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REVIEW ARTICLE

P. Stringer · C. M. Triggs · L. C. Baldwin · L. M. Melia
M. G. Savill

Distribution of HLA DQA.1 alleles in New Zealand Caucasian, Maori and Pacific Islander populations

Comparison with other population studies

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Abstract Allele and genotype frequencies for the HLA DQA.1 locus were determined for 127 unrelated Caucasians, 177 unrelated Maori and 98 unrelated Pacific Islanders from the New Zealand population. DNA from blood cells was analysed by polymerase chain reaction amplification of DNA followed by hybridization to allele specific oligonucleotide probes in a reverse dot-blot test. Allele frequencies at the HLA DQA.1 locus for New Zealand Caucasians, Maori and Pacific Islanders were compared with published data for other populations. The distribution of HLA DQA.1 genotype frequencies did not deviate from Hardy Weinberg expectations for the Caucasian and Maori populations. The power of discrimination was 0.93 for Caucasians and 0.86 for Maori. The total Pacific Islander population tested was analysed as was data obtained from Western Polynesians contained within that larger group. Both the total Pacific Islander group analysed, and the Western Polynesians contained within that larger group, failed Hardy Weinberg expectations for the distribution of HLA DQA.1 genotypes. This significant deviation was due to excess homozygotes. The power of discrimination for the total Pacific Islander group and for Western Polynesians was 0.86 and 0.85 respectively. Comparison of Caucasian population studies from New Zealand, the United Kingdom, South Australia, Norway, the United States and Sweden showed these populations have similar HLA DQA.1 allele frequency distributions. Maori and Pacific Islanders have HLA DQA.1 allele frequency distributions that are more similar to each other than any of the other populations studied.

Key words HLA DQA.1 · Forensic DNA Typing · New Zealand population · Polymerase chain reaction (PCR) · Genotype frequencies

P. Stringer (✉)
Institute of Environmental Science and Research Limited,
Wellington Science Centre, PO Box 30547, Lower Hutt,
New Zealand

C. M. Triggs
Department of Statitics,
School of Mathematical and Information Sciences,
The University of Auckland, Private Bag 92019, Auckland,
New Zealand

L. C. Baldwin · M. G. Savill
Institute of Environmental Science and Research Limited,
Christchurch Science Centre, PO Box 29 181, Christchurch,
New Zealand

L. M. Melia
Institute of Environmental Science and Research Limited,
Mt Albert Science Centre, Private Bag 92021, Auckland,
New Zealand

Introduction

The polymerase chain reaction (PCR) is increasingly being used in many laboratories throughout the world. New Zealand forensic laboratories have introduced PCR technology into casework analyses and are now routinely using the human leucocyte antigen (HLA) DQA.1 locus for identity testing for forensic purposes.

The location of the HLA DQA.1 locus in the human genome and its function has been well documented [1–3]. Thirteen alleles have been identified in the HLA DQA1 locus [4], 6 of which can be distinguished with the method applied here [5].

The New Zealand population consists of a variety of ethnic groups but is predominantly composed of Caucasians (73.2%), Maori (12.3%), Pacific Islanders (3.9%) and others (10.6%) [6]. This paper is the first study that presents data on the frequencies of the 6 detectable HLA DQA.1 alleles and the distribution of the different genotypes in samples taken from the pincipal ethnic groups present in the New Zealand population (Caucasians, Maori and Pacific Islanders).

This data was also compared with published data for HLA DQA.1 allele frequency distributions for other populations.

Table 1 Distribution of HLA DQA.1 alleles in New Zealand Caucasian, Maori, Pacific Islander* and Western Polynesian populations

DQA.1 Allele	Caucasian		Maori		Pacific Islander*		Western Polynesian	
	n	Frequency (%)	*n*	Frequency (%)	*n*	Frequency (%)	*n*	Frequency (%)
0101	35	13.8	45	12.7	17	8.7	8	6.6
0102	52	20.5	20	5.7	10	5.1	8	6.6
0103	12	4.7	31	8.8	26	13.3	20	16.4
0201	38	15.0	9	2.5	2	1.0	1	0.8
0301	45	17.7	85	24.0	85	43.4	55	45.1
0401 & 0501 & 0601	72	28.3	164	46.3	56	28.6	30	24.6
Total	254		354		196		122	

*This is all Pacific Islanders surveyed (including Western Polynesians)

Materials and methods

Population samples

Blood was obtained from 127 unrelated Caucasian, 177 unrelated Maori and 98 unrelated Pacific Islander donors and from casework blood samples. Ethnic origin was determined by self declaration.

The 98 Pacific Islanders comprised individuals with the following self-declared ethnic origins; 5 Pacific Islanders, 1 Western Samoan, 40 Samoans, 4 Cook Islanders, 17 Polynesians, 12 Tongans, 1 Niuean/Tongan, 7 Niueans, and 11 Rarotongans.

Western Polynesians were separated out from the total Pacific Islander group and analysed separately. Groups that were included in the Western Polynesian category consisted of Samoans, Western Somoans, Tongans, Niueans and Niuean/Tongans.

"Pacific Islanders" and "Polynesians" were not inlcuded in the Western Polynesian group for analysis as their actual origin was unknown except for this descriptor.

DNA extraction

Samples were processed either as whole blood or blood stains.

DNA was obtained from 3 µl whole blood or 2 mm^2 blood stains by Chelex extraction [5].

Amplification and typing of the HLA DQA.1 locus

DNA (10 µl) extracted by the chelex method [5] was added to each amplification reaction. Amplification and typing reactions were performed by strictly following the recommended protocol [5] and using the commercially available Cetus Amplitype HLA DQα Forensic DNA Amplification and Typing Kit. This kit does not directly detect the DQA1*0501 and DQA1*0601 alleles.

Statistical analysis

Allele frequencies for each population were calculated from genotype numbers.

The power of discrimination was calculated from genotypic data using the formula $PD = 1 - \Sigma P_j^2$ where P_j = observed frequency of each genotype [17].

An estimate of heterozygosity (allelic diversity) was calculated from the equation $h = N(1 - \Sigma P^2)/N - 1$ where P = observed frequency of each allele and N = number of allele observations [8].

Results and discussion

Data obtained for Pacific Islanders has been analysed and include data from any non-Maori person of Polynesian origin. Data for Western Polynesians has been extracted from this larger group and analysed separately.

Western Polynesians have been described as those originating from Samoa and Tonga [9] and associated islands.

Allele and genotype frequencies

The observed allele frequencies for New Zealand Maori, Caucasians, and Pacific Islanders and Western Polynesians are presented in Table 1.

The most common allele in Caucasians was HLA DQA1 allele 4 (*0401, *0501 and *0601) at 28.3%, and the least frequent allele was HLA DQA1*0103 at 4.7%. This has also been observed in other Caucasian populations [1, 10, 11].

The most common genotype was HLA DQA1*0102/*(0401,0501,0601) (also observed in [1]). Genotype HLA DQA1*0103/*0301 was not observed.

Amongst New Zealand Maori the most common allele was also HLA DQA1 allele 4 (*0401,*0501,*0601) and the least frequent allele was HLA DQA1 *0201.

The most common genotypes were HLA DQA1 *0301/*(0401,0501,0601) and HLA DQA1*(0401,0501,0601)/*(401,0501,0601), both at 23.2%. HLA DQA1 *0201/*0201 was not observed at all.

The most common allele in all Pacific Islanders, and in Western Polynesians was HLA DQA1*0301, at 43.4% and 45.1% respectively.

Similar to Maori, the least common allele in all Pacific Islanders and Western Polynesians was HLA DQA1*0201 at 1% and 0.8% respectively.

The most common genotype in both groups was HLA DQA1*0301/*0301 (23.5% for all Pacific Islanders and 27.9% for Western Polynesians).

In all Pacific Islanders the following genotypes were not observed; HLA DQA1*0101/*0101, *0101/*0102,

Table 2 Observed HLA DQA.1 genotype frequencies, power of discrimination, allelic diversity, observed heterozygosity and p values for New Zealand Caucasian, Maori, Pacific Islander and Western Polynesian populations. Pacific Islanders relates to all Pacific Islanders surveyed (including Western Polynesians), Western Polynesians relates to only Samoans, Western Samoans, Tongans, Niueans, Niuean/Tongans. Standard errors for power of discrimination and allelic diversity [8] are given in brackets ()

HLA DQα Genotype	Caucasian (n = 127)		Maori (n = 177)		Pacific Islanders (n = 98)		Western Polynesians (n = 61)	
	Observed N	(%)	Observed N	(%)	Observed N	(%)	Observed N	(%)
DQA1*0101	3	2.4	4	2.3	0	0	0	0
DQA1*0101/*0102	3	2.4	3	1.7	0	0	0	0
DQA1*0101/*0103	1	0.8	3	1.7	2	2.0	0	0
DQA1*0101/*0201	4	3.1	1	0.6	0	0	0	0
DQA1*0101/*0301	11	8.7	15	8.5	14	14.3	8	13.1
DQA1*0101/*0401 DQA1*0101/*0501 DQA1*0101/*0601	10	7.9	15	8.5	1	1.0	0	0
DQA1*0102	4	3.1	1	0.6	0	0	0	0
DQA1*0102/*0103	2	1.6	5	2.8	2	2.0	2	3.3
DQA1*0102/*0201	10	7.9	0	0	0	0	0	0
DQA1*0102/*0301	11	8.7	3	1.7	6	6.1	4	6.6
DQA1*0102/*0401 DQA1*0102/*0501 DQA1*0102/*0601	18	14.2	7	4.0	2	2.0	2	3.3
DQA1*0103	1	0.8	1	0.6	4	4.1	4	6.6
DQA1*0103/*0201	3	2.4	0	0	1	1.0	1	1.6
DQA1*0103/*0301	0	0	7	4.0	6	6.1	4	6.6
DQA1*0103/*0401 DQA1*0103/*0501 DQA1*0103/*0601	4	3.1	14	7.9	7	7.1	5	8.2
DQA1*0201	4	3.1	0	0	0	0	0	0
DQA1*0201/*0301	2	1.6	3	1.7	0	0	0	0
DQA1*0201/*0401 DQA1*0201/*0501 DQA1*0201/*0601	11	8.7	5	2.8	1	1.0	0	0
DQA1*0301	6	4.7	8	4.5	23	23.5	17	27.9
DQA1*0301/*0401 DQA1*0301/*0501 DQA1*0301/*0601	9	7.1	41	23.2	13	13.3	5	8.2
DQA1*0401 DQA1*0401/*0501 DQA1*0401/*0601 DQA1*0501 DQA1*0501/*0601 DQA1*0601	10	7.9	41	23.2	16	16.3	9	14.8
Power of Discrimination (PD)	0.92 (0.007)		0.86 (0.016)		0.86 (0.014)		0.85 (0.024)	
Allelic diversity (h)	0.81 (0.009)		0.70 (0.017)		0.71 (0.020)		0.71 (0.027)	
Observed heterozygosity	0.78		0.69		0.56		0.51	
p-values for exact test for Hardy Weinberg equilibrium [12]	0.102		0.496		0.00012		0.0003	

*0101/*0201, *0102/*0102, *0102/*0201, *0201/*0201, and *0201/*0301.

In Western Polynesians the following genotypes were not observed; HLA DQA1 *0101/*0101, *0101/*0102, *0101/*0103, *0101/*0201, *0101/*(0401,0501,0601), *0102/*0102, *0102/*0201, *0201/*0201, *0201/*0301 and *0201/*(0401,0501,0601).

The fact that so many genotypes in all Pacific Islanders and Western Polynesians were not observed may simply be a function of the small number of individuals surveyed.

Table 3 Comparison of recorded populations for the HLA DQA.1 System. Chi squared statistics: 5% point 11.07, 1% point 15.07, 0.1% point 20.52. Data obtained from published reports: UK Caucasians [10], South Australian Caucasians (Sth. Aust. C) [14], Norway [15], US Caucasians [11], South Sweden (S. Sweden) [16], Mid Sweden (M. Sweden) [16], German [20], Finnish [2], Dutch [1], Galicia (Portugal) [19], Spain [18], Coimbra (Portugal) [19], North Italy (Nth. Italy) [17], Italians [3], Japan [21]

	NZ Cauc	UK Cauc	STh. Aust. C	Norway	US Cauc	S Sweden	M Sweden	German	Finnish	Dutch	Galicia	Spain	Coimbra	Nth Italy	Italian	Pacific Isl.	Maori	Japan
NZ Cauc	0.0																	
Uk Cauc	1.3	0.0																
Sth. Aust. C	2.4	4.7	0.0															
Norway	2.6	3.1	2.5	0.0														
US Cauc	7.0	6.5	8.2	3.0	0.0													
S Sweden	11.5	11.2	21.0	7.1	10.1	0.0												
M Sweden	12.6	9.8	15.0	7.7	6.1	3.8	0.0											
German	6.1	11.0	7.3	6.0	8.9	15.5	15.2	0.0										
Finnish	12.3	13.8	13.0	10.8	8.6	13.7	8.4	4.9	0.0									
Dutch	12.7	18.3	11.2	12.2	15.0	29.8	19.4	5.4	7.8	0.0								
Galicia	19.8	30.5	21.9	22.3	26.6	42.1	33.2	7.0	11.5	4.6	0.0							
Spain	14.0	19.8	15.0	17.4	18.1	32.0	26.2	8.3	11.9	8.1	3.7	0.0						
Coimbra	14.7	16.5	13.2	15.8	13.3	30.5	19.0	15.2	14.2	10.9	15.4	5.9	0.0					
Nth. Italy	21.0	36.7	35.9	35.3	52.3	62.5	52.6	17.9	26.8	23.0	13.2	6.1	24.7	0.0				
Italians	20.6	29.3	22.7	26.5	34.0	46.5	44.2	15.1	25.9	14.4	8.5	10.6	25.8	7.3	0.0			
Pacific Isl.	80.4	78.1	106.8	78.9	79.2	63.3	40.2	92.6	59.9	96.3	116.8	79.4	59.5	149.2	123.2	0.0		
Maori	75.5	79.5	102.2	83.9	82.1	77.7	49.4	74.3	43.6	81.1	80.9	50.0	45.1	91.0	96.9	31.1	0.0	
Japan	122.4	130.9	164.0	108.3	125.9	92.4	45.6	118.0	72.4	110.8	140.2	125.0	106.5	227.4	159.7	53.2	135.1	0.0

Hardy-Weinberg equilibrium

The observed numbers of genotypes obtained from the population survey are shown in Table 2. The values for power of discrimination, allelic diversity and observed heterozygosity are also shown.

As noted above some alleles are very rare in some of the populations considered and hence some genotypes are extremely rare. This can cause problems in that the distribution of the statistic to test for Hardy-Weinberg equilibrium may not follow the chi-sqaured distribution if several genotypes have expected numbers less than 5. The exact combinatorial test for Hardy-Weinberg equilibrium was used using the method of Guo and Thompson [12].

It can be concluded that the Pacific Islander and Western Polynesian populations are not in Hardy-Weinberg equilibrium (p-values for the exact test 0.00012 and 0.0003 respectively). This is due to the high number of *0301 and *0401 homozygotes observed. For these 2 populations it would not be valid in forensic work to quote genotype frequencies calculated under the assumption of Hardy-Weinberg equilibrium.

An excess of homozygotes has also been observed in some other populations (UK Asians [10], Hispanic and South East Asians [11]). There are a number of factors which may be responsible for this; non-random mating in the sampled population, mixing of populations (Wahlund effect [13]), or the presence of a "null"-allele.

The data suggests that the New Zealand Caucasian and Maori populations are in Hardy-Weinberg equilibrium (p-values for the exact test 0.102 and 0.496 respectively), and hence genotype frequencies can be estimated from allele frequencies. In populations in Hardy-Weinberg equilibrium, allelic diversity should be equivalent to the frequency of observed heterozygotes, as observed (see Table 2). Since no departures from Hardy-Weinberg equilibrium could be detected, the effects of population substructure on Hardy-Weinberg estimates, although potentially small, would be minimal.

Comparisons of population studies

New Zealand data for the HLA DQA.1 system was compared with published data for several populations [1–3, 10, 11, 14–21]. Chi-squared statistics were computed for each pair of populations (Table 3). This data formed the input to a hierarchical cluster analysis using average linkage and the chi-squared metric [22]. The resulting clusters are shown in the dendrogram in Fig. 1.

We see that the Caucasian populations from New Zealand, the United Kingdom, South Australia, Norway, the United States, and Sweden are tightly clustered and cannot be distinguished on the basis of allele frequencies.

The Caucasian populations from Galicia, Spain, the Netherlands, Germany and Finland are also tightly clustered, with that from Portugal distinguishable at the 5% but not at the 1% level of significance.

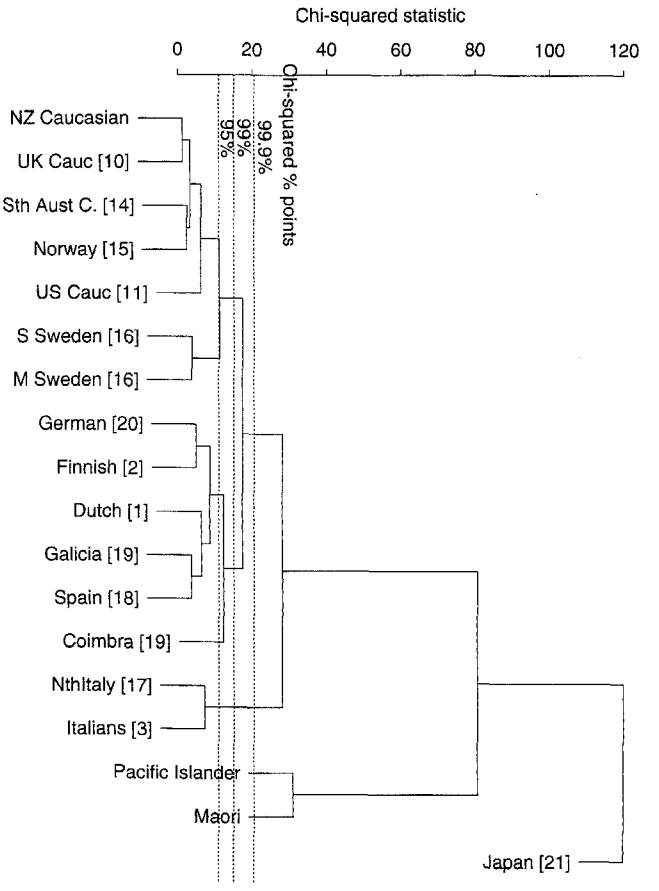

Fig. 1 Clustering Tree: Chi-squared distance, average linkage

The two populations from Italy cannot be distinguished from each other. Maori and Pacific Islanders are more similar to each other than to any other population. The Japanese are very different from all the populations in the study.

In conclusion we have established population databases of the HLA DQA1 locus for forensic use. The New Zealand Caucasian and Maori databases do not deviate from Hardy-Weinberg equilibrium. Pacific Islander and Western Polynesian data displayed significant deviation due to excess homozygotes.

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