

## Genetic polymorphism in C8 beta-chains. Evidence for two unlinked genetic loci for the eighth component of human complement (C8).

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### Research Article

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# Genetic Polymorphism in C8 $\beta$ -Chains

## EVIDENCE FOR TWO UNLINKED GENETIC LOCI FOR THE EIGHTH COMPONENT OF HUMAN COMPLEMENT (C8)

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**ABSTRACT** Genetic polymorphism in the  $\beta$ -subunit of the eighth component of human complement, C8, was defined by isoelectric focusing of serum in polyacrylamide gel in the presence of urea and development of specific patterns of hemolysis in an overlay gel containing antibody-sensitized erythrocytes and C8  $\beta$ -chain-deficient serum. Bands of hemolysis induced by serum from unrelated Caucasians suggested autosomal codominant inheritance of three structural alleles at a single locus, C82: C82°A (acidic), C82°B (basic), and C82°A1 (very acidic) with frequencies of 0.952, 0.044, and 0.004, as well as the probable null allele C82°Q0. The distribution of phenotypes agreed with the Hardy-Weinberg equilibrium.

The previously described genetic polymorphism in human C8 defined with the use of "complete" C8 (C8  $\alpha$ - $\gamma$ -chain)-deficient serum was distinct from and independent of the inherited structural variation at C82. Therefore, the locus for C8  $\alpha$ - $\gamma$ -chains has been redesignated C81, and has the alleles C81°A, C81°B, C81°A1, and C81°Q0. Linkage studies failed to show close linkage between the two loci for C8, C81, and C82, and between C82 and the major histocompatibility complex or C6.

### INTRODUCTION

The eighth component of human complement (C8)<sup>1</sup> is a 151,000-D molecule consisting of three subunits (1, 2). The  $\alpha$ - and  $\gamma$ -subunits are bound covalently

through a disulfide linkage, whereas the  $\beta$ -subunit is associated via weaker, noncovalent bonds.

Inherited deficiency of C8 occurs in two forms. In the first, or "complete" type, the serum of affected homozygotes lacks antigenic material reactive with antiserum to human C8 (3-5) as well as C8 functional activity. It has been shown that such patients are deficient in C8  $\alpha$ -chains but have normal C8  $\beta$ -chains in their serum (6-8). In the second form, although the serum of affected homozygotes lacks C8 function, it contains material reactive with antiserum to human C8, but antigenically deficient in comparison with normal C8 (9, 10). It has been demonstrated that such serum contains normal  $\alpha$ - $\gamma$ -subunits, but is deficient in the  $\beta$ -subunit of C8 (11).

Using serum from a patient with C8 deficiency of the complete type (C8  $\alpha$ - $\gamma$ -chain deficient) as a functional reagent, extensive inherited structural polymorphism in human C8 was demonstrated (12). Patterns given by human sera after isoelectric focusing in polyacrylamide gels consisted of major clear-cut bands of hemolysis that constituted the polymorphism and a poorly defined cathodal zone of hemolysis. Although no explanation for this second zone of hemolysis was at hand, it was suggested that protein-protein complexes containing C8 were involved (12). The C8 genetic types of members of the families of two unrelated homozygous deficient subjects provided clear-cut evidence that the deficiency was determined by a null allele (Q0) at a structural C8 genetic locus (12).

The two kinds of inherited deficiency of C8 could have arisen by a number of possible mechanisms, including some operating on a single cistron for the whole human C8 molecule. If there were inherited structural polymorphism in C8  $\beta$ -chains, it could be used to determine if C8  $\alpha$ - $\gamma$ -chains and C8  $\beta$ -chains

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<sup>1</sup> Abbreviations used in this paper: BF, properdin factor B; C, complement.

are synthesized by a single gene or two distinct genes, and, if two genes, whether these are linked or unlinked.

The present study utilizes serum from patients with deficiency of the  $\beta$ -subunit of C8 to define a second common genetic polymorphism in this protein, to show that this variation is distinct from and independent of the previously described C8 polymorphism and therefore controlled by a separate genetic locus for C8, to show that the C8  $\beta$ -deficiency state may represent a null allele at the structural locus for the  $\beta$ -subunit, to demonstrate the absence of close linkage between the C8  $\beta$ -locus and the C8  $\alpha$ - $\gamma$ -locus or the loci for the major histocompatibility complex or C6 and to provide evidence that the ill-defined zone of hemolysis observed in C8  $\alpha$ - $\gamma$ -subunit typing may represent complete C8 molecules undergoing association and/or dissociation during isoelectric focusing. A preliminary report of these findings has been made (6).

## METHODS

**Serum and plasma samples.** All sera were obtained by centrifugation from blood allowed to clot for up to 1 h at room temperature and were stored at  $-70^{\circ}\text{C}$  or (during shipment) on dry ice. Alternatively, plasma from blood collected in EDTA was similarly stored. One family with C8  $\alpha$ - $\gamma$ -chain deficiency (3), two with C8  $\beta$ -subunit deficiency (11), and families studied for unrelated purposes were tested for genetic polymorphism of the C8  $\beta$ -subunit. In addition, serum samples from randomly selected unrelated Caucasian individuals were analyzed for C8  $\beta$ -subunit polymorphism.

**"C8" genetic typing.** Sera were analyzed for "C8" polymorphism as previously described (12). The method consists basically of isoelectric focusing in polyacrylamide gel of 5  $\mu\text{l}$  of serum diluted 1:4 using a gradient of ampholytes between pH 6 and pH 8. Patterns were developed in a 0.6% agarose overlay gel in veronal-buffered saline at pH 7.4 containing  $1.5 \times 10^{-4}$  M  $\text{Ca}^{++}$  and  $1 \times 10^{-3}$  M  $\text{Mg}^{++}$ , 3% antibody-sensitized sheep erythrocytes and 3% serum from a patient homozygous for C8  $\alpha$ - $\gamma$ -subunit deficiency. In some experiments, 3.1 M urea was incorporated into the isofocusing gel. In that case, urea was removed by soaking the gel in veronal-buffered saline with  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  for 1 h before pouring the overlay gel. Typing for the C8  $\beta$ -subunit was carried out in identical fashion except that, because only an extended smudge of hemolysis was observed in the absence of urea, urea at 3.1 M was incorporated routinely and removed before pouring the detection gel, as outlined above. Moreover, 15  $\mu\text{l}$  of undiluted serum was applied to the isofocusing gel.

**Genetic typing of properdin factor B (BF), C2, C3, C4, and C6.** Typing of BF by agarose gel electrophoresis and immunofixation was described previously (13). C3 types were determined by agarose gel electrophoresis and protein staining (14). Neuraminidase-treated serum or plasma was analyzed for C4 types by electrophoresis in agarose gel and immunofixation as described previously (15). C2 and C6 variants were detected by isoelectric focusing in polyacrylamide gels and specific agarose gel overlays, as described earlier (16, 17).

**Linkage studies.** Linkage was analyzed by hand, by standard methods (18, 19). Logarithms of the odds at different  $\theta$  (recombination fractions) were calculated.

**Purified C8  $\beta$ -chains.** Purified C8  $\beta$ -chains >90% free of  $\alpha$ - $\gamma$ -chains were a gift of Dr. James Sodetz, University of South Carolina. They were prepared as described by him (2).

**Antiserum to C8.** Antiserum to human C8 produced in goats was obtained from Atlantic Antibodies, Scarborough, ME. It produced a single precipitin line on Ouchterlony analysis against normal human serum.

## RESULTS

**Genetic polymorphism in C8  $\beta$ -chains in normal individuals.** Patterns of C8  $\beta$ -chain-induced hemolysis developed from random sera after isoelectric focusing are shown in Fig. 1. Most samples gave a pattern we have called B (for basic), consisting of a major band of hemolysis and two less intense flanking bands. Some individual sera, however, showed an anodal duplication of the major and minor bands (A), as also shown in Fig. 1. In rare instances, this second set of bands was even more anodal than A bands (A1). We hypothesized that the B pattern resulted from homozygosity for the common form of C8  $\beta$ -chains at a locus designated C82 and such donors were thus C82 $^{\circ}$ B/C82 $^{\circ}$ B. Those with sera producing the additional set of anodally duplicated bands would be C82 $^{\circ}$ B/C82 $^{\circ}$ A heterozygotes, and those rare individuals with the rare anodal variant would be C82 $^{\circ}$ B/C82 $^{\circ}$ A1. These hypotheses were tested in family studies (Table I) and in a population of randomly selected unrelated individuals (Table II). From the family studies, it is evident that C82 variants are inherited as autosomal codominant traits and the ratios of offspring were close to those predicted by Mendelian inheritance. The distribution of types among Caucasians was close to that predicted by the Hardy-Weinberg equilibrium, also consistent with the proposed genetic

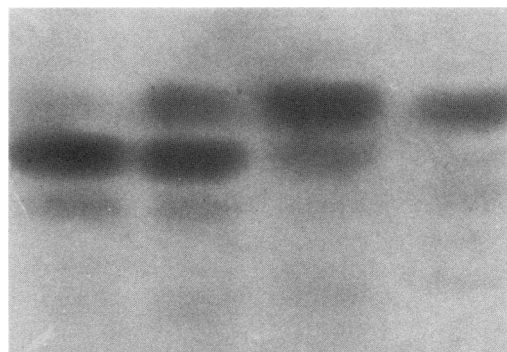


FIGURE 1 Patterns produced by isoelectric focusing of sera in 3.1 M urea and development in an overlay gel containing antibody-sensitized erythrocytes and C8  $\beta$ -chain-deficient serum. From left to right, types are A, AB, B, and B (from an individual homozygous deficient for complete C8 deficiency).

TABLE I  
C82 (C8  $\beta$ -Chain) Types in Families

Parental types	No. of families	Offspring types		
		AB	BB	A1B
BB $\times$ BB	7		18 (18)*	
AB $\times$ BB	11	16 (12)	8 (12)	
A1B $\times$ BB	1		3 (2)	1 (2)

\* Expected number is given in parentheses.

model. C82 types occurred entirely independent of previously defined C8 (C8  $\alpha$ - $\gamma$ ) types in sera from random unrelated persons.

*C8 (C81 or  $\alpha$ - $\gamma$ ) types in C8  $\beta$ -chain-deficient families.* When tested using C8  $\alpha$ - $\gamma$ -subunit-deficient serum, samples from heterozygous and homozygous C8  $\beta$ -chain-deficient family members gave normal C81 typing patterns, except that the cathodal blurred zone of hemolysis was reduced in intensity in heterozygous C8  $\beta$ -chain-deficient serum and absent from homozygous deficient serum (Fig. 2). There was also a suggestion that the typing bands themselves were reduced in intensity compared with normal, particularly in homozygous  $\beta$ -subunit-deficient sera, but this was not further investigated.

*C82 types in families with C8  $\beta$ -chain deficiency.* C82 types in two families with C8  $\beta$ -chain deficiency were determined. In almost every instance, serum from relatives of heterozygous C8  $\beta$ -chain-deficient subjects gave C82 B patterns. In one case, a serum from a paternal grand uncle of the propositus gave a C82 A pattern. Thus, most family members were either C82\*B/C82\*B or C82\*B/C82\*Q0. The grand uncle was either C82\*A/C82\*A or C82\*A/C82\*Q0.

*C82 types in a family with C8  $\alpha$ - $\gamma$ -deficiency.* Sera from a previously reported family (3, 12), one of whose members was homozygous for C8  $\alpha$ - $\gamma$ -subunit deficiency were subjected to C82 typing. All samples, including that from the proposita, gave C82 B patterns indistinguishable from those of random normal individuals, consistent with the presence of normal  $\beta$ -chains in C8  $\alpha$ - $\gamma$ -deficient serum. Reexamination of

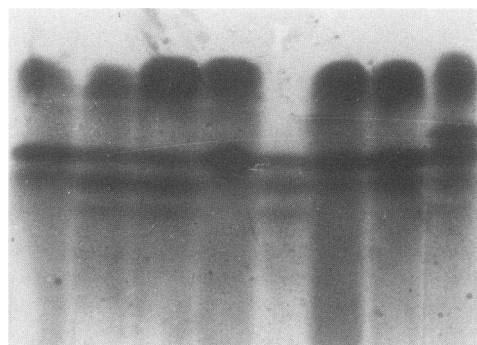


FIGURE 2 C8 (C81) types in C8  $\beta$ -chain-deficient sera. Patterns were obtained by isoelectric focusing of serum samples in polyacrylamide gel and development in an agarose gel overlay containing antibody-sensitized sheep erythrocytes and C8  $\alpha$ - $\gamma$ -chain-deficient serum. From left to right, patterns are A: heterozygous deficient; AA1: heterozygous deficient; AA1: normal; A: normal; AA1: homozygous deficient; A: normal; A: normal; AB: normal.

such C8  $\alpha$ - $\gamma$ -deficient serum in Ouchterlony analysis with a potent goat anti-human C8 serum provided further evidence for the presence of C8  $\beta$ -chains (Fig. 3). A line of identity between purified C8  $\beta$ -chains and serum from the individual homozygous for complete C8 deficiency was obtained. This line fused with the C8 line produced by normal human serum, but with spur formation over the isolated  $\beta$ -chains or the deficient serum, as expected. Thus, C8  $\beta$ -chains of normal isoelectric point could be detected functionally and immunochemically in C8-deficient serum, confirming that the deficiency in such individuals is in C8  $\alpha$ - $\gamma$ -chains only. Given the complete independence of the genetic polymorphisms and deficiency states in C8  $\alpha$ - $\gamma$ - and C8  $\beta$ -subunits, we propose to rename the genetic locus controlling C8  $\alpha$ - $\gamma$ -chains as C81. Its

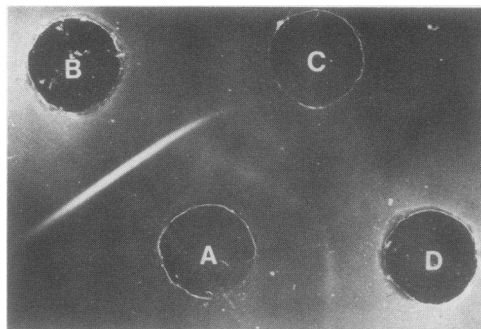


FIGURE 3 Ouchterlony analysis of sera with anti-C8 (A). Samples were normal human serum (B), purified  $\beta$ -chains (C), and serum from a patient with C8  $\alpha$ - $\gamma$ -chain deficiency (D).

TABLE II  
C82 (C8  $\beta$ -Chain) Variants among Random Unrelated Caucasian Individuals

	C82 B	C82 AB	C82 A1B	C82 AA
Observed ( $n = 125$ )	114	9	1	1
Expected	(113.3)	(10.5)	(0.95)	(0.2)

TABLE III  
Logarithms of the Odds (Lod) for Linkage between C82  
and Several Complement Loci

Linkage between C82 and:	Informative meioses		Recombination fraction $\theta$		
	Phase known	Phase unknown	0.01	0.05	0.10
C81	4	2	-2.512	-1.184	-0.673
C2-BF-C4A-C4B	4	2	-2.512	-1.184	-0.673
C3	0	4	0.584	0.516	0.430
C6	4	2	-2.512	-1.184	-0.673

known alleles would be C81<sup>A</sup>, C81<sup>B</sup>, C81<sup>A1</sup>, and C81<sup>Q0</sup> (the null allele defining the deficiency state) in accord with previous findings (12).

**Linkage studies of C82.** The results of linkage studies between C82 and some other complement loci are given in Table III. Although informative meioses were few, there was no evidence of close linkage of C82 to C81, C6, or the major histocompatibility complex loci BF, C2, C4A, and C4B. Although no recombinants were noted for C82-C3, the number of informative meioses was too small to permit a definitive conclusion.

**Studies of the ill-defined zones of hemolysis seen in C8  $\alpha$ - $\gamma$ -typing.** It seemed likely from the foregoing that the ill-defined zones of hemolysis seen in C8  $\alpha$ - $\gamma$ -typing represented complete C8 molecules, some of which were in the process of dissociating into  $\alpha$ - $\gamma$ - and  $\beta$ -chains or associating from these subunits during isoelectric focusing. To test this hypothesis, 3.1 M urea was added during isoelectric focusing and removed before pattern detection. In a separate exper-

iment, purified C8  $\beta$ -chains were added to  $\beta$ -chain-deficient serum before analysis for C8  $\alpha$ - $\gamma$ -types. The results were as follows. Inclusion of urea during isoelectric focusing eliminated the ill-defined zone of hemolysis (Fig. 4). The addition of  $\beta$ -chains to C8  $\beta$ -chain-deficient serum resulted in the appearance in isofocusing without urea of an ill-defined zone of hemolysis indistinguishable from that produced by normal serum.

**Effects of storage on C81 and C82 patterns.** On storage of EDTA plasma (but not serum) samples for more than 5 h at 37°C, there was an anodal shift in the patterns produced by C82 variants (Fig. 5), complete by 3 d. This was also true of C81 variants.

## DISCUSSION

The results of this study demonstrate that there are two genetic loci for human C8 and that these loci are not closely linked to each other or to loci for the major histocompatibility complex-linked complement proteins B, C2, or C4, or that for C6. This was definitively shown in formal linkage analysis using the two distinct inherited structural polymorphisms in C8. The demonstration that there are free  $\beta$ -chains (6-8) in what had previously been thought to be complete C8 deficiency (3-5) is consistent with this model for C8 genetics. To distinguish the genetic locus (loci) producing

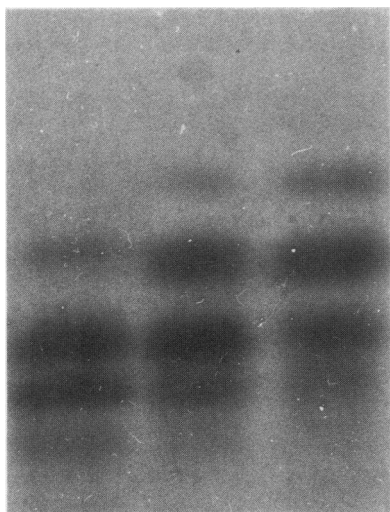


FIGURE 4 C8 (C81) types on isoelectric focusing of serum in the presence of 3.1 M urea. From left to right, the types are A, AB, and B. Note the absence of the cathodal ill-defined zone of hemolysis visible in Fig. 2.

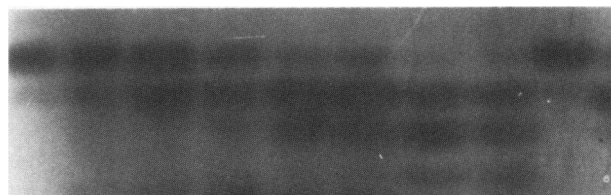


FIGURE 5 C82 patterns produced by EDTA plasma samples, fresh, aged at 37°C, and frozen and thawed. The patterns are, from left to right, B fresh, B aged 5 h, 16 h, 24 h, 3 d, 5 d, AB aged 5 d, A aged 5 d, B thawed and frozen five times, AB fresh. It is evident that changes are produced after 5 h and appear to be complete by 3 d. Freezing and thawing five times produced no change.

C8  $\alpha$ - $\gamma$ -chains from that synthesizing C8  $\beta$ -chains, we have introduced the designations C81 and C82, respectively. The allele specifying the first-described complete C8 deficiency is thus C81<sup>°</sup>Q0 (for quantity zero) and that for the antigenically deficient form is C82<sup>°</sup>Q0, in conformity with the recently proposed human gene nomenclature (20). The previous failure to observe material reactive with anti-C8 in complete (C81) C8-deficient serum appears to reflect the fact that most antisera previously produced against whole C8 react only or almost entirely only with C8  $\alpha$ - $\gamma$ -chains.

There are a number of other plasma proteins comprising subunits produced by distinct and unlinked genes. Examples are the immunoglobulins (21) and haptoglobin (22). The absence of covalent bonds between the  $\alpha$ - $\gamma$ -chains on the one hand and  $\beta$ -chains of C8 on the other facilitates the separate analysis of these polypeptides for structural polymorphism. We had been puzzled earlier (12) by the presence of the cathodal blurred zones of hemolysis observed when C8-induced patterns of hemolysis were obtained after the isoelectric focusing of normal sera by use of C8  $\alpha$ - $\gamma$ -deficient serum. It is now probable that these zones represent complete C8 molecules in the process of associating and/or dissociating. Since  $\beta$ -chain patterns consist only of such blurred zones whereas  $\alpha$ - $\gamma$ -patterns include both clear bands and blurred zones, it appears that there is normally an excess of C8  $\alpha$ - $\gamma$ -chains relative to C8  $\beta$ -chains. By incorporating 3.1 M urea in the isoelectric focusing gels, dissociation is favored and clear-cut bands without blurred zones are obtained.

The "conversion" of both C81 and C82 polymorphic patterns to somewhat more acidic positions creates a problem in typing that is best solved by examining only serum or only fresh EDTA plasma samples and reference samples. We have not further analyzed the nature and cause of this change in isoelectric point with storage.

The fact that a paternal grand uncle of a C8  $\beta$ -chain-deficient subject expressed only a relatively uncommon variant of the  $\beta$ -chain, C82 A, suggests, but certainly does not prove, that the deficiency state is an allele at the structural locus. Unfortunately, this individual did not have children and his parents were dead. From the fact that one of his siblings was a grandparent of two homozygous deficient children and therefore had an increased risk for being a heterozygote for C8  $\beta$ -chain deficiency, one may infer that he also had a greater than normal (1:4) chance of also being heterozygous for the deficiency. His C8  $\beta$ -type was C82 A, an ambiguous phenotype in this case, representing either the genotype C82<sup>°</sup>A/C82<sup>°</sup>A or the genotype C82<sup>°</sup>A/C82<sup>°</sup>Q0. The first has a frequency of (0.044)<sup>2</sup>

or 0.0019 and the latter in him has a probability of  $\frac{1}{4} \times 0.044$  or 0.011. Thus, it is approximately six times more likely that his genotype is C82<sup>°</sup>A/C82<sup>°</sup>Q0. A complication is the possibility that this person's type is C82 B but "aging" has made it appear to be C82 A. This is rendered unlikely because all samples from the family were handled in identical fashion and all other sera typed as C82 B.

#### ACKNOWLEDGMENT

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