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Genetic Polymorphism of the PCR-Based Locus HLA-DQA1 in Jordanians

Mawieh Hamad*, Salem R. Yasin and Ali Elkarmi

Department of Biological Sciences, Hashemite University, Zarqa 13133, Jordan

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ABSTRACT

Genotype and allele frequencies for the PCR-based locus HLA-DQA1 were determined in a randomly selected sample population consisting of 163 unrelated Jordanians. Genotype 4.1 - 4.1 was the most common with a frequency of 0.1779, followed by genotype 1.1 - 4.1 occurring at a frequency of 0.1104. HLA-DQA1 locus genotype distribution in Jordanians was in agreement with the expectations of the Hardy-Weinberg equilibrium. The most common HLA-DQA1 allele in Jordnians was allele 4.1. The least common allele was 4.2/4.3. Distribution of the HLA-DQA1 locus in Jordanians is similar to those in other Arab populations as revealed by G test statistic *P* values. Based on these findings, the calculated P_I value of the HLA-DQA1 locus in Jordanians was 0.08200. These data could be useful for forensic analysis and paternity testing procedures in the Jordanians society.

Key words : Allele frequency, HLA-DQA1 locus, PCR, Jordanian Population.

INTRODUCTION

Since the introduction of molecular DNA typing in 1985 by Jeffreys and co-workers (Gill et al., 1985), the power of discrimination provided by molecular analysis of biological samples has greatly improved. Molecular analyses of DNA samples based on restriction fragment length polymorphism (RFLP) were initially used. However, introduction of the Polymerase Chain Reaction (PCR) DNA typing techniques has revolutionized the whole field of forensic medicine. PCR-based typing of DNA samples is becoming the procedure of choice for such purposes (Kan and Dozy, 1978; Tahir and Watson, 1995). This is especially true where minute or degraded samples are involved. The Polymarker (PM) system which incorporates several genetic markers (Baird, 1998) and the HLA-DQA1 genetic marker system (Gyllensten and Erlich, 1988) are two prime examples of polymorphic loci that have been successfully typed by PCR. Nowadays, molecular typing of these and other genetic marker systems is being used routinely by different forensic

* To whom correspondence should be addressed.
E-mail : mawieh@yahoo.com.

laboratories worldwide.

It is well accepted that molecular DNA markers yield greater power of discrimination when compared with more conventional methods for analysis of biological samples. However, determination of the allelic frequency for a specific locus or group of loci is essential for accurate estimation of the rarity of the corresponding DNA profile. Allele frequency distribution for the HLA-DQA1 locus has been reported for a number of populations (Hayes et al., 1995; Woo et al., 1995; Tahir et al., 1997). Furthermore, we have recently reported on the allele frequency distribution for the PM loci in Jordanians (Yasin et al., 1999). To the best of our knowledge, however, no population data concerning the HLA-DQA1 allelic frequency distribution in Jordanians has been reported. In this study, genetic variation of the HLA-DQA1 locus in Jordanians was studied in terms of genotype and allele frequency distribution. Furthermore, the potential to use this genetic marker for DNA typing in identity testing procedures was investigated.

MATERIALS AND METHODS

Sample collection and DNA isolation

Blood samples were collected in EDTA vacutainer tubes from 163 randomly seleted unrelated Jordanians living in different parts of the Kingdom. DNA extraction was achieved by the phenol-chloroform method described elsewhere (Budowle and Baechtel, 1990). DNA extract per sample was quantified following the method of Waye and colleagues (Waye et al., 1989). 3 ~ 5 ng of each DNA sample was used for amplification.

Amplification of the HLA-DQA1 locus

Amplification and typing of all DNA samples were carried out using the AmpliType PM + DQ α forensic DNA Amplification and Typing kit which utilizes allele-specific oligonucleotide probes plus reverse dot blot methodology (Perkin Elmer corporation, Norwalk, CT, USA). All procedures were done following the manufacturer's instructions. A 480 Perkin Elmer thermal cycler was used to carry out the PCR reaction.

Statistical analysis

Allelic frequencies were calculated directly from the observed genotype frequencies in the sample set. Expected genotypic frequencies were calculated based on the Expectations of the Hardy-Weinberg equilibrium (HWE). Possible departures from the HWE were evaluated using the Chi-Square (x^2) test statistic. Methods to calculate the power of identity (P_I) and the power of discrimination (P_D) for the HLA-DQA1 locus have been described elsewhere (National Research Council Committee on DNA Forensic Science, 1996). G-statistic homogeneity (*P value*) test (Yasin et al., 1999) was used to calculate the discrepancy in HLA-DQA1 allelic frequency in the sample population and other sample groups; a $P\ value \geq 0.05$ is considered significant.

RESULTS

In this study, genotype and allele frequency distribution for the PCR-based locus HLA-DQA1 in Jordanians were determinded. Table 1 shows the observed and expected genotype distribution of the HLA-DQA1 locus in 163 individuals. Observed heterozygosity was 0.718 compared with 0.776 expected heterozygosity. As shown in table 1, 4.1 - 4.1 was the most common HLA-DQA1 genotype in that, it occurred at a frequency of 0.1779. Genotypes 1.1 - 4.1, 2 - 4.1, 1.2 - 4.1 and 3 - 4.1 were strongly represented in the Jordanian population occurring at α frequency of 0.1104, 0.098, 0.0798 and 0.0798, respectively. It should be noted that genotypes 1.3 - 1.3, 4.2/4.3 - 4.2/4.3, 1.1 - 4.2/4.3,

Table 1. Genotype Distribution for the HLA-DQA1 Locus in a Sample Population of 163 Unrelated Jordanians

Genotype	Observed	Expected
1.1-1.1	3	2.755
1.2-1.2	6	3.668
1.3-1.3	0	0.587
2-2	3	3.195
3-3	5	2.755
4.1-4.1	29	23.537
4.2/4.3-4.2/4.3	0	0.016
1.1-1.2	6	6.357
1.1-1.3	3	2.542
1.1-2	5	5.933
1.1-3	4	5.509
1.1.-4.1	18	16.104
1.1.-4.2/4.3	0	0.424
1.2-1.3	2	2.934
1.2-2	11	6.846
1.2-3	5	6.357
1.2-4.1	13	18.582
1.2-4.2/4.3	0	0.489
1.3-2	4	2.738
1.3-3	7	2.543
1.3-4.1	4	7.433
1.3-4.2/4.3	0	0.196
2-3	2	5.933
2-4.1	16	17.343
2-4.2/4.3	0	0.456
3-4.1	13	16.104
3-4.2/4.3	1	0.424
4.1-4.2/3	3	1.234
Total	163	162.994

Observed homozygosity = 0.282; Observed heterozygosity = 0.718.
Expected homozygosity = 0.224; Expected heterozygosity = 0.776.
χ^2 at 21 df and 95% level of significance = 29.06.

1.2 - 4.2/4.3, 1.3 - 4.2/4.3 and 2 - 4.2/4.3 were not represented in the Jordanian population.

Allele frequency distribution for the HLA-DQA1 locus in Jordanians is shown in table 2. Allele 4.1 was the most frequent HLA-DQA1 allele occurring at a frequency of 0.383, followed by allele 1.2 occurring at a frequency of 0.151. Allele 4.2/4.3 was the least common HLA-DQA1 allele, it occurred at a frequency of 0.013. This was clearly reflected in the finding that genotypes with 4.2/4.3 allelic combination were very low or not represented in the Jordanian population. Based on these findings, the power of identity (P_I) for the HLA-DQA1 locus in Jordanians is 0.08200 and the power of discrimination, P_D, is 0.91800.

The x^2 for the HLA-DQA1 locus as calculated by the Chi-square test was 29.06 at 21 degrees of freedom ($P \leq 0.05$). Accordingly, no significant departures from HWE expectations were detected at the HLA-DQA1 locus in Jordanians. Furthermore, the observed to expected heterozygosity ratio for the HLA-DQA1 locus was 0.93. As shown in table 3,

Table 2. Allele Frequency Distribution for the HLA-DQA1 Locus in 163 Unrelated Jordanians

Allele	Frequency
1.1	0.129
1.2	0.151
1.3	0.060
2	0.135
3	0.129
4.1	0.383
4.2/4.3	0.013
Total	1.000

Table 3. G Test Statistic for Homogeneity (*P* value) Based on the HLA-DQA1 Allele Frequencies in Jordanians versus Other Populations

Populations Compared (Reference)	*P* value
Jordanians vs. Arabs living in Israel (Hayes et al., 1995)	0.002
Jordanians vs. Arabs living in Dubia (Tahir et al., 1997)	0.005
Jordanians vs U.S. Caucasians (Budowle, et al., 1995)	0.058*
Jardanians vs. Japanese (Perkin Elmer User Guide; 1995)	0.240*

* Significant discrepancy.

the G-statistic homogeneity test (*P* value) analysis has shown that allelic frequencies for the HLA-DQA1 locus in Jordanians are statistically similar to those reported for Israeli Arabs (Hayes et al., 1995) and Arabs living in Dubai, UAE (Tahir et al., 1997). They showed for the HLA-DQA1 locus remarkably different allele distribution from that of the Japanese population (Perkin Elmer User Guide; 1995).

DISCUSSION

As PCR-based typing of biological samples in criminal settings and paternity testing takes central stage, the necessity to establish allele frequency data banks for the world populations is becoming increasingly apparent. In this study, we have examined the allele frequency distribution for the HLA-DQA1 locus in Jordanians. In a previous study (Yasin et al., 1999), we have reported on the genetic variation of the PCR-based Polymarker (PM) genetic loci, in which the P_I was calculated to be 0.00513. Consistent with previous studies (Perkin Elmer User Guide, 1995), the combined P_I for both PM (Yasin et al., 1999) and HLA-DQA1 loci in Jordanians is 0.00042 and the combined P_D is 0.99958. In other words, inclusion of these two genetic marker systems in forensic analysis should yield acceptable levels of discrimination between Jordanian individuals taking into consideration the relatively small size of the Jordanian population; less than 5 million according to the most recent Jordanian census bureau data. Nonetheless, it must be stressed that inclusion of additional genetic marker systems is imperative should more satisfactory levels of discrimination be achieved.

Genotype distribution of the HLA-DQA1 locus in Jordanians was in agreement with the HWE. This is interesting given the fact that consanguineous and close relative marriages are still commonly practiced in the Jordanian society. It is worth mentioning that the distribution of other genetic loci in the Jordanian population was reported to be in agreement with the HWE (Saha, 1985; Yasin et al., 1999; Hamad and Awadallah, 2000). These findings suggest that although consanguineous and close relative marriages are common in the Jordanian society, they are not common enough to produce detectable genetic disequilibrium. Absence of a significant degree of discrepancy between Jordanians and other Arab population is interesting, especially in the case of Jordanians versus Arabs living in Israel. It should be noted that the ethnogenetic relationship between these two groups is very well established as the two populations used to be parts of the same population prior to the establishment of the state of Israel in 1948. Establishment of ethnogenetic relationships between Jordanians and other Arab populations requires further analysis of addi-

tional genetic loci. In conclusion, this study has established the genotype and allele frequency distribution for HLA-DQA1 locus in Jordanians. Data presented here can be used for identification purposes in forensic medicine and paternity testing disputes that may arise in the Jordanian society.

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