TECHNICAL NOTE

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HLA-DQA1 and Polymarker Allele Frequencies in Two New York City Jewish Populations

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ABSTRACT: Allele and genotype frequencies were determined for the HLA-DQA1 and Amplitype® Polymarker loci (low density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gammaglobin (HBGG), D7S8, and group-specific component (Gc)) in Hasidic and non-Hasidic Ashkenazi New York City Jewish subpopulations. For all loci tested, except HBGG, the 2 subpopulations meet the assumption of Hardy-Weinberg equilibrium. Comparison of various allele and genotype frequencies for the Hasidic and the non-Hasidic groups showed no significant differences. Comparison of the various allele frequencies in the two subpopulations to another Caucasian group revealed significant differences at the HLA-DQA1 and D7S8 loci in the Hasidic group. These frequency data can be used for comparison to other populations and for frequency estimates in DNA profiling.

KEYWORDS: forensic science, DNA typing, Jewish populations, New York City, HLA-DQA1, polymarker, LDLR, GYPA, HBGG, D7S8, GC, population genetics, allele frequencies, polymerase chain reaction

There are many violent crimes (murder, rape, etc.) committed where no witnesses are available but biological (circumstantial) evidence (blood, semen, etc.) is obtained at the crime scene. Under these conditions, it becomes necessary to determine a genetic profile, usually based on 5–7 polymorphic genetic markers, of the evidence and of any subsequently identified suspect. Because a given genetic profile is usually not unique to a particular individual but rather is characteristic of a group of people, it becomes critical to estimate how large or small the group of matching individuals is in order to decide what "weight" to assign to a genetic match between evidence and suspect. Estimating the size of the genetically profiled group is dependent on the frequency of occurrence of each individual genotype in the population to which the alleged perpetrator of a crime belongs. The task is complicated by the fact that the "relevant geographic population" (usually that of the state [California, New York, etc.] in which the crime occurred) is composed of many ethnic groups. Such groups may have originated from diverse regions of the world, and may have distinctive allele frequencies, hence genotype frequencies, for the traits contained in any determined genetic profile. Wherever possible, it is important to ascertain allele frequencies of forensically important genes in as many ethnic groups as possible to define the likely range of allele frequency variation and so that databases will be available should they be required at some future time. This is especially true for endogamous groups which may have unusual allele frequencies brought about by their restrictive marriage patterns.

TABLE 1—*HLA-DQA1 4 allele subtype frequencies as determined by restriction enzyme digestion of PCR product (7).*

	Frequency	
Allele	Hasidic Population	Non-Hasidic Population
0501	.23	.25
0401	.03	.02
0601	.01	.01

Materials and Methods

Samples

This study presents HLA-DQA1 and Amplitype PM allele and genotype frequency data for samples drawn from the New York City Hasidic and non-Hasidic Jewish subpopulations. Both subpopulations are parts of a more general Jewish group known as the Ashkenazi which migrated to the United States from Eastern and Central Europe. The Ashkenazi are distinguished from the group known as the Sephardi which came from Spain (1).

DNA samples were obtained from unrelated donors provided by the Department of Human Genetics, Mount Sinai School of

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TABLE 2—*Distribution of observed and expected HLA-DQA1 genotype frequencies.*

	Hasidic Population $n = 107$			Non-Hasidic Population $n = 101$		
Genotype	Observed	Frequency	Expected	Observed	Frequency	Expected
1.1-1.1	1	.01	2.0	5	.047	2.4
1.1-1.2	2	.02	2.8	3	.028	3.9
1.1-1.3	5	.05	4.2	2	.019	2.6
1.1-2	5	.05	3.7	2	.019	4.8
1.1-3	5	.05	5.9	6	.056	6.7
1.1-4	9	.089	7.6	10	.093	9.3
1.2-1.2	0	0	1.0	2	.019	1.5
1.2-1.3	1	.01	3.0	3	.028	2.1
1.2-2	2	.02	2.6	5	.047	3.9
1.2-3	8	.079	4.2	4	.037	5.4
1.2-4	6	.059	5.5	7	.065	7.4
1.3-1.3	4	.04	2.3	1	.009	0.7
1.3-2	6	.059	3.9	4	.037	2.6
1.3-3	5	.05	6.4	6	.056	3.6
1.3-4	6	.059	8.2	1	.009	5.0
2-2	1	.01	1.7	1	.009	2.4
2-3	5	.05	5.5	8	.075	6.7
2-4	6	.059	7.1	10	.093	9.3
3-3	3	.03	4.5	3	.028	4.7
3-4	14	.139	11.5	15	.14	13.0
4-4	7	.069	7.4	9	.084	9.0
	$X^2 = 11.21$	DF = 9(15 − 6)	$p = .26$	$X^2 = 11.45$	DF = 11(17 − 6)	$p = .41$
	Exact Test	SD = 0.016	$p = .77$	Exact Test	SD = 0.016	$p = .44$

TABLE 3—*Distribution of observed and expected polymarker loci genotype frequencies.*

	Hasidic Population $n = 111$			Non-Hasidic Population $n = 109$		
Genotype	Observed	Frequency	Expected	Observed	Frequency	Expected
LDLR AA	20	.18	21.5	17	.17	16.6
LDLR AB	58	.52	54.7	51	.47	51.9
LDLR BB	33	.30	34.8	41	.38	40.5
	$X^2 = .33$	$p = .57$	with 1 DF	$X^2 = .04$	$p = .84$	with 1 DF
	Exact Test	$p = .57$	SD = 0.04	Exact Test	$p = .84$	SD = 0.01
GYPA AA	36	.32	36.1	36	.33	36.7
GYPA AB	55	.50	54.4	55	.51	53.1
GYPA BB	20	.18	20.5	18	.17	19.2
	$X^2 = .07$	$p = .80$	with 1 DF	$X^2 = .16$	$p = .69$	with 1 DF
	Exact Test	$p = 1.00$	SD = 0	Exact Test	$p = .85$	SD = .01
HBGG AA	19	.17	13.6	21	.19	20.1
HBGG AB	40	.36	49.7	51	.47	51.6
HBGG AC	0	0	0.8	1	.01	1.9
HBGG BB	51	.46	45.5	34	.31	33.0
HBGG BC	1	.01	0.6	1	.01	2.4
HBGG CC	0	0	0	1	.01	0
	Exact Test	$p = .045$	SD = .01	Exact Test	$p = .05$	SD = .01
D7S8 AA	29	.26	30.0	38	.35	39.2
D7S8 AB	57	.51	55.4	54	.50	52.3
D7S8 BB	25	.23	25.6	17	.16	17.5
	$X^2 = 0.09$	$p = 0.77$	with 1 DF	$X^2 = .11$	$p = .74$	with 1 DF
	Exact Test	$p = .85$	SD = .014	Exact Test	$p = .84$	SD = .01
Gc AA	5	.05	8.7	9	.08	8.5
Gc AB	14	.13	8.7	10	.09	10.4
Gc AC	39	.35	36.1	34	.31	33.6
Gc BB	0	0	2.2	1	.01	3.2
Gc BC	16	.14	18.0	25	.23	20.4
Gc CC	37	.33	37.3	30	.28	33.0
	$X^2 = 7.54$	$p = .06$	with 3 DF	$X^2 = 2.87$	$p = .41$	with 3 DF
	Exact Test	$p = .066$	SD = .004	Exact Test	$p = .49$	SD = .02

Medicine in New York City. The Hasidic samples were from the Chevra Dov Yeshivum Program conducted at the Mount Sinai Center for Jewish Genetic Diseases during 1983 and 1984. Samples previously had been screened for Tay-Sachs and other disorders such as Gaucher's disease. Both these inherited diseases have a high incidence in Eastern European Jewish populations (2,3). All samples were collected with informed consent that they may be used anonymously for other research purposes.

Sample Preparation

Genomic DNA was extracted from peripheral blood samples. Briefly, 0.5 mL of blood was mixed with 0.5 mL of lysis buffer (0.32 M sucrose, 10 mM Tris-HCl [pH 7.5], 5 mM $MgCl_2$, 1% Triton X-100) in a 1.5 mL microcentrifuge tube, followed by centrifuging at 13,000 × g for 20 s. The supernatant was discarded and the pellet resuspended in 1.0 mL of lysis buffer. The pellet was centrifuged and resuspended in 1.0 mL lysis buffer twice more. After centrifuging again, the supernatant was removed and the pellet resuspended in 0.5 mL PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 0.1 mg/mL Sigma Porcine gelatin, 0.45% Nonidet P40, 0.45% Tween 20). Six μL of Proteinase K (1 mg/mL) in PCR buffer was added to each 100 μL of the DNA extract solution and the tubes were incubated at 55°C for 1 h. The samples were then incubated at 95°C for 10 min to inactivate the protease and then the DNA solutions were stored frozen.

Amplification and Typing

Samples underwent multiplex amplification and typing according to the manufacturer's protocol (4,5). Genomic DNA (1 μL) was added to the amplification reaction mixture. Amplification was conducted for 32 cycles, each of which consisted of denaturation at 94°C for 1 min, annealing at 60°C for 30 s, extension at 72°C for 30 s followed by a delay of 7 min at 72°C. A Perkin-Elmer model 480 thermal cycler was used for amplification. Samples were then typed using reverse dot blot hybridization (6). The HLA-DQA1 and Amplitype PM kits used in this study do not distinguish several alleles found at either the HLA-DQA1 locus or at the GYPA locus (4,5). The HLA-DQA1 4 allele subtypes and their frequencies, as determined by restriction enzyme digestion of the PCR product (7), are given in Table 1.

Results and Discussion

The New York Metropolitan area has a Jewish population of 1.45 million. At least 70% of this population, or one million, are of Ashkenazi descent (Personal Communication, Dr. Bethany Horowitz, Director of Planning and Research, United Jewish Appeal of Greater New York). This group constitutes 5.8% of the New York State population and 0.4% of the United States population (8,9).

Members of the Hasidic subpopulation are largely descendants of a religious sect that grew out of the Ashkenazi population in Eastern Europe during the 18th century. After the Second World War many Hasidic Jews relocated to New York City. They live in close-knit communities and practice an ultra-orthodox form of Judaism. Their lifestyle emphasizes social separatism from the general society and from other Jews. Hasidim will accept new members into their group, but only those who assimilate completely. New members usually come from the non-Hasidic Ashkenazi community. There are approximately 125,000 Hasidim in the New York area. They constitute 0.7% of the New York State and 0.48% of the United States populations (1,8–11).

TABLE 4—*Observed HLA-DQA1 allele frequencies*.*

Allele	Frequency: Hasidic Population n = 101	Non-Hasidic Population n = 107	FBI Caucasian Population (14) n = 148
1.1	.14	.15	.12
1.2	.10	.12	.18
1.3	.15	.08	.04
2	.13	.15	.12
3	.21	.21	.22
4	.27	.29	.33

*See Table 1 for the 4 allele subtype frequencies.

Table 2 presents the distribution of observed and expected HLA-DQA1 genotypes for the Hasidic and non-Hasidic ethnic groups. Table 3 presents comparable data for the Amplitype PM genotypes. With the exception of HBGG (Table 3), all loci were tested by both Chi-Square and the Exact Test to see if they meet the assumption of Hardy-Weinberg equilibrium. In the case of HBGG, only the exact test results for this locus are listed. It was not possible to do Chi-square analysis on the HBGG locus due to the limitations imposed by Cochran's Rule, when the number of expected classes with 0 or 1 observations exceed 20% (12)

In the Chi-Square analysis of HLA-DQA1, it was necessary to combine a number of the small expected classes in order to meet the restrictions of Cochran's Rule (12). The number of classes for the Hasidic group was reduced from 21 to 15, whereas the non-Hasidic group was reduced to 17. For the exact test, calculations were made using Monte Carlo estimations as specified by Guo and Thompson (13). Tests were performed using 17,000 iterations in 20 groups of 850 iterations per group. The standard deviation (SD) is that of the group results. With the exception of HBGG, all loci were found to meet the assumption of Hardy-Weinberg equilibrium by both tests. For the HBGG locus, failure to meet Hardy-Weinberg equilibrium at about 5% significance levels may be the result of sampling variability.

Table 4 presents the frequencies of the various alleles at the HLA-DQA1 locus from Hasidic, non-Hasidic Ashkenazi, and, for comparison purposes, a group of Caucasians associated with an FBI database (14). Table 5 presents comparable data for the alleles at the PM loci. A Chi-Square analysis (see Table 6), omitting

TABLE 5—*Observed polymarker allele frequencies.*

	Hasidic Population n = 111	Non-Hasidic Population n = 109	FBI Caucasian Population (14) n = 148
LDLR A	.44	.39	.45
LDLR B	.56	.61	.55
GYPA A	.57	.58	.58
GYPA B	.43	.42	.42
HBGG A	.35	.43	.47
HBGG B	.64	.55	.52
HBGG C	.01	.02	.01
D7S8 A	.52	.60	.62
D7S8 B	.48	.40	.39
Gc A	.28	.28	.26
Gc B	.14	.17	.17
Gc C	.58	.55	.57

TABLE 6—*Chi Square test comparison of allele frequencies of samples taken from various subpopulations.*

		Hasidic vs. Non-Hasidic	Hasidic vs. FBI Caucasian (14)	Non-Hasidic vs. FBI Caucasian (14)
HLA-DQA1 (DF = 5)	X^2 value	5.40	25.02	8.72
	P value	.37	<.001*	.12
LDLR (DF = 1)	X^2 value	1.22	.06	2.03
	P value	.27	.81	.15
GYPA (DF = 1)	X^2 value	.06	.08	.001
	P value	.81	.77	.98
D7S8 (DF = 1)	X^2 value	2.73	4.88	.18
	P value	.10	.03*	.67
Gc (DF = 2)	X^2 value	1.10	1.48	.50
	P value	.58	.37	.78

*Significantly different.

HBGG (due to HWE test results, see Table 3), shows no significant difference between the Hasidic and non-Hasidic groups in allele frequency at any locus. This is not surprising, considering the origin of the Hasidic group and the fact that, over 250 years since its establishment, there has been a certain amount of gene flow into the Hasidic subpopulation from the non-Hasidic subpopulation.

A comparison of the non-Hasidic population with the FBI group shows no significant difference in allele frequency at any locus. However, there are significant differences in allele frequencies between the Hasidic and FBI groups at both the HLA-DQA1 and D7S8 loci. In the case of the HLA-DQA1 gene, it is a relatively low frequency of alleles 1.2 and 4, together with an unusually high frequency of allele 1.3 that accounts for the significant difference in the chi-square test. The 15% frequency of allele 1.3 may very well be the highest recorded of any ethnic group in the United States (15). The significant difference in allele frequency at the D7S8 locus appears to be due to a very low 52% frequency of the A allele and a very high 48% frequency of the B allele. Even more extreme allele frequency differences for this locus have been reported for a Sioux population (A = 40.6%, B = 59.4%) (16).

Significant differences in allele frequencies may reflect the chance presence of particular alleles in unusual numbers among the founding members of the Hasidic group (founder effect). Any such differences in allele frequencies could be further enlarged by the inevitable inbreeding which occurs in any endogamous group. Alternatively, it should be noted that sampling variance may explain some of the observations and should not be discounted. The results of this study have provided databases for two related subpopulations.

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