakers from Mozambique, or in 1614 Negroes from Zimbabwe, Malawi, Zambia and Mozambique.

Jenkins also reported an absence of the E1' allele in a sample of 419 South African Negroes and, in addition, failed to detect it in 263 Khoisan and 51 Cape Coloureds.

Although no clear evidence for the presence of the E1' allele was found during the population screening carried out in this study, it is found in the non-caucasoid populations, since E1'/E1' homozygotes are encountered regularly during routine laboratory investigations of individuals who have scoline apnoea. The first negroid E1'/E1' homozygote was described from South Africa and all the Negro and Coloured individuals who have been investigated for scoline sensitivity in this laboratory have been found to have the E1'/E1' genotype. There is no reason to assume at this stage that the E1' allele assumes higher frequencies in black and coloured South Africans than it does elsewhere in the world. What is important to note is that in these populations, the E1' allele occurs at higher frequencies than the E1' allele, which is unusual.

Variation at the E2 locus was found in all 7 populations studied, though the frequencies of the E2' and E2' alleles vary considerably. When calculating the frequency of the E2' allele, all individuals having the C5' phenotype were assumed to be E2'/E2' heterozygotes. The E2' allele frequency in the Ashkenazi sample was within the caucasoid range (6 - 12% C5' individuals). No comparative data are available for Ashkenazim living elsewhere. The C5' phenotype frequency in the Afrikaner sample was also found to be within the quoted caucasoid range. It appears that the E2' allele frequency is consistently higher in Caucasians than it is in Negroes and Cape Coloureds. The C5' phenotype is rare in 'pure' Negro populations, in which it occurs with a frequency of 0.3 - 2.9%. Jenkins, in keeping with this, found a C5' phenotype frequency of about 2% in South African blacks. In the present study, however C5' phenotype frequencies of 1.5 - 3.3% were observed.

The C5' phenotype frequency in the San sample (10.5%) was significantly higher than that found in the Negro sample and is in agreement with that of Jenkins, who suggested that the E2' allele is probably commoner in the San and Khoikhoi than in the Negro populations. The frequencies in the Negroes in this study tend to be higher than those previously reported in Negroid populations and could reflect the Khoisan admixture which is known to have occurred.

The high frequency of the E2' allele in the Ngwato may well be due to San admixture. They are cattle-herders, and the area they inhabit spreads westwards into the Kalahari. There is considerable contact between the two groups and thus San gene flow into the Bantu-speakers is very likely.

The Cape Coloured population would be expected to have relatively high C5' phenotype frequencies compared with Negroes because of their origin. Their gene pool has been estimated to have a total Khoikhoi, San and caucasoid contribution of 75% and since these three populations all have reasonably high frequencies of the E2' allele, the occurrence of C5' in 3.3% of the individuals is to be expected.

The factors contributing to the polymorphisms at the E1 and E2 pseudo-cholinesterase loci are unknown. A clear elucidation of the physiological function of pseudo-cholinesterase, and the alteration of this normal function caused by variant alleles, may help to explain the striking differences in gene frequencies at both loci in the populations of southern Africa.

We would like to acknowledge the assistance of Dr D. Dunn, Mr A. A. de Bruyn; Dr A. R. P. Walker; Dr M. Reby; Mr J. Ntlaule, headmaster, and the staff of St Paul's School, Taung; Mr S. P. P. Khanya, headmaster, and the staff of Mangwawana High School, Ubombo; in the obtaining of samples. In addition, we would like to thank all the individuals who kindly donated blood for this study. One of us (A. K.) wishes to acknowledge the receipt of a South African Medical Research Council scholarship and a senior bursary from the University of the Witwatersrand, during this study.

REFERENCES
The assessment of permanent disability following injury to the hand

J. H. FLEMING, J. H. YOUNGLESON

Summary

Each year approximately 290,000 workmen are injured on duty, 24,000 of whom will suffer some permanent disability and will merit financial compensation for their injuries. An accurate doctor’s report is the single most important factor in ensuring that a workman receives fair compensation.

0° Measurement into flexion extends up to a theoretical possibility of 180° or 200° depending on the joint involved. Lack of extension is also lack of movement and should be recorded. Please note that impairment of function is comparable with the loss of the part by amputation.

Sensation. While loss of dorsal sensation is not considered disabling, complete loss of palmar sensation is calculated as equal to 50% of an amputation. Two-point discrimination exceeding 2 cm is generally regarded as complete loss of sensation or anaesthesia.

Power. The assessment of the patient’s power grip and pinch power is very important in assisting the Commissioner to decide on the percentage of disability. The doctor can help the Commissioner very considerably in coming to a fair evaluation by testing the patient with a hand dynamometer for power grip. A satisfactory alternative is a blood pressure cuff (Baumanometer) rolled up and inflated to 30 mmHg which is then squeezed by the patient and the grip compared between the good and the injured hand.

Similarly pinch power is assessed with a pinch meter or, if this is not available, the same Baumanometer can be used to measure pinch power.

Finally, cosmetic disfigurement: a photograph is worth a thousand words in helping the Commissioner reach a just decision.

Assessment of disability

The doctor is not required to assess permanent disability. However, he is required to give a factual description of the patient’s physical impairment. The determination of the degree of a workman’s permanent disablement rests entirely with the Commissioner. He naturally takes due regard of conclusive medical evidence of such permanent anatomical defects and/or impairment of function as may have resulted from the accident, on a basis not inconsistent with the First Schedule to the Act.

Should the patient’s condition deteriorate at a later date and he require further surgery, then the case will have to be re-opened. For this purpose, the patient is required to submit to the Commissioner at his own expense, a detailed medical report in which his condition, how this relates to the accident, and the nature of the medical treatment/surgery envisaged are described in detail. Upon receipt of the necessary medical

In the RSA during 1982, 289,000 workmen were injured on duty and 23,920 of these suffered permanent disability. It is interesting to note that of the total figures, over 100,000 suffered hand injuries and 11,000 sustained some permanent hand disability. The most important link in the chain which brings compensation to these people is the doctor’s report. It is hoped that this article will help our colleagues to ensure that these patients receive adequate compensation.

Delay in payment can be caused by the employer either not reporting the accident or not forwarding the doctor’s reports to the Workmen’s Compensation Commissioner. The records can be mislaid by the hospital or the doctor may inaccurately assess the patient’s permanent disability.

‘Disablement’ is defined as disablement for employment or permanent injury or serious disfigurement. It is usually easy to describe amputations but loss of function is more difficult. The Commissioner assesses function on the basis of loss of active movements in degrees compared with the normal movement of the joint. Thus, it is very important that the range of active movement should be measured and recorded on the special hand chart (W.C.I.31) provided by the Workmen’s Compensation Commissioner. The method used by the Commissioner is that a joint in the neutral (straight) position is at

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