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Polymorphism of Erythrocyte Acid Phosphatase and Adenosindesaminase in Galicia (N.W. Spain) by AGIF and PAGIF

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Summary. A total of 1,086 individuals from Galicia have been typed for red-cell acidphosphatase (AcP) and adenosindesaminase (ADA) using isoelectric focusing in polyacrylamide (PAGIF) and agarose gels (AGIF). The following gene frequencies were detected:

for AcP: AcP^a: 0.278; AcP^b: 0.686; AcP^c: 0.036

for ADA: ADA^1: 0.950; ADA^2: 0.050

The use of AGIF in typing AcP for forensic purposes is discussed.

Key words: Blood groups, AcP-ADA polymorphism – AcP gene frequencies – ADA, gene frequencies

Zusammenfassung. Die Phänotypen der AcP und der ADA wurden bei 1086 Personen aus Galizien durch AGIF und PAGIF bestimmt.

Die ermittelten Genfrequenzen sind:

für AcP: AcP^a: 0.278; AcP^b: 0.686; AcP^c: 0.036

für ADA^1: 0.950; ADA^2: 0.050

Schlüsselwörter: Blutgruppen, AcP-ADA-Polymorphismus – AcP, Genfrequenzen – ADA, Genfrequenzen

Methods for the determination of isoenzyme polymorphism by starch, cellulose acetate, agarose, and acrylamide electrophoresis have been previously described for typing AcP and ADA [3, 4, 7].

Isoelectric focusing (IEF) as a technique for AcP phenotyping was described by Ishimoto and Kuwata [5] in 1972 using PAGIF at low voltage. Analysis by PAGIF at high voltage appears to be a technique which gives superior resolution to the conventional ones [1].

Simultaneous isoelectrofocusing of ADA and AcP can be carried out easily [6].

In this paper we report the results of a survey of red cell AcP and ADA by AGIF and PAGIF on lysates from 1,086 donors with four Galician grandparents. The use of AGIF in typing of AcP for forensic purposes is also discussed.

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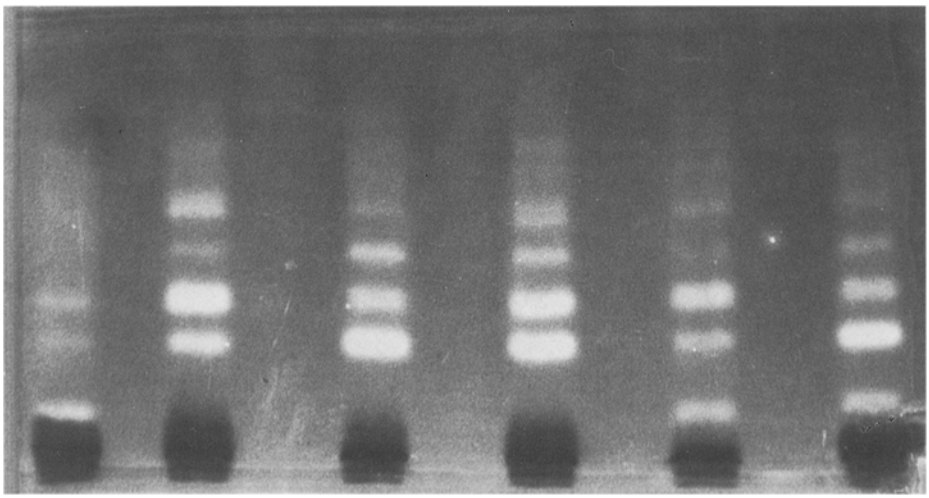

Fig. 1. AcP types by AGIF: from left to right A, B, C, BC, AB, and AC

Materials and Methods

Blood samples from 1,086 healthy adults proportionally representative of regional districts of Galicia were examined.

Red cells were washed three times in physiologic saline solution and subsequently frozen at − 20° C. The AcP and ADA type determinations were carried out within 2 months of taking the samples.

PAGIF was carried out with equal amounts of Ampholine (LKB) pH 4–6 and pH 6–8 with a final concentration of 3% (v/v) at a gel concentration of T = 5% and cross-linking of C = 3%. The electrode solutions were 1 M NaOH for the cathode and 1 M H_3PO_4 for the anode. Lysates were pretreated with fresh Clelland's reagent (0.05 M dithiothreitol) one drop to one drop for at least 15 min. Immediately before application they were diluted with distilled water (1:2) and placed near the anode using Whatman 3 MM (0.5 × 0.5 cm) paper. Electrofocusing was carried out at a maximum of 1,500 V, stabilized at 20 W for 90 min.

For AGIF, 1% agarose IEF (Pharmacia) and 13% (w/v) D-sorbit were used. pH gradient was prepared as PAGIF. The electrode solutions were 0.05 M H_2SO_4 (anode) and 1 M NaOH (cathode). Samples were treated and applied as for PAGIF. Electrofocusing was carried out at maximum of 1,200 V, stabilized at 15 W for 75 min.

Isoenzyme visualizations were done according to Spencer et al. [8] for ADA and according to Burdett and Whitehead [1] for AcP.

Results and Discussion

Figure 1 demonstrates the AcP phenotypes as observed by analysis of hemolysates by AGIF. All the six common phenotypes are depicted.

The results are shown in Tables 1 and 2, and it can be seen that there was a good agreement between the observed and expected Hardy-Weinberg phenotypes numbers.

A comparison with a previous Galician population genetic study [2] shows no significant differences.

Table 1. AcP phenotype and gene distribution in Galicia

Phenotypes	B	BA	A	BC	AC	C	Total
Observed	508	420	81	54	22	1	1086
Expected	511.07	414.35	83.48	53.51	21.69	1.40	1086

$\chi^2 = 0.325$; $df = 3$; $P > 0.90$
Gene frequencies AcP^b 0.686, AcP^a 0.278, AcP^c 0.036

Table 2. ADA phenotype and gene distribution in Galicia

Phenotypes	1	2-1	2	Total
Observed	980	104	2	1086
Expected	980.68	102.63	2.69	1086

$\chi^2 = 0.194$; $df = 1$; $P > 0.50$
Gene frequencies ADA^1 0.950, ADA^2 0.050

In our opinion both AGIF and PAGIF have a similar resolution. The preparation of agarose gel for AGIF requires notoxic products, the prefocusing is unneccessary and the running time is shorter. We believe AGIF could be applied successfully to the typing of ageing stains. However, for routine use PAGIF seems preferable as it is possible to make thinner gels using much smaller quantities of the expensive ampholytes.

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