Genetic Analysis of C4 Deficiency

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ABSTRACT The inherited structural polymorphism in the fourth component of complement was studied in the family of a child with homozygous deficiency of this protein. It was shown that a number of family members, including the child's parents, carried a C4 haplotype, *C4A*QO C4B*QO*, that produced no detectable protein at either the Chido (C4B) or Rodgers (C4A) locus. The family contained individuals with one, two, three, or four expressed C4 genes, and the mean serum C4 levels in such individuals roughly reflected the number of structural genes.

INTRODUCTION

Inherited deficiency of C4 has been well documented in two individuals, both of whom had systemic lupus erythematosus (1, 2). Using C4 concentrations in serum to identify carriers, evidence has been presented that C4 deficiency is inherited as an autosomal Mendelian trait, with affected persons homozygous for a deficiency "allele" very closely linked to the major histocompatibility complex (MHC)[1] (2, 3). However, such analysis is very much complicated by the extremely wide range of serum C4 levels in normal persons (2) as well as the fact that C4 is produced by genes at two distinct but very closely linked loci. Moreover, "half null" haplotypes (deletions at one or the other but not both C4 loci on any given chromosome) are common in Caucasians (4, 5).

We have recently developed methods for detecting the half null haplotypes in individuals who also carry "full" C4 haplotypes (6). We have also introduced a typing system that allows the detection of six common structural alleles at the Rodgers (C4A) locus and two or three at the Chido (C4B) locus (7) in whites. The present report presents an analysis of C4 types in members of a family with a C4-deficient propositus (2) and provides evidence that in this family C4 deficiency results from homozygosity for a rare, double null haplotype of the two linked C4 structural loci.

METHODS

Samples. The individuals in this study are from a family with inherited C4 deficiency described previously (2), except for one additional child (III-6), born since that study was published. Blood was collected into EDTA and plasma was promptly stored at −80° and thawed just before analysis.

Human factor B(BF) and C2 typing. For BF, plasma samples were subjected to agarose gel electrophoresis and immunofixation (8) with goat antiserum to human factor B (Atlantic Antibodies, Scarborough, Maine). Plasma samples were subjected to isoelectric focusing in thin-layer polyacrylamide gel, and C2 patterns were developed as previously described (9).

Analysis of C4. Concentration of C4 in the plasma samples was measured immunochemically as previously described (10) and the results expressed as percentage of that in normal pooled serum. Analysis of C4 genetic polymorphism was carried out on desialated samples. Plasma samples were incubated with neuraminidase from *Clostridium perfringens* (Type VI, Sigma Chemical Co., St. Louis, Mo.) at a concentration of 10 mU of enzyme/μl of plasma for 15 hr at 4°C while dialyzed against 0.1 M phosphate buffer, pH 6.8, containing 0.005 M Na_2 EDTA.

Desialated plasma samples were subjected to crossed immunoelectrophoresis for detection of half-null haplotypes as described previously (6). For the detection of C4 structural variants, desialated plasma samples were subjected to immunofixation electrophoresis in 0.75% agarose (ICN Nutritional Biochemicals, Cleveland, Ohio) using a discontinuous Tris/glycine/barbital buffer (7) and 0.005 M Na_2 EDTA in the gel. Electrophoresis was carried out on a cooled plate using a circulating water bath at 30 V/cm for 6 h. C4 bands were visualized by immunofixation with anti-human C4 antiserum (Atlantic Antibodies).

Nomenclature. The nomenclature for genetic polymorphism of human C4 used in this work is the same as that proposed earlier (7), which was designed to conform to the recently formulated International System for Human

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Dr. Ochs is an Investigator of the Howard Hughes Medical Institute.

Received for publication 28 May 1980 and in revised form 2 September 1980.

[1] *Abbreviation used in this paper*: MHC, major histocompatibility complex.

Gene Nomenclature (1979) (11). This recommends that gene locus names consist of capital letters or letters and Arabic numerals up to four characters in length and all written on the same line. Alleles are written on the same line as the gene and separated from it by an asterisk. Genes and alleles are underlined or italicized. Phenotypes are similarly designated except that a space is used rather than an asterisk to separate gene name and variant and phenotypes are neither underlined nor italicized. Null alleles and variants are designated "QO." The locus controlling the acidic Rodgers positive [Rg(a+)] C4 variants was designated as *C4A* and that controlling the basic Chido positive [Ch(a+)] variants as *C4B*. Among unrelated Caucasians, six structural variants and one null variant were detected at the A locus, designated C4A 1–6 and C4A QO. At the B locus, there were two structural variants and one null variant, B 1, 2 and B QO.

RESULTS

The C4 types of all members of the family studied are shown in Fig. 1 together with their MHC, glyoxalase (GLO), BF, and C2 types. The plasma C4 concentrations of each individual are given at the upper right of each symbol and were calculated as the mean of two to four immunochemical estimations as previously reported (2). Fig. 2 shows the crossed immunoelectrophoretic and immunofixation patterns on which the types for each individual were based.

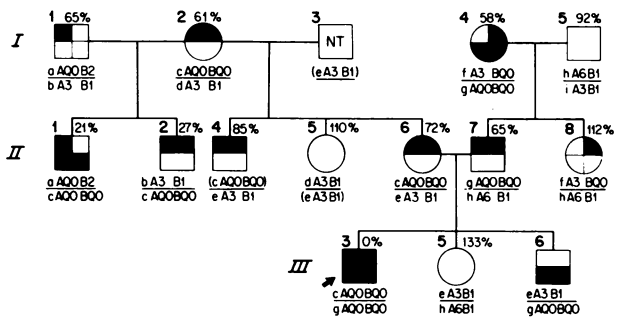

FIGURE 1 Pedigree of the family with inherited deficiency of C4. Males are shown as squares, females as circles. Serum C4 levels as determined immunochemically and reported previously (2) are given at the upper right of each symbol as percentage of the mean normal serum level. C4 genetic types are given below each symbol with their linked, coded MHC haplotypes. Deficiencies at each of the four C4 genetic loci in any single individual are shown as a blackened quadrant, the upper portion of each symbol representing the two loci on one chromosome (*C4A* on the left, *C4B* on the right), the lower portion the two loci on the other chromosome. Presumed types are given in parentheses. The MHC haplotypes were as follows:

	HLA-A	B	D	BF	C2	GLO
a	2	w22	×	S	C	2
b	11	5	×	S	C	2
c	2	12	w2	S	C	1
d	2 or ×	12 or ×	w2 or ×	S	C	1
e	w23	w15	w1	F	C	1
f	2	12	w4	S	C	2
g	2	w15	ND108	S	C	1
h	1	w17	×	S	C	1
i	9	w27	×	S	C	1

It is seen that the parents of the C4-deficient propositus (II-6 and II-7) express only a single *C4A* and *C4B* structural gene each and are apparent C4 homozygotes. However, since one of the parents of II-7 carries *C4A*6* and *C4B*1* on the same chromosome as the haplotype *C4A*6,B*1*, his other chromosome must carry *C4A*QO,B*QO*, transmitted from his mother, I-4, who expresses only a single C4 gene *C4A*3*.

The individual I-2 must also be a carrier of *C4A*QO B*QO* since her child, II-1, expresses only one of his father's C4 types, *C4B*2*, the other haplotype therefore must be *C4A*QO B*QO*. This same haplotype is carried by his half sister, II-6, the mother of the propositus.

The only remaining ambiguity in this family is in individuals II-4 and II-5. They could either be A3B1/A3B1 or A3B1/AQOBQO. The C4 structural variants in these family members were not informative since both parents, I-2 and I-3, carry A3B1. As deduced from the C4 levels in the plasma of these two individuals, they were assigned the C4 types shown.

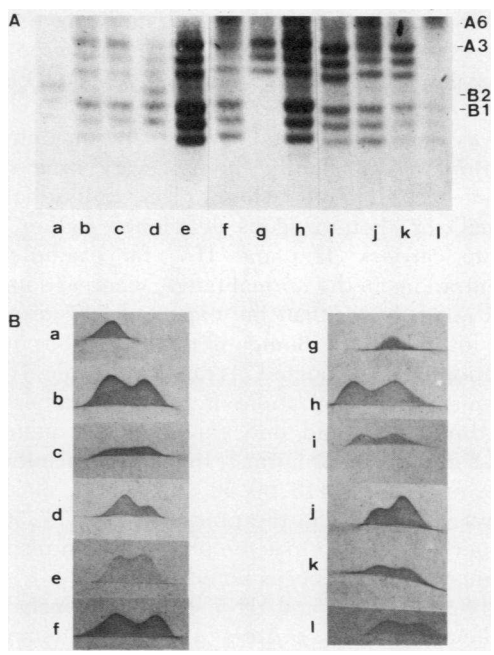

FIGURE 2 Electrophoretic (A) and crossed immunoelectrophoretic (B) patterns of neuraminidase-treated plasma samples from the members of the C4-deficient family of Fig. 1. (a) II,1; (b) I,2; (c) II,2; (d) I,1; (e) II,5; (f) I,5; (g) I,4; (h) III,5; (i) II,6; (j) II,8; (k) II,4; (l) II,7. The anode for the separation in A was at the top and that for the initial electrophoresis in B at the right.

DISCUSSION

The deficiency states for C3 (12), C2 (13), C6 (14), and C8 (15) have been previously shown to be the result of inheritance of null or "blank" alleles at the respective structural loci for these complement proteins. Such null alleles at single structural loci are demonstrated by the inheritance in heterozygous carriers (preferably parents or children of markedly deficient subjects) of no more than one structural allele present in only one of the carrier's parents. Because there are two closely linked loci controlling synthesis of C4 (5, 7), the analogous blank haplotype would be seen in heterozygotes as the presence of only one expressed structural haplotype and by inheritance of only one expressed structural haplotype present in only one parent. Such evidence is available in the present study in the instance of II-7, father of the homozygous C4-deficient child, and in II-1. Individual II-7 has only two expressed structural C4 genes, *C4A*6* and *C4B*1*. From the types of his parents, it is clear that both genes are on one haplotype, *C4A*6,B*1*, inherited from his father, I-5. His mother has only a single expressed C4 structural gene, *C4A*3*. Therefore, both II-7 and his mother have inherited a doubly deficient C4 haplotype, *C4A*QO, B*QO*. A similar argument applies to II-1, on the other side of the family. All heterozygotes for C4 deficiency on both sides of the family have only a single expressed C4 haplotype. Thus, the type of the homozygous C4-deficient child is *C4A*QO,B*QO/ C4A*QO,B*QO*.

It was previously noted (2) that C4 concentrations in serum in this family did not vary in a simple fashion according to whether an individual was "normal" or "heterozygous deficient." Rather, some obligate carriers (II-6 and II-7, for example) had concentrations in the normal range, whereas some had levels much lower than the expected 50% of normal (II-1, for instance). Some of this variation in concentration from "expected" is now explicable. If mean antigenic C4 concentrations in family members with four, three, two, and one expressed C4 genes are expressed as percent normal, then the concentrations rank as expected at 113, 89, 56, and 39. The means are somewhat higher than the predicted 100, 75, 50, and 25%, perhaps related to acute phase protein responses in some of the subjects as noted earlier (2).

Individuals II-4 and II-5 had previously been classified as being carriers of C4 deficiency and normal. In fact, it was not possible to determine from other MHC markers whether they had inherited the deficiency gene from their mother, I-2, because the latter was apparently homozygous for all markers and the father, I-3, was dead. The C4 haplotypes in I-2 were different (A3B1 and AQOBQO) and distinguished the MHC on one chromosome from that on the other, further suggesting that II-2 was homozygous for HLA, BF, and GLO. Nevertheless, II-4 and II-5 were ambiguous for C4 genotype. Phenotypically, they were C4A3 B1 and this could represent either of two genotypes: *C4A*3,B*1/C4A,*3,B*1* or *C4A*3,B*1/C4A*QO,-B*QO*. That the *C4A*QO,B*QO* haplotype was present in II-4 and II-6 was inferred from their children, III-1 (with 27% normal C4 concentration), and the homozygous-deficient subject, III-3.

The C4 haplotype giving rise to "complete" C4 deficiency when present in the homozygous state is clearly rare. There are less than half a dozen known cases and only two or three instances that have been documented in detail (1, 2). It may at first seem odd that this is so, since null alleles at one or the other of the two C4 genetic loci are rather common with frequencies of 0.10 to 0.15 in whites. The explanation lies in the very close linkage between *C4A* and *C4B* and the resultant extreme linkage disequilibrium between alleles at the two loci (7). For example, the C4A allele *C4A*4* was found to be in linkage disequilibrium with *C4B*2* to form the haplotype *C4A*4,C4B*2* on each of >25 chromosomes on which *C4A*4* occurred, and *C4B*QO* occurs in linkage with *C4A*3* in almost each instance. In the same manner, one may presume marked linkage disequilibrium between *C4A*QO* and *C4B*QO* in the haplotype leading to complete deficiency.

The situation appears to be precisely analogous to α-thalassemia in blacks and Orientals. In blacks, only one of the two α-globin genes on a given chromosome may be deleted, giving rise to the silent carrier state in the heterozygote and α-thalassemia trait in the homozygote (16). In southeast Asia, the double deletion occurs on some chromosomes leading to α-thalassemia trait in the heterozygote and total α-globin deficiency (hydrops fetalis) in the homozygote (17).

ACKNOWLEDGMENTS

This work was supported by the following grants from the National Institutes of Health: AI 14157, AM 16392, AI 15033, and RR-37 (Clinical Research Center Facility of the University of Washington).

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