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Genetic polymorphism of complement component C8

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Summary. Extensive genetic polymorphism of complement component C8 was demonstrated by isoelectric focusing of serum or plasma samples followed by immunoblotting procedures. Using these methods, we could detect both α-γ (C81) and β (C82) chain polymorphisms in the same gel. Two-dimensional (2D) electrophoresis of C8 immunoprecipitates was used to obtain further information of the C8 patterns. Evidence was obtained that the C81 polymorphism resides in the structural gene of the C8 α chain. Both C8 systems show autosomal, chiefly codominant inheritance, and the distribution of phenotypes agrees with the Hardy-Weinberg equilibrium. Our findings suggest at least five different alleles in the C81 system; the gene frequencies of the two most common ones, *C81*A* and *C81*B* being 0.59 and 0.39, respectively. In C82 we found evidence for at least three codominant alleles, the gene frequencies for the two most common ones, *C82*B* and *C82*A* being 0.94 and 0.05, respectively. In addition, family studies disclosed the existence of a null allele, *C82*Q0*.

Introduction

Complement component C8 is a globular protein consisting of three chains, α, β, and γ. The α and γ chains are covalently linked while the β chain is more loosely attached to the α-γ complex. The total molecular weight is about 151,000–153,700 daltons, the γ chain being 13,700–22,000 daltons, the α chain 64,000–77,000 daltons, and the β chain 63,000–64,000 daltons (Kolb and Müller-Eberhard 1976; Steckel et al. 1980).

Early studies of serum samples without C8 haemolytic activity, and without proteins reacting with anti-C8, have been published (Merritt et al. 1976; Petersen et al. 1976; Giraldo et al. 1977; Jasin 1977). These sera were thought to represent total C8 deficiency, but later studies (Marcus et al. 1982; Tedesco et al. 1982; 1983b) have shown that these patients have normal β chains, but have a complete lack of the α-γ complex.

In 1980, Tedesco and co-workers described a C8 dysfunctional state in two siblings, wherein the C8 molecule was haemolytically inactive and antigenically altered. They later showed that this type of C8 dysfunction was due to β chain deficiency (Tedesco et al. 1983a; Tschopp et al. 1981). Structural polymorphisms in both α–γ (Raum et al. 1979; Rittner et al. 1984) and β chains (Alper et al. 1983) have been demonstrated by isoelectric focusing in polyacrylamide gels followed by haemolytic overlay, using deficiency sera to detect functional C8 activity. Preliminary studies from our group have shown that the C8 polymorphisms can be detected by isoelectric focusing in polyacrylamide gels, passive blotting onto nitrocellulose sheets, and detection of C8 by immunological methods (Rogde et al. 1984; Mevåg et al. 1984). These studies have now been extended. In addition, 2D electrophoresis has been established as a useful and sometimes necessary verification procedure with regard to both C81 and C82 variation.

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Materials and methods

Family and population material

Serum or heparin plasma samples from Norwegian families (the Oslo NHIK material) were used for this study. A total of 57 matings with 265 offspring were examined. Matings were selected primarily with regard to the size of the sibships, preferring families with many children. Sera from 86 unrelated individuals from the family material and 19 persons working in the Institute of Forensic Medicine were examined with regard to phenotype and gene distribution. The serum/plasma samples of the family material had been stored for a varying number of years at −75°C. C8 α-γ chain deficiency serum was kindly given to us by Dr. Merritt.

Isoelectric focusing and immunoblotting

Isoelectric focusing was carried out in 1 mm thick 5% polyacrylamide gels containing 5% ampholytes (Ampholine LKB, pH 3.5–10 1.5 ml, pH 4–6 0.1 ml, pH 5–7 0.1 ml) for 2½ h at 10 W, reaching the final voltage of 2 kV after approximately 2 h. Electrode solutions were 1 *M* H_3PO_4 and 1 *M* NaOH.

Approximately 5 µl of serum or plasma were applied anodally on the gel on $5 \times 5\,mm^2$ pieces of Whatman filter paper no. 3. When focusing was completed, a nitrocellulose paper presoaked in distilled water was placed on the gel and left in a humid atmosphere at 37°C for 1½ h. The nitrocellulose sheet was then washed for 30 min in a solution of phosphate buffered saline and Tween 20 (PBS Tween), according to the method described by Towbin et al. (1979) modified by Whitehouse and Putt (1983). The paper was left overnight in a 1:1000 solution of goat anti-human C8 (Atlantic Antibodies) in PBS Tween.

After washing for 2h in three changes of PBS Tween, the paper was placed in a 1:1000 solution of peroxidase conjugated rabbit anti-goat IgG (Hoechst) for 4h, after which it was washed for ½h in three changes of PBS Tween. Peroxidase positive bands were detected using 4-chloro 1-naphthol according to the method described by Hawkes et al. (1982), but replacing methanol by acetone. Bands of C8 activity developed within 10–15min. The reaction was stopped using tap water. The nitrocellulose papers were then dried and stored in the dark. Adding 2*M* urea to the focusing gel split the β chain from the α-γ complex. The urea gel focusing was carried out for 3½h at 20W, otherwise the procedure was as described above.

Two-dimensional electrophoresis

C8 immunoprecipitates were obtained by adding 50µl of anti-C8 (Atlantic Antibodies) to approximately 250µl of serum. The mixture was left overnight at +4°C and then centrifuged. The pellet was washed three times in a Tris HCl buffer, pH 7.4. The precipitates were then resolved in 25µl of a solution of 2% sodium dodecyl sulphate (SDS), 1% cyclohexaminoethane sulphonic acid (CHES), 1% dithiothreitol (DTT), and 10% glycerol.

2D electrophoresis was carried out on IsoDalt equipment (Electro Nucleonics Inc., Oak Ridge, Tenn.) as described by Anderson and Anderson (1977) (details as given by Mevåg et al. 1981). After isofocusing in the first dimension, the gels were equilibrated for 15min in a 2% SDS, 5% (w/v) dithioerythritol (DTE) glycerol solution before application to the SDS gradient gels. A slight modification of the abovementioned method was also used: The immunoprecipitates were resolved in a SDS solution devoid of the reduction agent DTT. The equilibrating buffer, on the other hand, contained 25% instead of 5% DTE, to reduce the molecule at this stage.

Initially, 2D electrophoresis was performed without reducing agent in either dimension. The β chain was then identified by a considerably lower molecular weight than the α-γ complex. Adding reducing agent in both dimensions demonstrated all three chains at their individual pI and molecular weight. The position of the β chain already being known, the α and γ chains were readily identifiable. Staining of the gels was performed according to the method of Mevåg et al. (1981). Sometimes the gels were submitted to electroblotting for 1h using an Electroblot Apparatus (EC Apparatus Corp) according to the instructions for use. The nitrocellulose paper was then washed in PBS Tween, treated with antibodies, and developed as described above. After the blotting procedure, gels were protein stained.

Strategy for characterization of C8 protein variation at the peptide chain level

All samples were run in the one dimensional (1D) urea-free electrofocusing procedure. Each C8 pattern thus obtained, was further characterized according to patterns in 1D runs in urea-containing gels and by individual C8 peptide patterns as revealed by 2D runs with (for α chain typing) or without (for β chain typing) reducing agent (DTT) in the first dimension.

Nomenclature

We have used the nomenclature suggested by Alper et al. (1983) where C81 refers to the α-γ complex and C82 refers to the β chain. The variants were named according to pI (A: acidic, B: basic).

Results

By isoelectric focusing of serum and immunoblotting, two distinct regions of immunologically detectable C8 bands appeared at pI values of approximately 6–6.5 and 7–8. They were called A (acidic) and B (basic), respectively (Figs. 1 and 2). Isoelectric focusing of β chain-deficient sera revealed only an A pattern, while the α-γ deficient samples had neither an A nor a B pattern. When normal samples were run in 2*M* urea gels, the B pattern disappeared. The B pattern represents the complete C8 protein. B region variation could thus be caused by changes in all three known C8 chains. The evidence indicated that the A pattern represented the α-γ complex. To confirm the identification of the A pattern as consisting of α-γ chains, 2D electrophoretic studies were performed. The common A region variants were studied by 2D electrophoresis using a reducing agent in the first dimension (Fig. 3). The A pattern variation was shown to be due to α-γ polymorphism, more specifically to charge variation in the α chain. γ Chain variation was not observed.

As indicated above, A region and B region patterns were often similar, but additional variation in the B patterns was

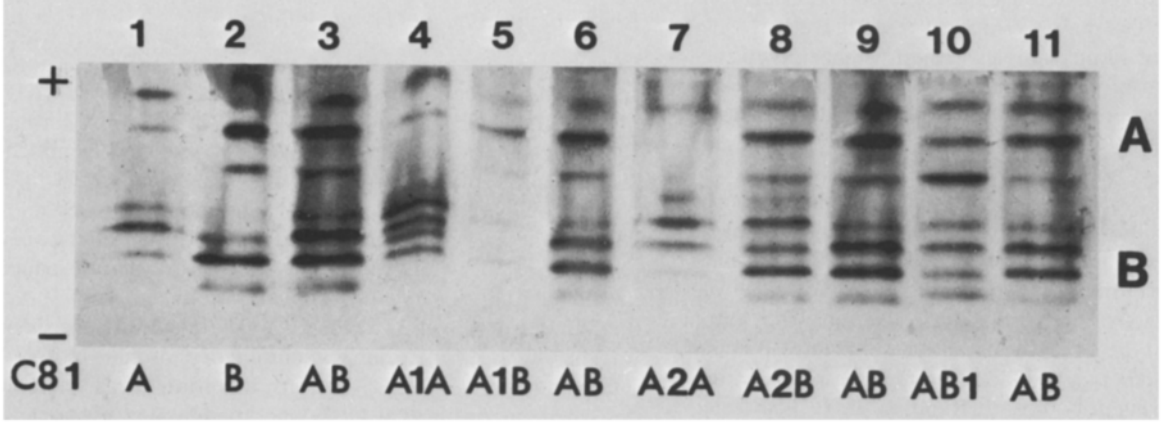

Fig. 1. C8 phenotype patterns demonstrating the α-γ polymorphism. In all these individuals the β chain is of type C82B. The B-region patterns are shown diagrammatically in Fig. 5

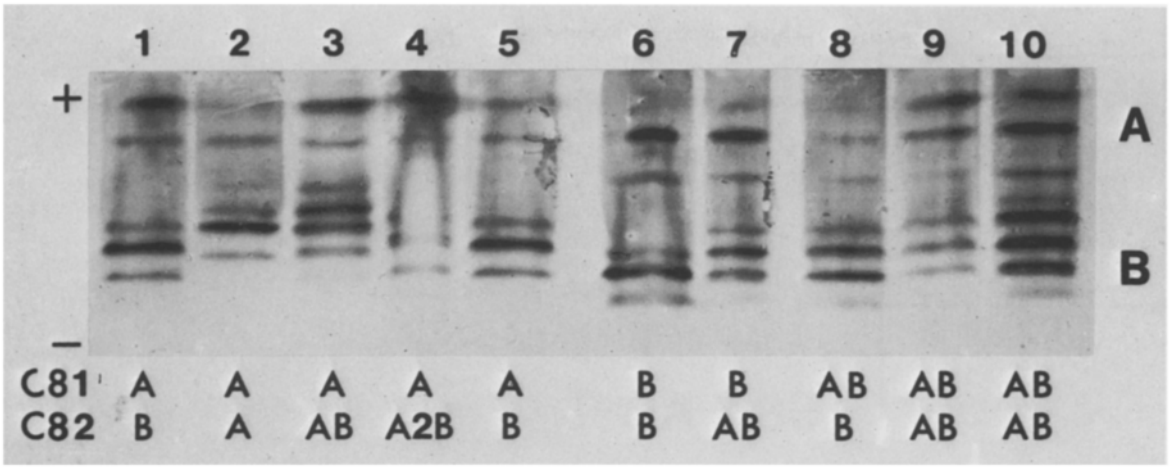

Fig. 2. C8 phenotype patterns demonstrating the β chain polymorphism. Patterns 1–5 show β chain variation in C81A individuals, patterns 6 and 7 in C81B individuals, and patterns 8–10 in C81AB individuals

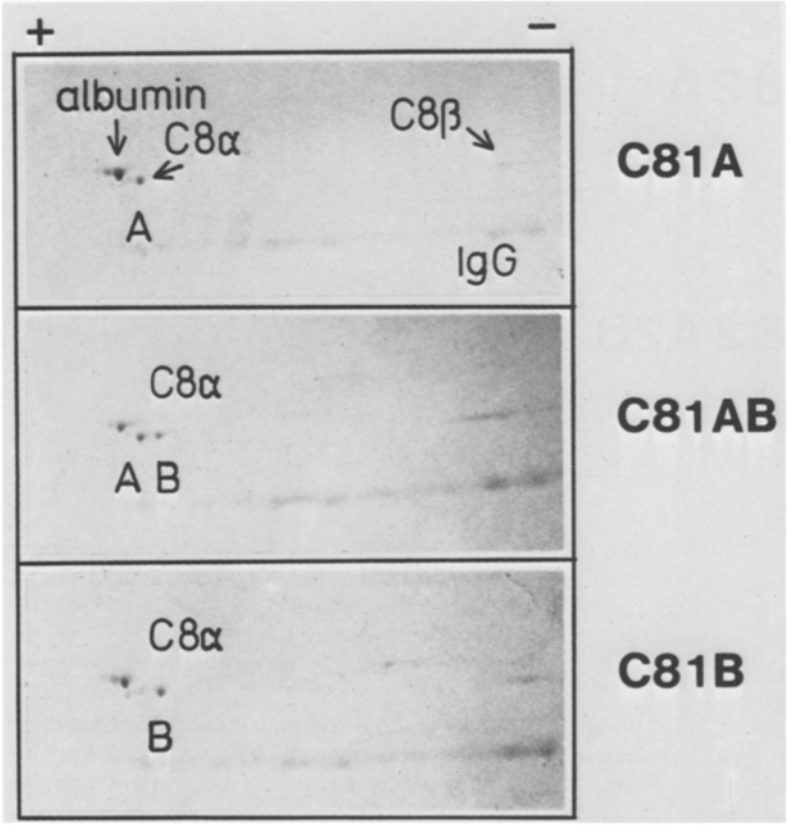

Fig. 3. 2D patterns of reduced C8 immunoprecipitates. Three different C81 patterns are presented; the designations are given to the right

often observed (Fig. 2). This variation was studied in detail with a modified 2D electrophoretic method without reducing agent in the first dimension. This procedure gave clear and reproducible β chain patterns (Fig. 4). The technique enabled us to show that the additional variation in the B region was due to varying charge of different C8 β chains.

A rather complex situation thus existed. In the A region a C8 polymorphism due to variations in the α chain was observed. In accordance with previous studies (Alper et al. 1983; Rittner et al. 1984) this has been designated the C81 polymorphism. In the B region the C81 polymorphism could also be observed, but in addition a polymorphism due to variation in the β chain could be observed. This was called the C82 polymorphism (Alper et al. 1983). The various C81 and C82 types are shown both in photographs and in diagrammatic presentations in Figs. 1, 2, 5, and 6.

Genetic studies

The C81 polymorphism

The formal genetics of the C81 variation were studied in families. An autosomal, codominant inheritance pattern was observed without exceptions (Table 1). The distribution of phenotypes and genes in unrelated individuals was studied.

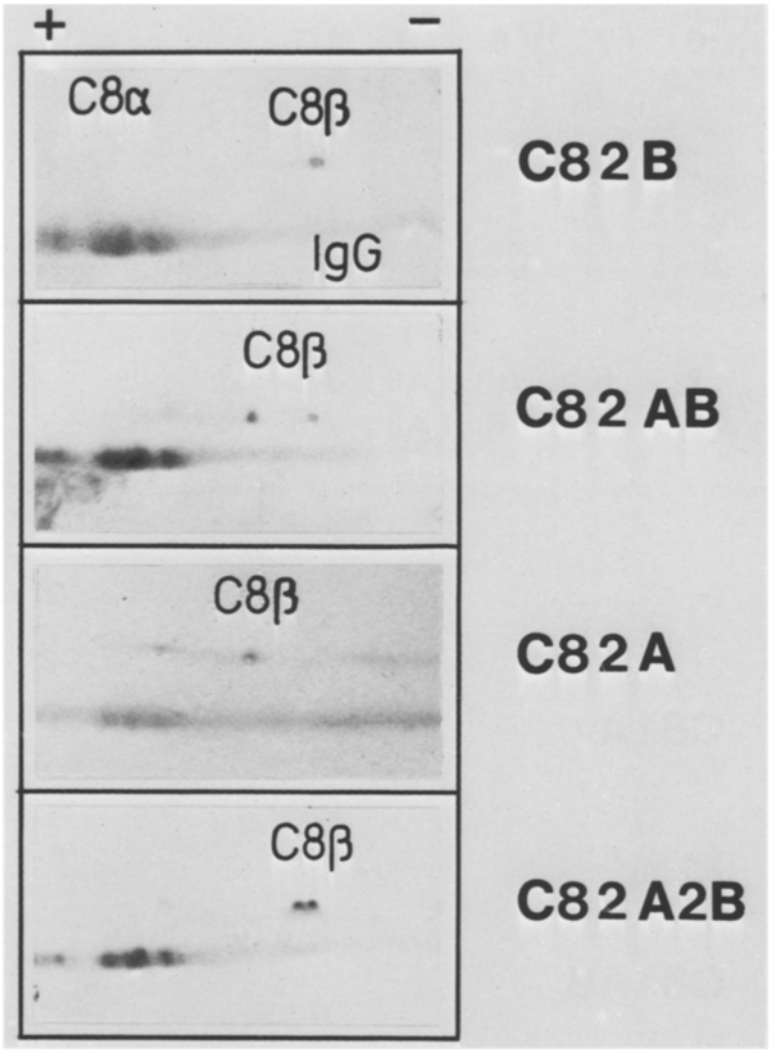

Fig. 4. 2D patterns of unreduced C8 immunoprecipitates. Four different C82 patterns are shown; the designations are given to the right

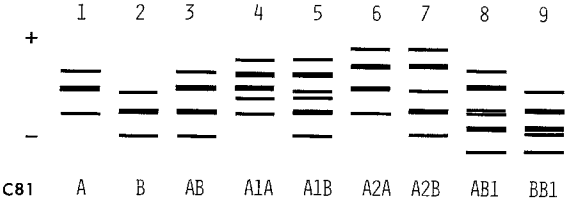

Fig. 5. Diagrammatic presentation of C8 (A-region omitted) phenotype patterns demonstrating the α-γ polymorphism. In all these individuals the β chain is of type C82B. Five different allotype products in various phenotypic combinations are shown: C81A in lanes 1, 3, 4, 6, and 8; C81B in 2, 3, 5, 7, and 9; C81A1 in 4 and 5; C81A2 in 6 and 7; and C81B1 in 8 and 9

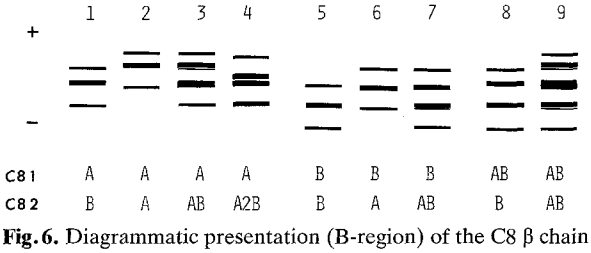

Fig. 6. Diagrammatic presentation (B-region) of the C8 β chain variation in combination with the most common C81 types. Patterns 1–4 show β chain variation in C81A individuals, 5–7 in C81B individuals, and 8 and 9 in C81AB individuals

The results are presented in Table 2. Variants not previously described are included. The phenotype distribution fits the Hardy-Weinberg equilibrium.

The C82 polymorphism

Variation observed in the B region, but not reflected in the A region, was shown to be due to β chain variation. An autosomal, ordinarily codominant, inheritance pattern was confirmed in family studies (Table 3). However, we have been able to show that a C8 β chain null allele *(C82*Q0)* segregates in two families (see Discussion). The distribution of phenotypes and the gene frequencies are shown in Table 4.

Discussion

By employing different separation procedures and immunological detection techniques we have shown that two different C8 polymorphisms exist, one in the α chain and the other in the β chain. This confirms and extends the information obtained in previous studies using deficiency sera in the study of C8 variation in man.

Most of the genetic variation in C8 can be detected and interpreted in a correct manner by isofocusing of serum in a

Table 1. C8 α chain (C81) types in families

Parental types	Number of families	Offspring types[a]									
		A	AB	B	A1A	A1B	A2A	A2B	AB1	B1B	*N*
A × A	5	25 (25)									25
A × B	8		34 (34)								34
B × B	2			5 (5)							5
A × AB	15	40 (35.5)	31 (35.5)								71
AB × AB	9	8 (7.75)	18 (15.5)	5 (7.75)							31
AB × B	7		13 (11.5)	10 (11.5)							23
AB × A1B	3		3 (3.75)	0 (3.75)	8 (3.75)	4 (3.75)					15
A × A2A	1	2 (4.5)					7 (4.5)				9
B × A2A	1		8 (8)					8 (8)			16
A × AB1	2	9 (11.5)							14 (11.5)		23
AB × AB1	1	3 (1.25)	0 (1.25)						1 (1.25)	1 (1.25)	5
AB × B1B	2		3 (1.5)	0 (1.5)					3 (1.5)	0 (1.5)	6
B × B1B	1			1 (1)						1 (1)	2
	57										265

[a] Expected numbers are given in parentheses

Table 2. C8 α chain (C81) phenotype and allele distribution in 105 Norwegians

	C81 phenotypes								C81 allele frequencies				
	AB	A	B	A1A	A1B	A2A	AB1	B1B	A	B	A1	A2	B1
Observed	46	37	16	1	1	1	1	2	0.586	0.386	0.010	0.005	0.014
Expected[a]	47.5	36.1	15.6	1.2	0.8	0.6	1.7	1.1					

[a] Expected numbers are calculated on the assumption of Hardy-Weinberg equilibrium

Table 3. C8 β chain (C82) types in families

Parental types	Number of families	Offspring types[a]				
		B	AB	A	A2B	*N*
B × B	41	205 (205)				205
B × AB	14	19 (21)	21 (21)	2 (0)[b]		42
AB × AB	1	1 (0.5)	1 (1)	0 (0.5)		2
B × A2B	1	4 (8)			12 (8)	16
	57					265

[a] Expected numbers are given in parentheses

[b] The two C82A offspring in a B × AB mating have probably inherited a C82 null allele from their mother

Table 4. C8 β chain (C82) phenotype and allele distribution in 105 Norwegians

	C82 phenotypes				C82 allele frequencies[b]		
	B	AB	A	A2B	B	A	A2
Observed	94	9	1	1	0.943	0.052	0.004
Expected[a]	93.4	10.3	0.3	0.8			

[a] Expected numbers are calculated on the assumption of Hardy-Weinberg equilibrium

[b] Allele frequencies are calculated assuming codominant inheritance, neglecting the silent alleles

non-urea containing gel whereupon C8 patterns are demonstrated by immunoblotting. There are, however, methodological difficulties with regard to the A region patterns; it may sometimes be difficult to get reproducible results.

α Chain typing (C81) must therefore usually rely on the interpretation of B region patterns. These patterns are clear and easy to reproduce between runs. When β chain variation is superimposed on α chain variation, the patterns may, however, be relatively complex as demonstrated in Figs. 2 and 6. Usually β chain (C82) types are established by B region typing. It is, however, very useful and sometimes necessary to resort to 2D electrophoresis for the interpretation of patterns and segregation in families. We thus conclude that 2D electrophoresis both with and without reducing agent in the first dimension, is sometimes a necessary adjuvant method in C8 typing.

In the present study we have demonstrated five different C81 (α chain) allotypes while three have been disclosed in previous studies (Raum et al. 1979; Rittner et al. 1984). The frequencies of the two most common alleles, *C81*A* and *C81*B* are quite similar in the three studies (Table 5). Both previous studies have included a rare *C81*A1* allele. We have, however, demonstrated two acidic variants, C81A1 and C81A2. The previously described C81A1 may represent either of the two—or both. Both acidic variants have been difficult to detect in urea gels, and in the A regions of ordinary runs, possibly because of heavy background staining. 2D studies have confirmed that the polymorphism resides in the α chain.

Table 5. C8 α chain *(C81)* allele frequencies in three studies

	A	B	Other alleles
This study	0.586	0.386	0.029
Raum et al. (1979)[a]	0.649	0.348	0.03
Rittner et al. (1984)	0.5536	0.4286	0.0178

[a] The figures refer to the Caucasian population

We have demonstrated three different C82 (β chain) variants. The frequencies of the two most common alleles, *C82*B* and *C82*A* are in accordance with the findings of Alper et al. (1983). They have described a rare variant called C82A1, more acidic than C82A. We have found another rare variant, C82A2, not previously described. It is less acidic than C82A. We have not yet typed any individuals expressing the *C82*A1* allele.

In addition we have found evidence for a C8 β chain null allele in two families. In one of our laboratory workers, a C82A pattern led to examination of two children who were C82B and C82AB, respectively. The other family group was found on examining our family material, where a C82B mother and C82AB father had two C82A children. These findings may indicate that *C82* null alleles are relatively frequent (5–10%) in the Norwegian population, and that total C8 β chain deficiency is correspondingly common. The present material is, however, too small to allow a reliable estimation of the *C82*Q0* allele frequency. Mother/child materials will be particularly important in this respect. Work is in progress to evaluate linkage relationships between the *C81* and *C82* gene loci, and the general linkage relationships for each of them.

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