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Population Frequencies of Carbonic Anhydrase II (CA II), Esterase D (EsD), and Glyoxalase I (GLO) in the Metropolitan Birmingham, Alabama Area

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ABSTRACT: Both black and white populations from Birmingham, Alabama were analyzed for the frequencies of carbonic anhydrase II (CA II), glyoxalase I (GLO) and esterase D (EsD) isozymes. The results compared favorably with published frequencies of these genetic markers in other populations.

KEYWORDS: pathology and biology, genetic typing, carbonic anhydrase, esterase D, glyoxalase

Genetic frequency data assist forensic serologists in establishing the probability of random matching between the isozymes (alternate forms of an enzyme) in a blood or bloodstain sample and those of an individual in the designated population. The results reported herein contribute to this process, not only for this particular population, but also for comparisons of regional, national, and worldwide population frequencies of these particular genetic markers.

Materials and Methods

Blood samples were collected from Community Blood and Plasma Service in Birmingham and were classified according to race, sex, and date of collection.

The whole-blood samples were washed three times each with 0.86% sodium chloride and 0.1% sodium azide in distilled water using 30-s centrifugations. The washed red blood cells were stored at 4°C for up to one month before electrophoresis.

Cellulose acetate electrophoresis was performed for carbonic anhydrase II (CA II) as described by Grumbaum [*1*]. The bands of CA II activity were observed 10 min after staining with 10 mg of fluorescein diacetate dissolved in acetone and 0.1 *M* phosphate buffer, pH 6.5. Under long-wave ultraviolet light, bands of CA II activity exhibited yellow fluorescence.

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Agarose gel electrophoresis was carried out for esterase D (EsD) as described by Kuhnl et al. [2]. Staining was performed according to the method of Hopkinson et al. [3]. EsD activity was visualized at approximately 5 cm anodal from the origin. The bands fluoresced under longwave ultraviolet light 5 to 10 min after staining.

Cellulose acetate electrophoresis of glyoxalase (GLO) was performed as described by Grunbaum [1] with minor modifications. The incubation time of the reaction mixture was approximately 15 to 20 min at 40°C. The samples were typed as soon as possible because of instability and rapid loss of activity observed by Kuhnl et al. [2].

For the statistical calculation of each locus, genotype frequencies were analyzed, separately for blacks and whites, for goodness-of-fit to the Hardy-Weinberg equilibrium. Chi-square tests utilized the Yates correction where appropriate.

Results

CA II

No genetic variation was observed at the CA II locus in 196 Birmingham whites (Table 1). Of 333 blacks, CA II phenotypes and gene frequencies of 333 blacks agreed with those expected by the Hardy-Weinberg equilibrium (Table 2). The CA II gene frequencies compared favorably with other black populations that have been studied [4–7] (Table 3).

EsD

The phenotypes and gene frequencies of EsD among 513 Birmingham whites and blacks (Tables 4 and 5) showed only slight differences between the two. This was consistent with

TABLE 1—*Carbonic anhydrase II phenotypes and gene frequencies in the white population of Birmingham, Alabama.*

	Phenotypes					
	Observed		Expected		Gene Frequency	
CA II	*N*	%	*N*	%	CA^1	CA^2
1-1	196	100	196	100	1.00	0.00
2-1	. . .	. . .	. . .	. . .	. . .	. . .
2-2	. . .	. . .	. . .	. . .	. . .	. . .

TABLE 2—*Carbonic anhydrase II phenotypes and gene frequencies in the black population of Birmingham, Alabama.*

	Phenotypes					
	Observed		Expected		Gene Frequency	
CA II	*N*	%	*N*	%	CA^1	CA^2
1-1	268	80.5	269	80.8	0.899	0.101
2-1	63	18.9	60.6	18.2	$X^2 = 0.267$	
2-2	2	0.6	3.3	1.0	(1 df) $p = 0.61$	
Total	333	100	333	100	. . .	. . .

TABLE 3—*Gene frequencies of carbonic anhydrase II.*

Population	CA^1	CA^2	Ref
BLACK			
Gambia, West Africa	0.90	0.10	*4*
African (British residents)	0.964	0.046	*5*
United States	0.8986	0.1014	*6*
United States (Western Pennsylvania)	0.9025	0.0975	*7*
United States (Birmingham, Alabama)	0.899	0.101	this study
Mean frequency	0.91	0.09	. . .
OTHER			
Asiatic indian	1.00	. . .	*5*
European white	1.00	. . .	*5*
United States white	1.00	. . .	this study
Mean frequency	1.00	. . .	. . .

TABLE 4—*Esterase D phenotypes and gene frequencies in the white population of Birmingham, Alabama.*

	Phenotypes					
	Observed		Expected		Gene Frequency	
EsD	*N*	%	*N*	%	EsD^1	EsD^2
1-1	148	75.51	149.37	76.21	0.873	0.127
2-1	46	23.47	43.46	22.17	$X^2 = 0.231$	
2-2	2	1.92	3.17	1.62	(1 df) $p = 0.63$	
Total	196	100	196	100	. . .	. . .

TABLE 5—*Esterase D phenotypes and gene frequencies in the black population of Birmingham, Alabama.*

	Phenotypes					
	Observed		Expected		Gene Frequency	
EsD	*N*	%	*N*	%	EsD^1	EsD^2
1-1	254	80.13	255.06	80.46	0.897	0.103
2-1	61	19.24	58.58	18.48	$X^2 = 0.284$	
2-2	2	0.63	3.36	1.06	$p = 0.59$	
Total	317	100	317	100	. . .	. . .

previous findings among white and black populations surveyed in Europe, America, and Africa [*5, 7-15*] (Table 6). Black populations have exhibited slightly higher EsD^1 frequencies compared with whites (0.90 versus 0.88), while the frequencies of EsD^1 in Asiatic populations were distinctly lower (0.77) (Table 6).

GLO

The gene frequencies of 0.434 for GLO^1 and 0.566 for GLO^2 were determined from the phenotypic distributions of GLO in Birmingham whites (Table 7). The allele frequency of

TABLE 6—*Gene frequencies of esterase D.*

Population	EsD^1	EsD^2	Ref
WHITE			
England	0.887	0.113	*8*
North Germany	0.882	0.118	*9*
West Germany	0.883	0.117	*10*
Belgium	0.89	0.11	*11*
United States (Minnesota)	0.911	0.089	*12*
United States (Miami)	0.892	0.108	*13*
United States (Los Angeles)	0.852	0.148	*13*
United States (Western Pennsylvania)	0.8862	0.1138	*7*
United States (Birmingham, Alabama)	0.873	0.127	this study
Mean frequency	0.88	0.12	. . .
BLACK			
Great Britian	0.90	0.10	*5*
Gambia, West Africa	0.91	0.09	*14*
Uganda	0.89	0.11	*11*
United States (Miami)	0.914	0.086	*13*
United States (Western Pennsylvania)	0.8816	0.1184	*7*
United States (Birmingham, Alabama)	0.873	0.127	this study
Mean frequency	0.88	0.12	. . .
ASIAN			
Nepal	0.649	0.351	*15*
India	0.773	0.227	*5*
Mean frequency	0.771	0.289	. . .

TABLE 7—*Glyoxalase I phenotypes and gene frequencies in the white population of Birmingham, Alabama.*

	Phenotypes					
	Observed		Expected		Gene Frequency	
GLO I	*N*	%	*N*	%	GLO^1	GLO^2
1-1	40	20.41	36.93	18.84	0.434	0.566
2-1	90	45.92	96.29	49.13	$X^2 = 0.832$	
2-2	66	33.67	62.78	32.03	(1 df) $p = 0.36$	
Total	196	100	196.00	100	. . .	. . .

GLO^1 in the black population (0.272) was lower than that observed for whites (0.434) (Table 8). These frequencies corresponded well with those found in American white, American black, and European white populations [*16-24*] (Table 9), except for the Lapps [*17*], whose GLO frequencies differed considerably from other white populations. This indicated that the Lapps are a genetically distinct population group. The inclusion of the Lapp data in European gene frequencies might result in misinterpretation of the rarity of a particular sample in the general population. Therefore, they are not included in the mean frequency calculated in Table 9. Regarding the Hardy-Weinberg equilibrium, the frequencies for both black and white populations in Birmingham provided an excellent fit to this statistical parameter.

TABLE 8—*Glyoxalase I phenotypes and gene frequencies in the black population of Birmingham, AL.*

	Phenotypes					
	Observed		Expected		Gene Frequency	
GLO I	*N*	%	*N*	%	GLO^1	GLO^2
1-1	19	5.92	23.68	7.4	0.272	0.728
2-1	136	42.50	126.72	39.6	$X^2 = 1.73$	
2-2	165	51.56	169.60	53.0	(1 df) $p = 0.19$	
Total	320	100	320	100	. . .	. . .

TABLE 9—*Gene frequencies of glyoxalase I.*

Population	GLO^1	GLO^2	Ref
	EUROPEAN		
Netherlands	0.4544	0.5456	*16*
Norway	0.442	0.558	*17*
Southwest Germany	0.427	0.573	*18*
Lapland	0.304	0.696	*17*
South Germany	0.4235	0.5765	*19*
Hessen, Germany	0.4391	0.5609	*2*
Switzerland	0.444	0.556	*20*
Denmark	0.4311	0.5689	*21*
Mean frequency	0.437	0.563	. . .
	MIDEASTERN		
Iraq	0.423	0.577	*22*
Israel, Iranian Jews	0.2294	0.7706	*23*
Israel, Iraqi Jews	0.2710	0.7290	*23*
Israel, Arabs	0.2951	0.7049	*23*
Mean frequency	0.305	0.695	. . .
	AMERICAN		
White	0.42	0.58	*24*
White, Birmingham	0.433	0.566	this study
Mean frequency	0.43	0.57	. . .
Black	0.28	0.72	*24*
Black, Birmingham	0.272	0.728	this study
Mean frequency	0.28	0.72	. . .

Summary

Cellulose acetate and agarose gel electrophoresis were used to determine the phenotypic distributions and allele frequencies of CA II, EsD, and GLO gene loci in the Birmingham area. Blood was analyzed from over 500 unrelated individuals, black and white, for each enzyme. The frequencies obtained from the Birmingham sample were similar to those of other population frequencies reported, around the mean of which there is little variation. Thus, these frequencies are useful as rough estimates for the general population.

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