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The Red Cell Acid Phosphatase Polymorphism in Sweden

Gene Frequencies and Application to Disputed Paternity [1]

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SUMMARY

Red cell acid phosphatase phenotypes were determined in 517 unrelated persons from Sweden (317 men, 200 women). The observed gene frequencies were $p^A = 0.372$; $p^B = 0.558$; $p^C = 0.070$.

Experiences in 101 cases of disputed paternity with 167 men involved are reported. On grounds of red cell acid phosphatase phenotypes, 19 men (in 18 cases) could be excluded.

In all 112 mother-child combinations studied the distributions of phenotypes were in agreement with the genetical hypothesis suggested by Hopkinson et al (1963).

A genetically controlled polymorphism of human erythrocytic acid phosphatase was first described by Hopkinson et al (1963). Using horizontal starch gel electrophoresis they distinguished five different phenotypes referred to as *A*, *B*, *BA*, *CA*, and *CB*. The finding could be explained assuming the occurrence of three codominant alleles: p^a, p^b, and p^c.

The predicted phenotype *C* (p^cp^c) subsequently was found by Lai et al (1964).

Furthermore, two other rare alleles p^d and p^r, occurring in Negro populations, have been described (Giblett and Scott, 1965; Karp and Sutton, 1967).

Material and Methods

The studied sample consisted of:

a) 9 F and 113 M: healthy, unrelated blood donors, all living in Stockholm;

b) 75 F and 3 M: unrelated medical outpatients, all living in Stockholm;

c) 13 M: healthy, unrelated medical students and members of the staff at Karolinska Institutet, Stockholm;

d) 116 F and 188 M involved in cases of disputed paternity, from several places across Sweden.

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In all, the sample consisted of 200 women and 317 men. Also investigated were 112 children from paternity-cases.

The blood donors and the outpatients were selected according to day of visit to the respective clinic.

About 4 ml of blood were collected by venipuncture into glass vials containing 2 ml of ACD-solution. The samples were analysed within one week. During the meantime they were stored in a refrigerator at +4°C.

The red blood cells were washed three times with physiological saline. Hemolysates were prepared by diluting the packed red cells with two volumes of distilled water, rapidly followed by freezing the samples in ethanol at —10°C, and subsequent thawing at +20°C.

This method yielded as good results as the more complicated technique described by Radam and Strauch (1966a), and better results than simple freezing and thawing. No difference was noticed in the results from fresh samples or samples stored in ACD-solution at +4°C. Indeed, freshly prepared hemolysates from samples stored for three months gave excellent results (Karp and Sutton, 1967).

As a bridge buffer we have utilized a citrate/phosphate buffer (0.15 M in trisodium-citrate and 0.245 M in NaH_2PO_4) of pH 5.9, containing 1.86 g of EDTA per liter (Karp and Sutton 1967).

The gel buffer was prepared by dilution of the bridge buffer (1 : 100). For making the gels we have used hydrolyzed starch (Connaught Medical Research Laboratories, Toronto) in a concentration of 12% in the gels.

The suspended starch was boiled and degassed according to the method described by Smith (1968, p. 225), poured into trays, and cooled at +4°C for 2 hours prior to electrophoresis. The gel trays consisted of perspex frames with tops and bottoms of glass plates. The gels measured 180 × 80 mm.

The samples were applicated on small strips of Whatman 17 filter paper, inserted into slits cut in the gel.

Electrophoresis was carried out in a horizontal apparatus at constant current, 12 mA-100 V/gel for 17 hours at +4°C.

Staining of the zones of enzyme activity was accomplished by the method of Hopkinson, Spencer and Harris (1964), using phenolphtalein diphosphate as substrate.

Results

Gene Frequency

The distribution of phenotypes is tabulated in Tab. I. The gene frequencies were calculated by the gene-counting method (Hopkinson et al, 1963).

As no significant differences in phenotype frequencies were observed neither between the different groups of the sample nor between the sexes, the different data were pooled.

It appears from Tab. I that the agreement with the expected Hardy-Weinberg distribution is good, indicating that the sample is unselected, and therefore can be considered a fair representation of the Swedish population.

The gene frequencies in some European populations are listed in Tab. II.

Tab. I. Distribution of red cell acid phosphatase phenotypes
[$N = 517$]

Phenotypes	*A*	*BA*	*B*	*CB*	*CA*	*C*	Total
Observed	79	205	162	48	22	1	517
Expected[a]	71.54	214.63	160.98	40.39	26.93	2.53	517.00

$\chi^2 = 4.48$; $df = 3$; $0.20 < P < 0.30$

[a] Expected values were calculated using the gene frequencies: $p^A = 0.372$; $p^B = 0.558$; $p^C = 0.070$.

Tab. II. Gene-frequencies in some European populations

Population	No. of individuals tested	Gene frequencies p^A	p^B	p^C	Reference
Denmark	470	0.342	0.602	0.056	Lamm, 1968
England	1010	0.359	0.595	0.046	Hopkinson, 1966
Germany (Berlin)	1188	0.362	0.577	0.061	Radam and Strauch, 1966b
Czechoslovakia	307	0.365	0.578	0.057	Herzog and Bohatova, 1969
Austria	410	0.365	0.573	0.062	Speiser and Pausch, 1967
Sweden	517	0.372	0.558	0.070	Present paper

Experience with the System in Cases of Disputed Paternity

Red cell acid phosphatase phenotypes were determined in 416 persons involved in 101 cases of disputed paternity. The number of exclusions based on the 3-allele hypothesis is recapitulated in Tab. III.

The average chance of a man in a given population to be excluded from a falsely alleged paternity can be calculated according to the formula of Speiser and Pausch (1967):

$pq\ (1 - pq) + pr\ (1 - pr) + qr\ (1 - qr) + 3\ pqr\ [1 - (pq + pr + qr)]$ where p, q, and r stand for p^A, p^B, and p^C respectively. The value for the studied sample is 0.2590, i.e., 26%.

From Tab. III it appears that, out of 56 cases of disputed paternity with two men involved in each case, one of the men was excluded in 14 cases.

This corresponds to a practical 25% incidence of exclusion.

Mother-Child Combinations

The results of a study in 112 mother-child combinations are given in Tab. IV.

In all the combinations studied the constellations of phenotypes were in agreement with the genetic hypothesis suggested by Hopkinson et al (1963).

Tab. III. Results in 101 cases of disputed paternity

No. of men involved in each case	No. of cases	Total no. of men involved	Excluded[a]
1	40	40	2
2	56	112	14
3	5	15	3[b]

[a] On grounds of red cell acid phosphatase phenotypes.
[b] In one case two men could be excluded.

Tab. IV. Distribution of phenotypes in mother-child combinations
[$N = 112$]

Mother/Child	*A*	*BA*	*B*	*CB*	*CA*	*C*	Total
A	6	15	—	—	2	—	23
BA	11	16	10	1	3	—	41
B	—	9	22	2	—	—	33
CB	—	0	3	5	0	0	8
CA	3	0	—	3	1	0	7
Total	20	40	35	11	6	0	112

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RIASSUNTO

I fenotipi della fosfatasi acida eritrocitaria sono stati esaminati in 517 svedesi non consanguinei (317 uomini, 200 donne). Sono state osservate le seguenti frequenze geniche: $p^A = 0.372$; $p^B = 0.558$; $p^C = 0.070$.

Si riferiscono i risultati di ricerche su 101 casi di incerta paternità, con 167 uomini implicati. Sulla base dei fenotipi della fosfatasi acida eritrocitaria, 19 uomini (in 18 casi) sono stati esclusi.

In tutte le ricerche compiute in 112 combinazioni madre-figlio, la distribuzione dei fenotipi è risultata conforme all'ipotesi genetica di Hopkinson et al (1963).

RÉSUMÉ

Les phénotypes de la phosphatase acide érythrocytaire ont été déterminés chez 517 suédois non apparentés (200 femmes et 317 hommes). Les suivantes fréquences géniques ont été observées: $p^A = 0.372$; $p^B = 0.558$; $p^C = 0.070$.

Sur 101 cas de paternité incertaine, avec un total de 167 hommes examinés, 19 hommes (en 18 cas) ont pu être exclus sur la base des phénotypes de la phosphatase acide.

Dans les 112 combinaisons mère-enfant examinées, la distribution des phénotypes est toujours en accord avec l'hypothèse de Hopkinson et al (1963).

ZUSAMMENFASSUNG

In der vorliegenden Arbeit werden die Phänotypen der sauren Erythrozytenphosphatase in der Schwedischen Bevölkerung an 517 nichtverwandten Merkmalsträgern (317 Männer, 200 Weiber), untersucht.

Die gefundenen Genfrequenzen sind folgende: $p^A = 0.372$; $p^B = 0.558$; $p^C = 0.070$.

Erfahrungen in 101 Fällen von fraglicher Vaterschaft an 167 begutachteten Männern, werden berichtet. Unter Zugrundelegung der Phänotypen der sauren Erythrozytenphosphatase wurden 19 Männer (in 18 Fällen) von der Vaterschaft ausgeschlossen.

Die Phänotypenkonstellationen zwischen Mutter und Kind sind in sämtlichen 112 untersuchten Fälle in Übereinstimmung mit der genetischen Hypothese von Hopkinson et al (1963).

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