

HLA POLYMORPHISMS IN SOUTHERN AFRICAN POPULATIONS

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INTRODUCTION

HLA is a powerful tool, as it is the most polymorphic genetic system known in man, determined by heredity in a known way, unchangeable during the life of an individual and observable by relatively simple blood tests. The knowledge of HLA polymorphisms may be applied to the study of the relationship between populations. The proportions of any particular set of antigens and those of the genes on which they depend, tend to remain constant from generation to generation, and any changes due to mutation, natural selection, and genetic drift are likely to be very slow. However, populations separated for many generations, such as the South African (SA) Negroes, the Caucasoids and the San, show variations in the frequencies of their HLA alleles and haplotypes.

The three major populations of the world are the Negroids, Mongoloids and Caucasoids. In this study the Negroids are represented by the Southern African Negroes (Xhosa) and the San. The SA Caucasoids belong to the Caucasoid group and the Cape Coloureds are a hybrid population with both Negroid and Caucasoid characteristics as well as some minor Mongoloid features.

The tremendous polymorphism of the HLA system and the low frequencies of the phenotypes make it extremely useful in solving problems of disputed parentage. It is, however, imperative that reliable gene frequencies exist for the population of the individual concerned. It is equally important that the investigator is familiar with the problems encountered in defining antigens in the population group to which a particular individual in a case of disputed parentage belongs.

The HLA-A,B,C and DR phenotypes of SA Caucasoids, Cape Coloureds, Xhosa and San were determined, and a comparison was drawn between their gene and haplotype frequencies. We also looked at the definition of some of the splits of the HLA antigens in these populations.

POPULATIONS STUDIED

a) South African Caucasoids

The SA Caucasoids, numbering 4 576 690 (1985 Census) originated from a relatively small genetic pool. Their ancestors were mainly the Dutch, who were the first settlers in 1652, with a substantial contribution from the German and French until 1700. The British immigrants arrived mostly in the 19th century. It has also been shown that SA Caucasoids have a further admixture of approximately seven percent Southern African and Asian genes (Botha et al. 1975).

b) Cape Coloureds

The Cape Coloureds, who now number over 2 825 094 (1985 Census), form an anthropologically distinct population group whose relatively recent origin is the result of early gene mixing at the Cape. They possess Western European, Southern African and South-East Asian genes, but in significantly different proportions from those seen in the SA Caucasoids. The indigenous Southern African component was derived mainly from the Khoikhoi (Hottentot) and to a lesser extent, from the San (Bushmen), as well as the Negro slaves who originated from Madagascar, Portuguese East Africa and the Gold Coast. Most of the South-East Asian genetic contribution came from the islands of the Dutch East Indies, Ceylon, and the shores and countries adjoining old India. The Caucasoid contribution was mainly from Western European settlers. The Cape Coloureds are therefore a classic example of a hybrid race of recent origin.

c) South African Negroes (Xhosa)

The Bantu-speaking Negroes of Southern Africa are divided into tribes. The two largest are the Xhosa and Zulu, each consisting of approximately 6 million, and they account for more than 50 percent of the Negro people of South Africa. From our own HLA data the Xhosa were shown to be representative of the majority of the other Southern African Negro tribes. The Xhosa belong to the Cape Nguni linguistic division of South-Eastern Bantu, and they have occupied the Transkei area in the south and south-east of the country for hundreds of years. There is evidence that Bantu-speaking Negroes crossed the Zambezi and journeyed south in a number of waves, between 500 and 1500 A.D. (Dart 1937). In 1652, when the first Dutch arrived at the Cape, they were still confined to the Transkei. Of all the Southern African Negroes the Xhosa are presumed to have had the greatest contact with the Khoikhoi and San.

d) San (Bushmen)

The present day San are descended from people of the later Stone Age living in Southern Africa as long ago as 10,000 years. They can be distinguished from the Southern African Negroes because of their shorter stature, more slender build, lighter skin colour, flatter faces and frequent steatopygia. There are also cultural,

linguistic, blood group and serological differences. However, it is virtually certain that the San share a common ancestry with the Negroes but have been separated for many centuries. Judging by the occurrence of their rock art, together with archeological and skeletal evidence, the San were spread over most of Southern Africa and are thought to have been the earliest known inhabitants. There are estimated to be 55,000 San still surviving, with about 15,000 in Namibia. They have had little contact with Caucasoids, and until recently lived as hunter-gatherers, like all human beings throughout the Pleistocene Age. The Negro influence has been limited mainly to a patron-client relationship.

RESULTS AND DISCUSSION

All the well recognized **HLA-A** locus antigens tested were present in SA Caucasoids, Cape Coloureds and SA Negroes, although striking variations in frequencies were seen (Table 1). The only HLA-A antigen not present in SA Caucasoids was the negroid' antigen Th. In the San, HLA-A11, A31, Aw34 and A25 were absent.

The classic Southern African Negro **HLA-B** antigens, Bw70 and Bw42 had their highest frequencies in the Xhosa, while Bw58 was highest in the San (Table 1). Although B8 has often been called a Caucasoid antigen (gf= 0.088), it was the most common in the San (gf=0.120) and also reached a relatively high frequency in the SA Negroes (gf=0.062). HLA-B27 was extremely rare in the Xhosa, present in only 3 of 1027 individuals, and absent in the San (Table 1).

Table 1 also shows the significantly different **HLA-C** gene frequencies in the different population groups. A feature in the Southern African Negroes and the San was the high frequency of Cw2 and Cw6 when compared to the SA Caucasoids and Cape Coloureds ($p<0.001$). HLA-Cw1 was rare in the Xhosa (gf=0.002) and absent in the San.

The commonest **HLA-DR** antigen in the SA Negroes was DR3 (gf=0.238) (Table 1), which occurs far more frequently than reported for all the race groups studied during the Eighth and Ninth International Histocompatibility Workshops (Baur and Danilovs 1980, Baur et al. 1984). The San have the highest frequency of DR4 (gf=0.241) which has a relatively low frequency in the Xhosa (gf=0.069).

When the HLA-A,B,C, and DR frequencies of the San and the Xhosa were compared, a typical "Southern African" pattern emerged (Table 2). This is best exemplified by the high frequencies of A30, Bw58, Cw6 and DRw6 and the virtual absence of A11, B27, Cw1 and DRw8. Most of the so-called classic Negro antigens reach their highest frequencies in the San, thus supporting the suggestion (Nurse et al. 1983; Mourant 1985) that the San share a common ancestry with, and may be even more "African" than the Negroes. On the other hand the San must have evolved for a considerable time in relative isolation without much contact with

Table 1. Examples of significantly different HLA-A,B,C and DR gene frequencies in Cape Coloureds, Xhosa and San compared with SA Caucasoids.

	SA Caucasoids	Cape Coloureds	Xhosa	San
Increased	n=1059	n=3716	n=1027	n=289
A23	.021	.059	.080	.120
A28	.050	.082	.140	.100
A30	.026	.096	.182	.181
Aw34	.006	.026	.035	0 ^a
Aw43	.002	.028	.030	.106
Th	0	.019	.044	.018
Bw42	.002	.032	.113	0 ^a
B45	.010	.027	.050	.002 ^a
Bw57	.031	.043	.054	.021 ^a
Bw58	.011	.086	.163	.292
Bw70	.012	.081	.158	.140
Cw2	.055	.087	.125	.148
Cw6	.098	.171	.219	.327
	n=365	n=549	n=322	n=195
DR3	.096	.110 ^a	.238	.054 ^a
DRw6	.082	.109 ^a	.181	.231
Decreased	n=1059	n=3716	n=1027	n=289
A1	.142	.086	.035	.026
A2	.269	.171	.130	.136
A3	.158	.077	.070	.144 ^a
A11	.060	.065 ^a	.002	0
A31	.023	.011	.002	0
B27	.040	.023	.002	0
B35	.083	.059	.021	0
B44	.150	.088	.065	.046
Bw60	.050	.023	0	0
Bw61	.012	.024 ^a	.002	0
Bw62	.081	.045	.005	.028
Cw1	.032	.017	.002	0
Cw3	.140	.082	.067	.032
Cw5	.075	.019	.004	0
	n=365	n=549	n=322	n=195
DR1	.106	.057	.043	.149 ^a
DR4	.152	.113	.069	.241 ^a
DR7	.132	.135 ^a	.074	.100
DRw8	.032	.027 ^a	.006	.008

^a Exception to the observed trend.

Table 2. The Typical "Southern African" pattern for the HLA-A,B, C and DR loci.

	San n=289 af	Xhosa n=1027 af
<u>Common</u>		
A28	.190	.276
A30	.329	.331
A23	.225	.154
Aw43	.201	.058
Th	.035	.086
Bw58	.498	.299
Bw70	.260	.291
Cw2	.273	.234
Cw6	.547	.391
	<u>n=195</u>	<u>n=322</u>
DRw6	.441	.329
<u>Rare</u>		
A1	.052	.068
A11	0	.003
A25	0	.001
B27	0	.003
Bw55	0	0
Bw56	0	0
Bw60	0	0
Bw61	0	.003
Cw1	0	.003
Cw5	0	.009
	<u>n=195</u>	<u>n=322</u>
DRw8	.015	.012

Table 3. Some HLA antigen frequencies which distinguish the San from the Xhosa.

	San n=289 af	Xhosa n=1027 af
<u>Increased</u>		
A3	.266	.135
Aw43	.201	.058
B8	.225	.120
Bw41	.170	.031
Cw4	.291	.194
Cw6	.547	.391
	<u>n=195</u>	<u>n=322</u>
DR1	.287	.084
DR4	.441	.134
DRw9	.154	.016
<u>Decreased</u>		
A26	.004	.123
Bw42	0	.213
Cw3	.067	.133
	<u>n=195</u>	<u>n=322</u>
DR2	.072	.217
DR3	.108	.419
DR5	.133	.319

the Negroes, accounting for the differences in HLA frequencies that exist between them. The differences in these two populations that were originally closely related, may be due to genetic drift and the selective advantage of particular HLA alleles. Some of the principal HLA features distinguishing the San from the Xhosa were that the classical SA Negro antigen Bw42 was absent in the San while Aw43, B8, Bw41, Cw6 and DR4 were more frequent in this group (Table 3).

Some of the most common HLA-A,B and B,DR haplotypes are shown in Table 4. The HLA-A,B and B,DR haplotypes that were in strong positive linkage disequilibrium in the SA Caucasoids were the same as in the European Caucasoids (Baur et al. 1984). Different HLA-A,B haplotypes were seen in positive linkage disequilibrium in the Xhosa, San and SA Caucasoids. The Cape Coloureds, on the

other hand, shared haplotypes such as HLA-A1,B8 and A3,B7 with the SA Caucasoids and A30,Bw42 with the Xhosa. Two HLA-B,DR haplotypes, B7,DR2 and B8,DR3 were in strong positive linkage disequilibrium in the Xhosa, Cape Coloureds and the SA Caucasoids. The four most common HLA-B,DR haplotypes in the San, however, were different from those seen in the other three populations studied.

Table 4. Haplotype frequencies (HF) and linkage disequilibrium (Δ) of significant HLA-A,B and B,DR associations ($p < 0.001$), in SA Caucasoids (SA Cauc), Cape Coloureds (Cape Col), Xhosa and San.

	Haplotype			HF	Haplotype			HF
	A	B	Δ		B	DR	Δ	
SA	1	8	.057	.066	8	3	.056	.059
Cauc	3	7	.048	.071	7	2	.047	.067
	2	44	.018	.058	35	1	.029	.038
					62	4	.024	.035
Cape	30	42	.016	.019	8	3	.025	.028
Col	1	8	.017	.021	7	2	.023	.042
	3	7	.010	.019	57	7	.021	.026
					42	3	.017	.018
Xhosa	30	42	.039	.058	42	3	.051	.076
	28	70	.034	.056	7	2	.043	.052
	2	45	.023	.028	8	3	.023	.035
					45	1	.018	.017
San	43	7	.048	.054	7	6	.046	.074
	23	58	.047	.072	8	4	.038	.063
	2	8	.041	.051	70	4	.034	.068
	24	58	.030	.034	58	5	.027	.046

Whereas HLA antigens in Caucasoids appear to be well defined, **new variants** are still being discovered in the Negroid and Mongoloid populations. One of the classic examples of an HLA-A variant apparently confined to Southern Africa is HLA-Aw43, originally defined in the San (du Toit et al. 1984). A split of Aw43, recently detected in a small group of San, was also seen in the Cape Coloureds. The antigen Th is another example of an HLA-A locus antigen virtually confined to the Negroid populations. Among the HLA-B alleles, the B15 complex still has to be unravelled. We have described the B15 variant B15Kemp (Campbell et al. 1983a) in Cape Coloureds and recent studies in our laboratory suggest further splits of B15 in the Xhosa. A split of the Bw22 complex, 621-CT, was defined in the Xhosa (Campbell et al. 1983b). During the Ninth International Histocompatibility Workshop, the division of Cw3 into Cw3.1 and Cw3.2 suggested by Chandanayingyong et al. (1981) was confirmed in our laboratory (Campbell and du Toit 1983). In the Xhosa only the Cw3.2 variant was present whereas in the SA Caucasoids Cw3.1 was most frequent. An additional complication in the definition of Cw3 was the segregation of Cw1 and Cw3.2 on the same haplotype in association with Bw46. Two HLA-DR2 splits have been observed in our

laboratory, DR2s and DR2LUM (du Toit et al. 1984), the former in Cape Coloureds and the latter in the Xhosa.

A striking feature of the **genetic distances** (Table 5), calculated by the method of Cavalli-Sforza and Bodmer (1971), was the extreme dissimilarity (0.024) between the San and the Xhosa, the other African group studied. This was in spite of shared "African" genes and of the more recent gene flow between the San and Xhosa, predominantly from the former to the latter. The genetic distance measurement between the Cape Coloureds and the San was high (0.029) confirming that gene flow has been minimal between these two groups. The low values obtained between the Xhosa and the Cape Coloureds (0.012) as well as the Cape Coloureds and SA Caucasoids (0.010) suggested a close relationship between these respective populations. The genetic distances between the Xhosa and the SA Caucasoids, and the Caucasoids studied during the Ninth Histocompatibility Workshop, as shown in Table 5, were 0.033 and 0.034 respectively, both values considerably lower than that between the San and Caucasoids (0.050). This may indicate closer genetic links between the Caucasoids and Negroes, and is also in keeping with the view that the San must have evolved for a considerable time in relative isolation (Nurse et al. 1985).

Table 5. Genetic distances were calculated from the gene frequencies for the total number of antigens at the HLA-A, B,C and DR loci for the Xhosa, Cape Coloureds (Cape Col), S A Caucasoids (SA Cauc), San and Caucasoids from the Ninth Histocompatibility Workshop¹ (9WS Cauc).

Xhosa	0				
Cape Col	.012	0			
San	.024	.029	0		
SA Cauc	.033	.010	.047	0	
9WS Cauc	.034	.011	.050	.002	0
	Xhosa	Cape Col	San	SA Cauc	9WS Cauc

1 Baur et al. (1984)

HLA and Disputed Parentage

In an analysis of 3662 cases of disputed parentage, the HLA system alone excluded 98.08 percent of the SA Caucasoid, 95.68 percent of the Cape Coloured and 93.04 percent of the Xhosa non-fathers. In all three population groups, a significantly larger number of cases were excluded as a result of the B locus alone compared to the A locus. Between 60 and 70 percent of the HLA exclusions were based on both HLA-A and B locus alleles being different in the alleged father.

We used two theoretical child-mother-alleged father trios (Table 6) to assess the Paternity Index (PI) and the likelihood of paternity (W) (Essen-Möller 1938; Pohl 1982). A large variation was observed when the frequencies of the four different populations were used in these calculations. In example 1 given in Table 6, we used classic Xhosa phenotypes and in example 2 classic Caucasoid phenotypes. The results clearly illustrate the importance of using the appropriate population frequencies. It is also interesting to note that in the Cape Coloureds, a hybrid population, where the HLA haplotype frequencies are all relatively low, even for the most common haplotypes, the PI and W values never reached the same levels as in the established populations.

These examples stress the necessity of the use of local gene frequencies, based upon reliable data. Bias due to insufficient knowledge of the HLA system and inadequate HLA gene frequencies may have an important effect upon the inclusionary estimates of the system.

Table 6. Two examples of the calculations of the PI and W (%) values in the four Southern African populations reported, using the phenotype frequencies of these populations.

	Example 1		Example 2	
Alleged Father	A1,3	B7,8	A30,28	Bw42,w70
Child	A1,X	B8,Y	A30,X	Bw42,Y
Mother	A24,X	B27,Y	A23,X	B45,Y
Population	PI	W(%)	PI	W(%)
SA Caucasoids	7.18	87.78	547.56	99.82
Cape Coloureds	21.80	95.61	27.17	96.45
Xhosa	46.89	97.91	7.46	88.18
San	881.58	99.89	2,176.79	99.95

CONCLUSION

The HLA haplotype and gene frequencies in the SA Caucasoid group are essentially the same as in European Caucasoids. However, it is clear that a certain amount of inter-population gene exchange has occurred in Southern Africa as is evident by the presence of the unique Khoisan allele Aw43 in SA Caucasoids.

The Cape Coloureds now have HLA frequencies that are unique. In some instances the phenotype frequencies are closer to the Caucasoids e.g. HLA-A11 is present in 12 percent of SA Caucasoids and Cape Coloureds, but only 0.3 percent of the Xhosa. In other instances the frequencies approximate the Negro frequencies e.g. Bw58 which is present in 16 percent of Cape Coloureds, 30 percent of Xhosa and virtually absent from SA Caucasoids. It should be noted that Bw48, said to be characteristic of early Mongoloid

cultures, is only present in the Cape Coloureds. This may be explained by the South-East Asian contribution to the Cape Coloured genome. The delta values of HLA haplotypes in strong positive linkage disequilibrium in the Cape Coloureds were approximately half that seen in SA Caucasoids, Xhosa and San, an indication of a hybrid population of recent origin. The genetic distances also indicate that the Cape Coloureds are a hybrid population derived from the SA Caucasoid as well as Southern Africans such as the Xhosa and San.

The HLA frequencies of the **Xhosa** are significantly different from either the Caucasoids or the Cape Coloureds. They have the HLA gene, antigen and haplotype frequencies characteristic of other Southern African Negroes, with evidence of the assimilation of the Khoisan, as seen in the relatively high frequency of Aw43.

In general the **San** resemble the Negroes or even surpass them in many of the features by which Africans generally differ from non-Africans e.g. the very high frequencies of Aw43, Bw58, Cw6 and DRw6. There are, however, also distinct differences in HLA frequencies between these two groups e.g. the raised A3, B8 and Bw41 frequencies and the complete absence of Bw42 in the San.

It has been shown that the HLA system is extremely powerful for the testing of disputed parentage in the varied populations of Southern Africa. Accurate and reliable estimation of an individual population's HLA gene frequencies is a prerequisite for the use of this system in disputed parentage testing, especially when the populations concerned exhibit marked heterogeneity, as is the case in Southern Africa.

REFERENCES

- Baur MP, Danilovs JA (1980) Population analysis of HLA-A,B,C,DR and other genetic markers. In: Terasaki PI (ed) Histocompatibility Testing 1980, UCLA Press, Los Angeles, p 955
- Baur MP, Neugebauer M, Albert ED (1984) Reference tables of two-locus haplotype frequencies for all MHC marker loci. In: Albert ED, Baur MP, Mayr WR (eds) Histocompatibility Testing 1984, Springer-Verlag, Berlin, Heidelberg, p 677
- Botha MC, Campbell E, Briggs B, du Toit ED (1975) Differences in serological definition and frequency of some HLA antigens among Southern African populations. In: Kissmeyer-Nielsen F (ed) Histocompatibility Testing 1975, Munksgaard, Copenhagen, p 151
- Campbell EM, du Toit ED (1983) Possible splits of Cw3. In: Ninth International Histocompatibility Workshop Conference, Newsletter, No 1
- Campbell EM, Taljaard DG, du Toit ED (1983a) B15Kemp/B15 Short Thai. In: Ninth International Histocompatibility Workshop and Conference, Newsletter No V, p 12
- Campbell EM, du Toit ED, Oudshoorn M (1983b) 621-CT: A possible new HLA-B locus antigen found in a Xhosa family. In: Ninth International Histocompatibility Workshop and Conference, Newsletter No V, p 27

- Cavalli-Sforza LL, Bodmer WF (1971) The Genetics of Human Populations. WH Freeman and Company, San Francisco, p 704
- Chandanayingyong D, Tait BD, D'Apice AJF (1981) Serum 537 - Does it recognize a Subtype of Cw3? In: Simons MJ, Tait BD, (eds) Proceedings of the second Asia and Oceania Histocompatibility Workshop Conference, Immunopublishing, Victoria, Australia, p 424
- Dart RA (1937) Racial Origins. In: Schapera I (ed) The Bantu-speaking tribes of Southern Africa. Routledge and Kegan Paul, London, p 1
- Du Toit ED, Briggs, BR, Botha MC, Campbell, EM (1980) Antigen Aw43. In: Terasaki PI (ed) Histocompatibility Testing 1980, UCLA Press, Los Angeles, p 344
- Du Toit ED, Oudshoorn M, Campbell EM (1984) Heterogeneity of HLA-DR2 and Dw2. In: Ninth International Histocompatibility Workshop and Conference, Book of Abstracts, p 4 ..
- Essen-Möller E (1938) Die Beweiskraft der Ähnlichkeit im Vaterschaftsnachweis; theoretische Grundlagen Mitt. Anthropol. Ges. ,Wien 68:9
- Mourant AE (1985) Blood relations, blood groups and anthropology. 1st edn. Oxford University Press, New York
- Nurse GT, Weiner JS, Jenkins T (1985) Research monographs on human population biology, No 3. The peoples of Southern Africa and their affinities. Clarendon Press, Oxford, p 103
- Pohl B (1982) Probability of paternity calculations: Logic II In: Silver H (ed) Probability of inclusion in paternity testing. The American Association of Blood Banks, Hartford, (Conn) p 61