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Genetic Analysis of Erythrocyte Uridine Monophosphate Kinase and Aminolevulinate Dehydrase and Its Application to Biological Paternity Testing

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ABSTRACT: Simultaneous determination of uridine monophosphate kinase (UMPK) and aminolevulinate dehydrase (ALAD) has been carried out after discontinuous starch gel electrophoresis in the Galician population (NW Spain), including 129 families with a total of 291 descendents. Formal genetic studies are in agreement with the autosomal codominant way of inheritance for each locus. No evidence of phenotype association between both loci among the offspring is observed. Chance of exclusion for non-fathers is 0.041 13 for UMPK and 0.0702 for ALAD configuring a total exclusion rate of 0.1085 when both systems are evaluated together.

KEYWORDS: pathology and biology, genetic typing, enzymes, aminolevulinate dehydrase, uridine monophosphate kinase, formal genetics, biological paternity testing

The uridine monophosphate kinase (UMPK, E.C. 2.7.4) phosphorylates the uridine monophosphate into uridine diphosphate as the first step in a metabolic pathway leading to production of pyrimidine nucleoside triphosphate required for ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) synthesis [*1*]. Genetic polymorphism of UMPK was initially reported using discontinuous starch gel electrophoresis [*2*] and its phenotypes are expressed as codominant autosomal alleles of a locus placed on chromosome 1 [*3*]. Delta aminolevulinate dehydrase (ALAD, E.C. 4.2.1.24) is the second enzyme in the heme biosynthetic pathway that catalyzes the asymmetric condensation of two molecules of delta aminolevulinic acid to form porphobilinogen. Likewise, ALAD exhibits a genetically controlled polymorphism [*4*] whose locus is regionally localized to chromosome 9 [5].

Unfortunately the number of formal genetic studies for both systems is limited. The main goal of this paper is to study the genetic polymorphism of UMPK and ALAD to provide further data of their genetic transmission in family groups based on the possibility of a simultaneous typing of both systems in one electrophoretic step.

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Materials and Methods

The statistical sample consisted of 500 unrelated healthy individuals from the population of Galicia (NW Spain). Family studies were carried out in 129 family groups including a total of 291 offspring.

From each individual the whole blood obtained was treated with ethylenediaminetetraacetate-disodium (EDTA-Na_2) as anticoagulant. Blood samples were centrifuged at 2500 rpm for 15 min. After the plasma supernatant was removed, erythrocytes were washed with physiological saline three times at 2500 rpm for 15 min and then stored at −20°C for no longer than six months, until use.

Hemolysates were made by freezing and thawing and then 50μL of hemolysate were mixed with 50 μL of a solution of 5% (w/v) Sephadex G-200 in 40% (w/v) sucrose. The mixture was poured into slots prepared in the starch gel matrix.

Simultaneous electrophoresis for both systems was done in horizontal starch gel (12% starch, w/v) [6]. The gel buffer consisted of 0.02*M* histidine-hydrochloric acid (HCl) and 0.01*M* sodium hydroxide (NaOH) adjusted to pH 6. The bridge buffer consisted of 0.04*M* citric acid and 1.13*M* NaOH adjusted to pH 6. Electrophoresis was carried out at 4°C at a constant 4 V/cm for 15 h. After this, the gel was sliced, following which we detected specifically for UMPK and ALAD in distinct slices. Zones of UMPK activity were stained in a buffer of 0.3*M* Tris (hydroximethyl aminomethane), 0.1*M* potassium chloride (KCl), 0.02*M* magnesium chloride ($MgCl_2$), and 0.2*M* histidine-HCl adjusted to pH 7.8 containing 9m*M* uridine-5′-monophosphoric acid, 3m*M* phospho*enol* pyruvate, 2m*M* adenosine triphosphate, 1m*M* nicontiamide adenine dinucleotide (reduced form) (NADH), 7 IU/mL pyruvate kinase, and 7 IU/mL lactic dehydrogenase. A No. 1 Whatman paper was soaked in the staining mixture and applied over the slice then incubated in a humid chamber at 37°C for 2 h until defluorescent zones or UMPK activity were observed against a fluorescent background under ultraviolet light at 365 nm.

Activity of ALAD was detected in a two-step staining. The first stage was made in an incubating buffer of 0.1*M* Tris, 0.1*M* histidine-HCl, 0.1*M* imidazole, and 5m*M* $MgCl_2$ adjusted to pH 7.4, containing 5m*M* dithiothreitol, 10m*M* delta aminolevulinic acid, and 10m*M* zinc acetate. The solution was applied on the sliced gel using a No. 1 Whatman paper. The gel was incubated at 39°C for 2 h after which the surface was briefly washed with distilled water and then incubated for 30 min at 37°C in the dark with a No. 1 Whatman paper which was soaked with a solution containing 6.4 mL of acetic acid, 3.2 mL of 70% (v/v) perchloric acid, and 1 mL of 0.5*M* mercuric chloride ($HgCl_2$) containing 200 mg of *p*-dimethylaminobenzaldehyde. After this, pink zones showing ALAD activity (as a result of the presence of porphobilinogen) appeared 4 to 5 cm from the origin moving towards the anode.

Results and Discussion

In Table 1 phenotype and gene frequencies of UMPK in the Galician population are summarized. The gene frequencies are $UMPK^1 = 0.955$ and $UMPK^2 = 0.045$. No rare variants have been found. A good fit between empirical and theoretical phenotype frequencies according to the Hardy-Weinberg law is observed ($\chi^2 = 0.000\ 17$; $0.98 < p < 0.99$, 1 degree of freedom [df]). Likewise, gene frequencies for ALAD in our population are $ALAD^1 = 0.917$ and $ALAD^2 = 0.083$. A different degree of polymorphism for both systems is observed. Thus, ALAD has a heterozygosity degree (h) ranging between 0.1974 and 0.1522 among Caucasian populations hitherto analyzed [7] and UMPK shows values of h between 0.047 and 0.109 [8]. In the Galician population, h is 0.086 for UMPK and 0.152 for ALAD.

Formal genetic analysis in 129 family groups for UMPK and ALAD is shown in Tables 2 and 3. The theoretical rare matings not found in this study represent only 0.036 and 0.68% for UMPK and ALAD, respectively. Segregation ratios among the 291 offspring examined

TABLE 1—*Phenotype distribution of UMPK among 500 individuals from the Galician population.*

Phenotype	Absolute Phenotype Frequencies		Allele Frequencies
	Empirical	Theorical	
1-1	456	456.0125	$UMPK^1 = 0.9550 \pm 0.0065$
2-1	43	42.9750	
2-2	1	1.0125	$UMPK^2 = 0.0450 \pm 0.0065$
Total	500	500.0000	

TABLE 2—*Distribution of UMPK phenotypes in 129 families from Galicia. Expected frequencies are put in parentheses.*

Matings		Families No.	Children No.	Phenotypes of Children			df	χ^2
Father	Mother			1-1	2-1	2-2		
1-1	× 1-1	104	229	229 (229.00)	... (0.00)	... (0.00)	...	...
2-1	× 1-1	13	32	9 (16.00)	13 (16.00)	... (0.00)	1	1.125
1-1	× 2-1	10	24	10 (12.00)	14 (12.00)	... (0.00)	1	0.666
2-1	× 2-2	2	6	2 (1.50)	2 (3.00)	2 (1.50)	2	2.148
Total		129	291	260	29	2		3.939

TABLE 3—*Distribution of ALAD phenotypes in 129 families from Galicia. Expected frequencies are put in parentheses.*

Matings		Families No.	Children No.	Phenotypes of Children			df	χ^2
Father	Mother			1-1	2-1	2-2		
1-1	× 1-1	92	203	203 (203.00)	... (0.00)	... (0.00)	...	...
1-1	× 2-1	17	35	21 (17.50)	14 (17.50)	... (0.00)	1	1.400
2-1	× 1-1	12	31	13 (17.50)	18 (17.50)	... (0.00)	1	0.926
2-1	× 2-1	5	15	6 (3.75)	5 (7.50)	4 (3.75)	2	2.200
1-1	× 2-2	2	4	... (0.00)	4 (4.00)	... (0.00)	...	...
2-2	× 2-1	1	3	... (0.00)	3 (1.50)	... (1.50)	1	3.00
Total		129	291	243	44	4		7.526

TABLE 4—*UMPK and ALAD phenotype distribution among 291 offspring. Expected values are put in parentheses.*

UMPK	Phenotype Frequencies			
ALAD	1-1	2-1	2-2	Total
1-1	220 (217.113)	21 (24.216)	2 (1.670)	243
2-1	38 (39.313)	6 (4.385)	... (0.302)	44
2-2	2 (3.574)	2 (0.399)	... (0.027)	4
Total	260	29	2	291

were in keeping with Mendelian expectations giving the following total values: $\chi^2 = 3.393$ ($0.2 < p < 0.3$, 3 df) for UMPK and $\chi^2 = 7.526$ ($0.1 < p < 0.2$, 5 df) for ALAD. These results indicate that each locus exhibits a single autosomal codominant mode of inheritance, which is concordant with the results obtained by Battistuzzi et al. [*4*], Kühn et al. [*9*], and Sachs et al. [*10*].

We should emphasize that the major deviations from observed and expected phenotype frequencies of descendents correspond with less frequent matings, which in large part could be due to statistical randomness observed among the low number of offspring. Therefore, in the case of UMPK, the least frequent pair (2-1 × 2-1), including only six children, has a partial value of $\chi^2 = 2.1479$ which represents more than 54% of the total value. For ALAD, the couple 2-2 × 2-1 determines a partial value of $\chi^2 = 3.00$ representing nearly 40% of the total value of χ^2.

In Table 4 the distribution of UMPK and ALAD phenotypes among the 291 descendents is summarized. We found no evidence of significant deviations between observed and expected phenotype frequencies under the hypothesis of independence in the expression of both loci ($\chi^2 = 8.626$, $0.05 < p < 0.1$, 4 df).

The chance of exclusion of non-fathers for these systems in the Galician population is 0.041 13 for UMPK and 0.0702 for ALAD. The total exclusion rate for both genetic markers is 0.1085 which is similar to that subtyped esterase D (EsD) after isoelectric focusing.

Acknowledgments

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