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Characterisation of HLA DQα for forensic purposes. Allele and genotype frequencies in British Caucasian, Afro-Caribbean and Asian populations

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Summary. Allele and genotype frequencies for British Caucasian, Afro-Caribbean and Asian populations were determined for a total of over 600 unrelated individuals at the HLA-DQα locus. These were analysed by polymerase chain reaction amplification of the DNA followed by hybridisation to allele specific oligonucleotide probes in a reversed dot-blot test. Six different alleles were detected and the allele distributions for the 3 populations analysed displayed significant differences. However, the British Caucasian genotypes were statistically very similar to previously published data from US Caucasians as were British Afro-Caribbean genotype frequencies with US Black data. In Caucasians the allele frequencies ranged from 5.2% to 26.9% with a power of discrimination of 0.93. DQα genotype frequencies of Caucasian and Afro-Caribbean populations do not deviate from Hardy-Weinberg equilibrium. However, the Asian data displayed significant deviation due to excess homozygotes.

Key words: HLA-DQα – Genotype frequencies – PCR

Zusammenfassung. Die Allel- and Genotyp-Frequenzen am HLA-DQα Locus wurden für britische Europäer, Afro-Karibier und für asiatische Bevölkerungen an mehr als 600 unverwandten Personen bestimmt. Die Bestimmung erfolgte durch Amplifikation der DNA mit Hilfe der Polymerase-Kettenreaktion, gefolgt von einer Hybridisierung mit allelspezifischen Oligonucleotid-Sonden in einem umgekehrten „Dot-Blot"-Test. Sechs verschiedene Allele wurden nachgewiesen, und die Allel-Verteilungen für die drei untersuchten Bevölkerungen läßt signifikante Unterschiede erkennen. Jedoch waren die britisch-europäischen Genotypen statistisch sehr ähnlich zu früher veröffentlichten Daten von den US-Europäern, wie auch die britischen afrokaribischen Genotyp-Frequenzen zu den Daten der US-Schwarzen sehr ähnlich sind. Bei Europäern variierten die Allel-Frequenzen von 5,2% bis 26,9%, die Diskriminationsmöglichkeit beträgt 0,93. Die DQαGenotyp-Frequenzen für europäische und afrokaribische Populationen weichen nicht vom Hardy-Weinberg-Gleichgewicht ab. Jedoch zeigen die asiatischen Daten eine signifikante Abweichung, welche durch einen Überschuß an Homozygoten bedingt ist.

Schlüsselwörter: HLA DQα – PCR – Populationsgenetik – Europäer – Afrikaner – Asiaten

Introduction

Since its introduction in 1985, DNA profiling has rapidly become a routine technique in forensic laboratories worldwide [1]. Three PCR strategies have been developed for forensic applications: dot-blot analysis of the human leucocyte antigen (HLA) DQα locus [2], amplification of variable number tandem repeat loci [3, 4], and direct sequencing of amplification products [5, 6, 7].

Dot-blot analysis of DQα, now redesignated as DQA1 [8], was developed by Cetus Corporation (Emeryville, CA, USA) [9]. This test enabled twenty-one different genotypes to be typed. Depending on the population under consideration, this corresponded to a discrimination power (DP) of approximately 0.93 [9]. Briefly, a dot-blot was prepared by hybridising (under high stringency) the amplified DQα product with allele-specific oligonucleotides (ASOs). By conjugating horseradish peroxidase (HRP) to the ASO, hybridisation could be detected non-radio-isotopically through the localized conversion of the dye tetramethylbenzidine to a blue precipitate.

This format was used successfully to amplify and type the HLA DQα locus of DNA from a single hair root [10]. Similarly, DNA sequences from single sperm and diploid cells were also amplified and analysed at the LDL receptor locus, and β-globin locus using a similar dot-blot detection system [11]. The test for HLA DQα typing was also evaluated for forensic applications [12]. This original work showed that typing was successful with DNA extracted from semen, liquid blood and hair roots, but showed that typing results could be altered by differences in the probe sensitivities, variations in hybridisation times, and in the amount of sample loaded.

An improved DQα 'reversed' dot-blot system has now been developed in which the ASO probes rather than the PCR product are immobilised on nylon strips [13].

To date, the DQα typing system has been used in over 200 cases in the USA and has been evaluated on populations worldwide [7, 14]. We report here the allele and genotype frequencies for the HLA DQα locus in British Caucasian, Afro-Caribbean and Asian (Indian subcontinent) populations and assess the applicability of the test for forensic analysis.

Materials and methods

Population and sample preparation. Blood samples were taken from unrelated individuals. These totalled 201 Caucasians (pooled from areas around Chorley, Aldermaston, Wetherby, Chepstow and Huntingdon) 202 Afro-Caribbeans (pooled predominantly from Chorely, Aldermaston and Birmingham areas) and 191 Asians originating from the Indian subcontinent (from Birmingham area). The DNA was extracted essentially as described previously [15]. Total DNA was extracted using the same method from cultures of *Escherichia coli* and *Candida albicans*. DNA samples from cat, rat, pig and dog were kindly provided by Dr. E. Naito.

DNA amplification and typing

Samples of between 2 and 100 ng were typically used for amplification. All amplification reactions and hybridisations were performed in 100–140 µl volume using components from the 'Amplitype' HLA DQα Forensic DNA Amplification and Typing Kit (Cetus Coporation, CA, USA). All conditions and parameters were as stipulated in the Amplitype User Guide (Cetus Corporation). Essentially this involved amplification of a segment of the HLA DQα locus using the biotinylated primers provided. Wells within rows A–D of a Perkin Elmer Cetus Thermal Cycler were used for amplification (as recommended by Cetus, Amplitype package insert guide p.16 note 8), with 32 programmed cycles of 94°C for 1 min, 60°C for 30s and 72°C for 30s. As a check for amplification, 5 µl of the PCR mix was electrophoresed in a 3% NuSieve agarose gel (FMC) containing ethidium bromide, and the products were visualised under UV light. Thirty-five µl of the remaining product was utilised in the subsequent hybridisation and colour development reactions.

To investigate the operational limitations of the amplification and typing procedure a DNA sample from a heterozygote 1.2, 3 was amplified with a series of cycling programs in which the annealing and extension temperatures were unchanged, but the 1 min denaturation step was varied between a programmed 94°C and 86°C. The effect on the typing result of altering the hybridisation temperature between 51°C and 59°C was also tested.

Population genetics. The expected allelic diversity values (heterozygosity) were determined according to the equation $h = [1 - \Sigma X_i^2]\,[n/n - 1]$, where h = allelic diversity, X_i = allele frequencies and n = the number of alleles [16]. The power of discrimination (PD) was determined from the genotype data using the equation $PD = 1 - \Sigma P_j^2$, where P_j is the genotype frequency [17]. Tests of significance were carried out using either chi square or G-test [18] if expected figures were low.

Results and discussion

Within ethnic group variation

Before pooling data, allelic frequencies were compared to determine whether significant differences could be observed within ethnic groups from different regions of the UK. None were observed (G-test = 16.4 (10 DF); $P = 0.07$ for Afro-Caribbean data. $G = 18.3$ (20 DF) for Caucasian data; $P = 0.6$). The analysed Asian population was from one location (Birmingham).

Variation between ethnic groups

The observed allele frequencies from this study for the 3 pooled British populations are listed in Table 1. Also included in Table 1 are data from a similar studies of US Blacks and Caucasians [14, 20]. Comparisons between different ethnic groups indicated that the ethnic distributions between the 3 British ethnic populations were significantly different from each other (chi square = 127.3 for 10 degrees of freedom; $P < 0.0001$): British Caucasian allele frequencies varied between 5.2% (allele 1.3) and 26.9% (allele A4), Afro-Caribbean varied between 4.7% (A1.3) and 32.9% (A1.2), and Asian from 8.6%

Table 1. Comparison of DQα allele distributions (%) in UK Caucasian, UK Afro-Caribbean, UK Asian, US Caucasian and US black populations

HLA-DQα Allele	Caucasian			Afro-Caribbean			Asian
	UK ($n = 402$)	USA		UK ($n = 404$)	USA		UK ($n = 382$)
		($n = 1174$) (a)	386 (b)		($n = 792$) (a)	306 (b)	
1.1	13.9	13.4	13.7	13.6	13.4	12.3	17.0
1.2	18.9	21.1	25.7	32.9	27.6	18.0	13.6
1.3	5.2	7.3	4.4	4.7	4.2	8.7	20.2
2	14.2	11.7	11.7	8.9	11.7	14.7	10.7
3	20.9	19.1	10.1	11.9	12.0	15.0	8.6
4	26.9	27.5	34.5	28.0	31.2	31.3	29.8
Chi square (10 degrees of freedom)		9.97 (ns)			12.3 (ns)		–

n = Number of alleles in population samples.
USA population data: (a) Helmuth et al. [15], (b) Comey and Budowle [20]
ns = Not significant

Table 2. Observed and expected HLA-DQα genotype frequencies (%) of British Caucasian, Afro-Caribbean, and Asian populations

HLA-DQα Genotype	Caucasian ($n = 201$)	Afro-Caribbean ($n = 202$)	Asian ($n = 191$)
1.1, 1.1	1.5 (1.9)	1.5 (1.9)	4.7 (2.9)
1.1, 1.2	5.0 (5.3)	7.9 (9.0)	6.3 (4.6)
1.1, 1.3	2.0 (1.5)	0.5 (1.3)	7.3 (6.9)
1.1, 2	5.0 (4.0)	3.0 (2.4)	3.1 (3.7)
1.1, 3	7.5 (3.2)	3.5 (3.2)	2.1 (2.9)
1.1, 4	5.5 (7.5)	9.4 (7.6)	5.8 (10.2)
1.2, 1.2	2.5 (3.6)	11.9 (10.8)	1.6 (1.9)
1.2, 1.3	2.0 (2.0)	3.5 (3.1)	6.3 (5.5)
1.2, 2	4.0 (5.4)	5.4 (5.9)	4.2 (2.9)
1.2, 3	9.0 (7.9)	8.4 (7.8)	1.6 (2.4)
1.2, 4	12.9 (10.2)	16.8 (18.4)	5.8 (8.1)
1.3, 1.3	0.0 (0.3)	0.5 (0.2)	6.3 (4.1)
1.3, 2	1.5 (1.5)	1.5 (0.8)	3.1 (4.3)
1.3, 3	2.0 (2.2)	1.0 (1.1)	2.6 (3.5)
1.3, 4	3.0 (2.8)	2.0 (2.6)	8.4 (12.0)
2, 2	1.0 (2.0)	1.5 (0.8)	2.6 (1.2)
2, 3	5.0 (5.9)	0.5 (2.1)	1.6 (1.9)
2, 4	10.9 (7.6)	4.5 (5.0)	4.2 (6.4)
3, 3	3.0 (4.4)	2.0 (1.4)	0.5 (0.7)
3, 4	12.4 (11.2)	6.4 (6.6)	8.4 (5.1)
4, 4	4.5 (7.2)	8.4 (7.8)	13.6 (8.9)
DP	0.93	0.91	0.93
Heterozygosity	0.88 (0.81)	0.74 (0.77)	0.71 (0.8)
Hardy Weinberg G (15 degrees of freedom)	15.34 (ns)	9.67 (ns)	30.05 ($P < 0.05$)

Expected values assuming the populations are in Hardy Weinberg equilibrium are given in parentheses
For Hardy Weinberg equilibrium, ns = Not significant

(A3) and 29.8% (A4). In contrast, frequencies determined for British and US Caucasians were very similar, as were frequencies for British Afro-Caribbeans compared with US Blacks (chi square showed no significant differences in both cases; Table 1).

The observed genotype frequencies and the expected frequencies based on the assumption of Hardy Weinberg equilibrium are listed in Table 2. A chi square comparison of these distributions showed no significant difference between observed and expected values for the Caucasian and Afro-Caribbean populations. Likewise the observed and expected heterozygosities for both these populations were very similar. However the Asian genotype frequencies deviated significantly from Hardy Weinberg equilibrium and the observed heterozygosity (0.71) was lower than that exptected (0.80). This finding may indicate that two or more populations are mixed (Wahlund effect) or could indicate that non-random mating is occurring within the sampled population. Another possibility could be that a 'null' allele was present (due to primer or probe mismatch) and this could be sufficiently rare (frequency = < 0.05) for a null homozygote not to be observed in the sample, yet still cause the effect.

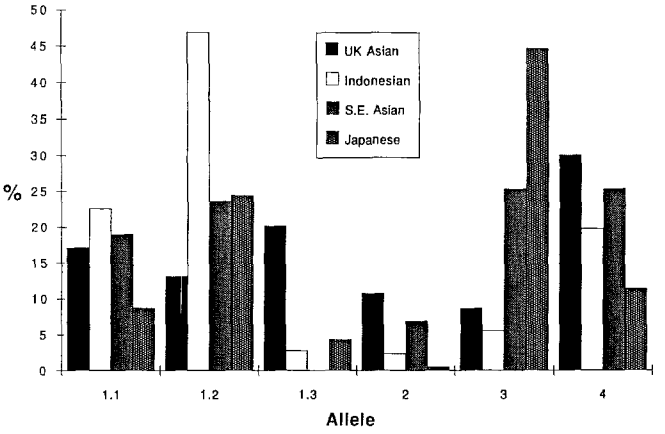

Fig. 1. Allele frequency variation of DQ alpha in Asian populations

Clearly in a forensic context it would not be valid to quote phenotype frequencies using formulae which assume Hardy Weinberg equilibrium, although frequencies could be estimated using the observed data only. Currently, it is not known whether the excess of homozygotes is peculiar to the Birmingham population or whether it is general in UK Asian populations. Helmuth et al. [14] reported slight homozygote excess (not significant) in populations from Southeast Asia (29% vs 22% expected). The excess was almost the same as that observed in the UK population, but it was noticeable that allelic frequencies from Southeast Asia, Indonesia, Japan and UK Asians (from the Indian subcontinent) were very different from each other (Fig. 1). Further work is being carried out to investigate the significance of allelic variation within Asian populations.

Amplification of non-human DNA

Samples of DNA (100 ng) from cat, rat, pig, dog and the micro-organisms *Escherichia coil* and *Candida albicans* failed to amplify (as previously shown by Comey and Budowle [20]). However, DNA from a Japanese Monkey yielded an amplification product that did not hybridise with ASOs '1, 2, 3' and '4' but did bind weakly to the positive control, '1.2, 1.3, 4' ASO, and the 'all but 1.3' ASO. These results are in accord with an earlier study [19] in which DNA from primates phylogenetically closely related to *Homo sapiens* were successfully amplified with the same primers used in this work [13], the more distantly related animals such as sheep, horse and fish were not. This indicates that likely sources of non-human DNA contamination in forensic samples will not interfere with the DQα typing result.

Robustness

Amplification of DNA from a known heterozygote under different denaturation conditions was tested at 2°C increments and did not induce preferential amplification of the 3 allele compared with the '1.2' allele between an indicated 94°C and 88°C. At 86°C neither allele amplified. Furthermore, measurement with a 'dummy' reac-

tion tube containing a thermocouple, indicated that even in the 'coolest' position within rows A–D of the thermal cycler a peak temperature of 94.0°C was achieved within the 1 min denaturation step at 94°C. Likewise no typing anomalies were observed when varying the hybridsation temperature between 54°C and 58°C. These results confirmed that the test is robust regarding these parameters and performs correctly when used according to the manufacturer's recommendations. Accordingly, care was taken to ensure that the working range of the hybridisation temperature was within the 55°C ± 1°C window suggested by Cetus (Amplitype Users guide).

In some instances where only a weak positive signal was obtained from the typing strips, some heterozygotes with a '3' allele gave a signal intensity with the '3' probe which was comparable to the signal of the positive control probe (present on every typing strip) and significantly weaker than for probes '1', '2' and '4'. Scoring of these samples was reserved since the positive control 'C' probe defines the minimum scorable signal which should be observed. Cetus Corporation subsequently provided a modified '3' probe which had been lengthened from 19 to 20 bases: an additional T at the 3′ end of the 19-mer TTCCGCAGATTTAGAAGAT. This significantly improved the signal of the 3 probe relative to the positive control and confirmed in every instance that the aforementioned samples were true heterozygotes containing type '3' alleles.

This study has indicated that the Amplitype™ test is both robust and reliable in the typing of human DNA samples. The main advantages of a PCR based system compared with DNA profiling are: speed, senstivity and the ability to type degraded DNA.

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