Genetic markers in glaucoma

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SUMMARY A number of genetic polymorphisms have been investigated in Negro and Caucasoid patients with glaucoma and the results compared with data obtained on healthy controls. A sample of 61 Negro patients was not significantly different from a sample of 238 controls with respect to 18 red cell and serum protein systems; no association between glaucoma and any of these systems could be demonstrated. The comparison among Caucasoids, using 14 systems, was made between 31 patients and 70 controls. The populations differed from each other in the rhesus and Duffy blood group systems, and a significant association between open-angle glaucoma and rhesus D (+) was found.

It has been known for many years that primary open-angle glaucoma (OAG) occurs more frequently in families in which another relative suffers from the disease. A recent study found a 50% positive family history among patients with primary OAG.¹ And the sibs and offsprings of glaucoma patients have been shown to have an increased incidence of glaucoma.²⁻⁴

Throughout the years many features related to OAG have been found to be heritable. Among these are the increased cup/disc ratio,⁵ the height of the intraocular pressure,⁶ and the hypersensitivity to topically administered corticosteroids.⁷ The accepted view today concerning the mode of transmission of primary OAG is that it is multifactorially inherited,^{4,8} and various workers have looked for associations between genetic polymorphisms and OAG.⁹

The present study presents the results of genetic marker studies on patients with primary OAG drawn from 2 distinct population groups and compares them with healthy control samples.

Materials and methods

Sixty-one black patients and 31 white patients with OAG constitute the material for the study. All the patients were attending the glaucoma clinics at the Johannesburg General Hospital and at St John's Eye Hospital, Baragwanath. The control population

used for the black population was the Rural Pedi, which had been carefully selected for the International Biological Programme (Human Adaptability) by Jenkins.¹⁰ In the case of the newer polymorphisms other populations studied by the MRC Human Ecogenetics Unit were used. The Caucasoid patients were compared with a population sample of Johannesburg Afrikaans university student volunteers who are part of an ongoing study into the genetics of hyperlipidaemia.

Blood was collected by venepuncture into 2 Vacutainers, one containing ACD and one containing no anticoagulant, and tested for a wide range of genetic characters. Blood grouping was performed by standard methods recommended by Race and Sanger,¹¹ the red cell enzyme systems and haptoglobins and transferrins by the methods summarised by Giblett.¹² In addition glutamic pyruvic transaminase phenotypes were determined by the method of Chen and Giblett,¹³ the esterase D by the method of Hopkinson *et al.*,¹⁴ the carbonic anhydrases by the method of Hopkinson *et al.*,¹⁵ and glyoxalase I by the method of Komph *et al.*¹⁶

Phenotype differences between the population with the disease and the control population were assessed by means of the χ^2 test. The method of Woolf¹⁷ as modified by Haldane¹⁸ was used to assess the significance of the association between a genetic character and the disease.

Results and discussion

The phenotypes among the black glaucoma patients and their normal controls are set out in Tables 1

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and 2. No polymorphism was encountered in the adenosine deaminase system (the ADA² allele attains very low frequencies in Southern African Negroids) and in the haemoglobins, though one individual was found to have the δ -chain variant, HbA₂' or HbB₂. (The gene for HbA₂' attains low frequencies in most Southern African populations and it is polymorphic among the Herero of South West Africa/Namibia and the Kgalagadi of Botswana (unpublished data).)

It will be seen that no significant differences could be found in the ABO, MNS, rhesus, Kell, Duffy, 6-phosphogluconate dehydrogenase (6PGD), adenylate kinase (AK), phosphoglucomutase (first and second loci) (PGM₁ and PGM₂), acid phosphatase (AcPh), peptidase A (PepA), glutamic pyruvate transaminase (GPT), glyoxalase (GLOI), carbonic anhydrase I and II (CA_I and CA_{II}), esterase D (EsD), haptoglobin (Hp) and transferrin (Tf) systems. There seemed to be an excess of glucose 6-phosphate dehydrogenase (G6PD) deficient individuals (Gd(-), A-) among the glaucoma patients, but it did not attain significance ($P > 0.05$).

The same range of investigations was carried out on Caucasoid patients suffering from glaucoma and the results compared with a normal control population (Tables 3 and 4). Significant differences

Table 1 Comparison of ABO, rhesus and MNSs blood groups among Negro glaucoma patients and healthy control population

System and phenotype	Glaucoma patients	Controls	χ^2	DF	P
ABO (n=61)	(n=238)				
A ₁	9	35	3.87	2	>0.50
A ₂	7*	26			
Abantu	3	4			
B	11	42			
AB	3†	7			
O	28	123			
Rhesus (n=61)	(n=237)				
ccDee	45	155	12.492	6	>0.50
CcDee	3	39			
ccDEe	9	33			
CCDee	1	0			
ccDEE	2	2			
ccdee	1	6			
CcDEe	0	2			
MNSs** (n=59)	(n=130)				
MMS	8	18	3.862	5	>0.50
MMss	10	11			
MNS	14	27			
MNnn	17	48			
NNS	2	7			
NNss	8	19			

*Three were A_{Int}. †Two were A₂B. **Tested with anti-M, -N, and -S sera only. †There were 2 (3.4%) Henshaw positives among the glaucoma patients: 1 MMss and 1 MNSs; among the controls there were 6 (4.6%): 2 MMS and 4 MNS.

Table 2 Comparison of Kell and Duffy blood group systems, as well as red cell enzymes glucose 6-phosphate dehydrogenase (G6PD), 6 phosphogluconate dehydrogenase (6PGD), adenylate kinase (AK), glutamic pyruvate transaminase (GPT), esterase D (EsD), phosphoglucomutase, first locus (PGM₁) and second locus (PGM₂), peptidase A (PepA), red cell acid phosphatase (AcPh), carbonic anhydrase, second locus (CA_{II}), glyoxalase I (GLOI); and the serum proteins haptoglobins (Hp), and transferrins (Tf) among Negro glaucoma patients and a healthy control population

System and phenotype	Glaucoma patients	Controls	χ^2	DF	P
Kell	(+)	0			
	(-)	61			
Duffy	a+b-	2	1.827	1	>0.10
	a-b+	4			
	a-b-	55			
G6PD**	B	25	4.661	2	>0.05 <0.10
	A	8			
	A-	5			
6PGD	A	41	1.681	1	>0.10
	AC	10			
	C	2			
AK	1	50	0.275	2	>0.80
	2-1	1			
GPT	1	45	2.485	2	>0.20
	2-1	8			
	2	1			
EsD	1	52	1.260	2	>0.50
	2-1	2			
	2	0			
PGM₁†	1	31	1.742	2	>0.30
	2-1	20			
	2	3			
PGM₂	1	52	1.018	1	>0.20
	2-1	2			
PepA	1	47	1.008*	3	>0.70
	2-1	6			
	8-2	1			
	2	0			
AcPh	B	38	5.092	5	>0.30
	AB	8			
	A	2			
	RB	4			
	RA	1			
	R	1			
CA_{II}	1	46	3.694	2	>0.10
	2-1	5			
	2	2			
GLO I	1	7	0.813	2	>0.50
	2-1	14			
	2	24			
Hp	1	14	4.24	4	>0.30
	2-1	13			
	2-1mod	3			
	2	17			
	O	0			
		2			
Tf	C	47	0.079	1	>0.70
	CD	3			

*Obtained by pooling PepA 8-2 and PepA 2 categories; control population from Kirk *et al.*²⁶ **Males only. †Excluding one individual from control population who was PGM₁ 6-1.

Genetic markers in glaucoma

Table 3 Comparison of ABO, rhesus, and MNSs blood groups among Caucasoid glaucoma patients and a healthy control population

<i>System and phenotype</i>	<i>Glaucoma patients (n = 31)</i>	<i>Controls (n = 70)</i>	χ^2	<i>DF</i>	<i>P</i>
ABO					
A ₁	9	21	0.878	5	>0.95
A ₂	3†	10			
B	3	11			
AB	1*	3††			
O	13	25			
Rhesus**					
CcDee	5	26	25.326	6	<0.001
ccdee	0	13			
CcDEe	6	10			
ccDEe	3	9			
CCDEe	13	5			
ccDEE	3	5			
ccDee	0	2			
MNSs					
MMS	3	7	12.566	5	<0.05 >0.02
MMss	1	7			
MNS	12	23			
MNss	12	15			
NNs	0	7			
NNss	3	11			

†Two individuals were A₁Int. *This individual was A₂B. ††One A₁B and two A₂B. **When Rhesus negative (ccdee) compared χ^2 (DF=1) = 3.404; 0.10 > P > 0.05.

were apparent with respect to 2 systems: Duffy (P<0.01), because of a striking deficiency of Fy(a+b+) and excess of Fy(a+b-) and Fy(a-b-) individuals among the OAG patients when compared with controls; and in the rhesus system (P<0.001), where the difference was due to a striking excess of CCDee and a decrease of CcDee individuals among the OAG patients. Equally striking is the absence of Rh negative (ccdee) individuals among the OAG patients when compared with the controls, but the difference just fails to reach significance (0.02 < P < 0.05).

There have been relatively few studies of the association of genetic polymorphic characters with glaucoma. Mourant *et al.*⁹ have recently summarised them. In the ABO system there appeared to be an excess of A over O [χ^2 (DF=1)=6.6127] but not of B over O [χ^2 (DF=1)=0.0164] in a pooled series representing 336 patients from 5 different studies. One Indian population (from Sitapur, North India) was studied for ABH secretor status, and there was a striking deficiency of secretors among a group of 56 wide-angle, 39 narrow-angle, and 5 unclassified cases of primary glaucoma when compared with controls [χ^2 (DF=1)=50.884]. In the phenylthiocarbamide tasting system 7 studies, when combined, gave an excess of nontasters among OAG when compared with normal control

populations [χ^2 (DF=1)=46.5]. Two studies not included in Mourant *et al.*'s book are known to us: Kubickova *et al.*¹⁹ found an increased association of OAG with blood group O, and Arezzi²⁰ found the association to be with blood group A. In neither study was statistical significance attained.

The association between OAG and the various polymorphic characters in the Negro and Caucasoid populations are shown in Table 5. It will be seen that a significant departure of the relative incidence from unity is obtained in the rhesus system among the Caucasoids: there is an absence of rhesus D(+) individuals among the glaucoma patients [χ^2 (DF=1) = 6.4805; 0.01 < P < 0.02]. There is a suggestion of

Table 4 Comparison of Kell, Duffy, and P blood group systems as well as red cell enzymes 6 phosphogluconate dehydrogenase (6PGD), adenylate kinase (AK), adenosine deaminase (ADA), glutamic pyruvate transaminase (GPT), esterase D (EsD), phosphoglucomutase, first locus (PGM₁), peptidase A (PepA), and acid phosphatase (AcPh) among Caucasoid glaucoma patients and a healthy control population

System and phenotype	Glaucoma patients (n=31)	Controls (n=70)	χ^2	DF	P
Kell	(+)	2	0.627	1	>0.30
	(-)	29			
Duffy	a+b+	7	12.438	3	<0.01
	a+b-	13			
	a-b+	7			
	a-b-	4			
P ₁	(+)	28	4.965	1	>0.02
	(-)	3			
6PGD	A	29	0.322	1	>0.50
	AC	2			
AK	1	30	0.250	1	>0.50
	2-1	1			
ADA	1	27	2.356	2	>0.30
	2-1	3			
	2	1			
GPT	1	8	5.995	2	>0.05
	2-1	19			
	2	4			
EsD	1	26	3.040	2	>0.20
	2-1	5			
	2	0			
PGM ₁	1	19	4.253	2	>0.10
	2-1	8			
	2	4			
PepA	1	31	1.766	1	>0.10
	2-1	0			
AcPh	B	11	8.576	4	>0.50
	AB	13			
	A	3			
	CB	1			
	CA	3			

Table 5 Associations between glaucoma and various polymorphisms in the Negro and Caucasoid populations (see text for statistical method used)

System and association tested	Negro		Caucasoid	
	Relative incidence	$\chi^2(DF=1)$ for difference from unity	Relative incidence	$\chi^2(DF=1)$ for difference from unity
ABO				
A/O	1.2901	0.6017	0.7496	0.3810
B/O	1.1726	0.1696	0.5749	0.6915
MNSs				
NNS/other phenotype	0.7159	0.2310	0.1344	3.4370
MMss/other phenotypes	2.2042	3.0972	0.4164	1.1407
Rhesus				
D+/D-	1.1325	0.0233	14.7900	6.4805*
Kell				
K+/K-			0.6231	0.4534
Duffy				
Fy(a+)/Fy(a-)	0.4366	1.5550	0.8819	0.0816
P				
(P ₁ (+)/P ₁ (-))			3.5368	4.5539
G6PD				
GdA-/GdB+GdA	3.8230	3.9243		
6PGD				
6PGD (C/6PGD A+6PGD AC)	1.5243	0.2544		
PGM ₁				
PGM ₂ /PGM ₁ 1 + PGM ₂ -1	3.3810	2.7449	4.4836	3.8580
Ac Phosphatase				
AcPh B/other phenotypes	0.9633	0.0095	0.7449	0.4623
Carbonic anhydrase II				
CA _{II} 2/CA _{II} 1 + CA _{II} 2-1	3.1553	1.5306		
Glyoxalase I				
GLO2/GLO1 + GLO2-1	1.2621	0.4310		
Haptoglobins				
Hp2/Hp1 + Hp2-1 + Hp2-1m	1.2574	0.3526		

*0.01 < P < 0.02.

association in the case of G6PD among the Negro patients and in the case of PGM₁ among the Caucasoid patients. No confirmation has been found for the observation by Arezzi²⁰ and Kubickova *et al.*¹⁹ for a greater prevalence of glaucoma among blood group A subjects, or for the observation by Kubickova *et al.*¹⁹ of an association with group O.

It is well known that the carbonic anhydrase inhibitors such as acetazolamide or methazolamide are the only diuretic agents of value in reducing

aqueous formation in patients with glaucoma and, hence, in daily use in the treatment of the disease. It was of some interest, therefore, to find a decreased proportion of CA_{II}2-1 heterozygotes among the black glaucoma patients when compared with the control group. This difference did not, however, attain significance (P > 0.10), and a comparison of relative incidence of CA_{II}2 with the other CA_{II} phenotypes failed to reveal a significant association [$\chi^2(DF=1)=1.5306$].

Mourant *et al.*'s⁹ very useful summary failed to show any association between glaucoma and the MN and rhesus blood group systems or haptoglobins. They drew attention to one study, that of Garg and Pahwa,²¹ which revealed an association between glaucoma and nonsecretors of ABH. They could locate 7 studies, representing a total of 746 cases, and association between PTC taste testing and OAG which revealed a raised frequency of nontasters in both Caucasoids and Negroids.

With the advent of tissue typing several investigations were carried out to study the association between the HLA antigens and OAG. At this stage, the reports in the literature are rather conflicting,²²⁻²⁵ and more research is needed before a meaningful conclusion with regard to this polymorphism can be reached.

We are not aware of any studies directed towards establishing association between OAG and the other serological polymorphisms, many of which have been included in the present study. We present our findings in detail in the hope that other workers might be encouraged to extend the search, pooling our results with theirs and in this way, hopefully, discovering the associations which might well exist.

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References

- ¹Shin DH, Becker B, Kolker AE. Family history in primary open-angle glaucoma. *Arch Ophthalmol* 1977; **95**: 598-600.
- ²Miller SJH, Paterson G. Studies on glaucoma relatives. *Br J Ophthalmol* 1962; **46**: 513-22.
- ³Leighton DA. Studies on relatives of glaucoma patients. *Proc R Soc Med* 1968; **61**: 542-4.
- ⁴Jay B, Paterson G. The genetics of simple glaucoma. *Trans Ophthalmol Soc UK* 1972; **94**: 161-71.
- ⁵Armaly MF. Genetic determination of cup/disc ratio of the optic nerve. *Arch Ophthalmol* 1967; **78**: 35-43.
- ⁶Armaly MF. The genetic determination of ocular pressure in the normal eye. *Arch Ophthalmol* 1967; **78**: 187-92.
- ⁷Armaly MF. Inheritance of dexamethasone hypertension and glaucoma. *Arch Ophthalmol* 1967; **77**: 747-51.
- ⁸Armaly MF. Statistical attributes of the steroid hypertensive response in the clinically normal eye. I. The demonstration of the 3 levels of response. *Invest Ophthalmol* 1965; **4**: 187-97.
- ⁹Mourant AE, Kopec C, Domaniewska-Sobczak K. *Blood*

- Groups and Diseases*. Oxford: Oxford University Press, 1978.
- ¹⁰Jenkins T. Some considerations in the study of Southern African populations undergoing environmental change. In: Vorster DJM, ed. *Human Biology of Environmental Change*. London: IBP, 1972: 121–6.
 - ¹¹Race RR, Sanger R. *Blood Groups in Man*, 6th ed. Oxford: Blackwell, 1975.
 - ¹²Giblett ER. *Genetic Markers in Human Blood*. Oxford: Blackwell, 1969.
 - ¹³Chen S-H, Giblett ER. Polymorphism of soluble glutamic-pyruvic transaminase: a new genetic marker in man. *Science* 1971; **173**: 148–9.
 - ¹⁴Hopkinson DA, Mestriner MA, Cortner J, Harris H. Esterase D: a new human polymorphism. *Ann Hum Genet* 1973; **37**: 119–37.
 - ¹⁵Hopkinson DA, Coppock JS, Mühlemann MF, Edwards YH. The detection and differentiation of the products of the human carbonic anhydrase loci CA_I and CA_{II}, using fluorogenic substrates. *Ann Hum Genet* 1974; **38**: 155.
 - ¹⁶Kompf J, Bissbort S, Gussmann S, Ritter H. Polymorphism of red cell glyoxalase I (E.C.: 4.4.1.5). A new genetic marker in man. *Humangenetik* 1975; **27**: 141.
 - ¹⁷Woolf B. On estimating the relation between blood group and disease. *Ann Hum Genet* 1954–5; **19**: 251–3.
 - ¹⁸Haldane JBS. The estimation and significance of the logarithm of a ratio of frequency. *Ann Hum Genet* 1955–6; **20**: 309–11.
 - ¹⁹Kubickova Z, Kloucek F, Kraus H, Dvorakova M. ABO blood groups, secretory behaviour and PTC tasting of glaucoma patients. *Klin Monatsbl Augenheilkd* 1972; **161**: 32–5.
 - ²⁰Arezzi R. Report on the ABO blood-group system and chronic simple glaucoma. *Minerva Optalmol* 1976; **18**: 77–81.
 - ²¹Garg MP, Pahwa JM. Primary glaucoma and blood groups. *J All-India Ophthalmol Soc* 1965; **13**: 127–9.
 - ²²Shin DH, Becker B. HLA-A 11 and HLA-Bw 35 and resistance to glaucoma in white patients with ocular hypertension. *Arch Ophthalmol* 1977; **95**: 423–4.
 - ²³Aviner Z, Henley WL, Fotino M, Leopold IH. Histocompatibility (HLA) antigens and primary open-angle glaucoma. *Tissue Antigens* 1976; **7**: 193–200.
 - ²⁴Grabner G, Mayr WR. Histocompatibility antigens (HLA) and open-angle glaucoma. *Albrecht von Graefes Arch Klin Ophthalmol* 1977; **202**: 75–9.
 - ²⁵David R, Maier G, Baumgarten I, Abrahams C. HLA antigens in glaucoma and ocular hypertension. *Br J Ophthalmol* 1979; **63**: 293–6.
 - ²⁶Kirk RK, Blake NM, Vos GM. The distribution of enzyme group systems in a sample of South African Bantu. *S Afr Med J* 1971; **45**: 69–72.