A Unique Recombination Event Resulting in a *C4A*Q0,C4B*Q0* Double Null Haplotype

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Abstract

The fourth component of complement (C4) is encoded by two closely linked genes (*C4A* and *C4B*) within the MHC. Null alleles at either locus (*C4AQ0* or *C4BQ0*) are relatively common, occurring at the *C4A* locus in ~ 10% of normal individuals and at the *C4B* locus in ~ 16% of normal individuals. However, the presence of the double null haplotype (*C4A*Q0,B*Q0*) on the same chromosome is extremely rare. We recently studied a 7-yr-old patient with recurrent sinopulmonary infections in whom we documented the mechanism by which the *C4A*Q0,B*Q0* double null haplotype arose. Evaluation revealed significantly reduced levels of both C4 antigen and C4 hemolytic activity. Analysis of extended haplotypes in the family was performed using MHC typing and genomic DNA analysis. The patient was found to have a *C4A*3,B*Q0* haplotype and a *C4A*Q0,B*Q0* haplotype. The *C4A*3,B*Q0* haplotype was contributed by the father. The mother possessed a *C4A*Q0,B*1* haplotype and a *C4A*3,B*1* haplotype. The first maternal haplotype was involved in a recombination event within the C4B locus on her other chromosome and resulted in a new C4B*Q0 null allele and the patient's *C4A*Q0,B*Q0* haplotype. Segregation analysis mapped the recombination to a region 3′ to the unique 6.4-kb TaqI restriction fragment of the maternal C4B locus. This is the first demonstration of a recombination event producing a C4 double null haplotype. (*J. Clin. Invest.* 1992. 90:1180–1184.) Key words: C4 deficiency • complement • C4A • C4B • major histocompatibility complex

Introduction

The fourth component of the human complement system (C4) is encoded by two closely linked genes within the MHC on the short arm of chromosome 6 (1). The protein products of these two loci are termed C4A and C4B. Although they share certain structural and functional characteristics that identify them as C4, they also differ with respect to other characteristics, such as electrophoretic mobility (2), hemolytic efficiency (3, 4), and unique epitopes (5).

There is a relatively high frequency (5–20%) of nonexpressed variants, termed *C4* null alleles (*C4*Q0*), at either C4 locus in normal individuals (6). As a result, homozygous C4A deficiency (C4AQ0,Q0) occurs in ~ 1% of the population and homozygous C4B deficiency (C4BQ0,Q0) is found in ~ 3% of individuals. Interestingly, even though deficiencies of C4A and C4B are relatively common, the double null haplotype (*C4A*Q0,B*Q0*), referring to the presence of a *C4A*Q0* and *C4B*Q0* on the same chromosome, is rare (6). Similarly, complete C4 deficiency (*C4A*Q0,B*Q0/C4A*Q0,B*Q0*) is extremely rare, with only about 20 cases reported worldwide (7).

The clinical manifestations of C4 deficiency are variable. Patients with complete C4 deficiency have presented with a lupus-like syndrome and increased susceptibility to infection (6, 7). Heterozygous and homozygous C4A deficiencies have been reported in association with systemic lupus erythematosus (SLE) and other autoimmune diseases (8, 9). Homozygous C4B deficiency has been reported in association with bloodborne bacterial infections (10, 11), Henoch-Schönlein purpura (12, 13) and IgA nephropathy (13, 14). Null alleles at C4 loci have been attributed to deletions, gene conversions, and nonexpressed alleles; however, the mechanisms by which the *C4A*Q0,C4B*Q0* double null haplotype arise have not been elucidated. We present here the results of a study in which the *C4A*Q0,C4B*Q0* double null haplotype appeared within an informative family, and was related to a maternal recombination event within the *C4B* locus.

Methods

Case report

The patient is a 7-yr-old white male with a history of recurrent sinopulmonary infections, asthma, and diarrhea since infancy. He was the product of a full term gestation that was complicated by preeclampsia. The patient was well until 7 mo of age, when he developed recurrent episodes of diarrhea, frequently resulting in dehydration and requiring several hospital admissions. Episodes of wheezing, recurrent otitis media, pharyngitis, sinusitis, and pneumonia began at 16 mo of age and have persisted to the present, with only some decrease in frequency. Physical examination was normal except for scarred tympanic membranes and swollen nasal turbinates. Height and weight were at the 50th percentile for that age. Maternal family history was positive for juvenile rheumatoid arthritis and hyperthyroidism in the mother, and SLE in a maternal aunt.

Laboratory evaluation revealed normal serum immunoglobulin levels and normal antibody responses to diphtheria, tetanus, and *H. influenza* type b vaccines. A CH50 was 22 U/ml (normal = 20–55 U/ml). Although serum levels of C4 antigen were markedly diminished (< 5.4 mg%; normal = 12–42 mg%), C4 antigen was still detectable by double immunodiffusion. Further studies were then conducted to determine the basis for the C4 deficiency.

Procedures

Methods

Complement assays. Hemolytic titrations of C1 (15), C4 (16), C2 (17), and C3 (18) were performed by established methods using red

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cell intermediates and purified complement components (Diamedix Corp., Miami, FL). Normal ranges for individual components were determined in 25 healthy adults. Purified C4 was prepared using a modification of a technique previously described (19).

Complement and HLA phenotyping. Phenotyping of C4 was performed on frozen plasma samples using a standard technique (2, 20). Samples were treated with carboxypeptidase B (Sigma Chemical Co., St. Louis, MO) and type VIII neuraminidase (Sigma) and analyzed by high voltage electrophoresis in agarose gel, followed by immunofixation with goat anti-human C4 antiserum (Atlantic Antibodies, an Incstar Co., Stillwater, MN). In addition, SDS-polyacrylamide gel electrophoresis was performed on immunoprecipitated C4 from the proband in order to determine the molecular weight of the C4 alpha chain (21). Phenotyping of C2 was performed by isoelectric focusing in polyacrylamide gels (22). Factor B phenotyping was done using agarose gel electrophoresis (23). HLA typing for antigens at the *HLA-A, -B,* and *-C* loci was performed by a microlympho-cytotoxicity assay (24). *HLA-DR, -DQ* typing was done by a double fluorescence cytotoxicity assay (25).

Preparation of genomic DNA and Southern blotting. DNA was prepared from individual blood samples anticoagulated with EDTA. The DNA was extracted from white blood cells and purified by sucrose sedimentation according to standard procedures (26). Individual DNA samples of 1.5 μg were digested with restriction enzymes (TaqI, NlaIV, Eco0109) at concentrations recommended by the manufacturer (Bethesda Research Laboratories, Gaithersburg, MD). Southern blotting was performed by standard techniques, and the blots were probed with a 0.6-kb 5′ end fragment of C4 cDNA (pAT-A) (27) and a 2.1-kb cDNA fragment of 21-hydroxylase (*CYP21;* p21/3c) (28).

Results

Complement assays. The concentration of functional C4 in the patient's serum was 11% of normal. The addition of purified C4 to the patient's serum reconstituted total hemolytic activity in a dose-dependent fashion, thus confirming the absence of an inhibitor. The functional activities of C1, C2, C3, and C1 esterase inhibitor, as well as antigenic levels of C3 and C1 esterase inhibitor were normal.

Complement phenotyping. The results of the C4 phenotyping performed on the proband and immediate family members are shown in Fig. 1. No C4B allotypes were seen in the proband. Only a single, somewhat faint C4A3 band could be seen on the gel. An aliquot of the patient's serum was also concentrated 10-fold by precipitation with 16% polyethylene glycol and reconstituted with PBS (19). This material was subjected to C4 phenotyping and again demonstrated the presence of a single C4A3 band, but no C4B allotypes. An SDS-PAGE performed on an immunoprecipitated serum sample revealed only a 96-kD band representative of the C4A alpha chain. No detectable 94-kD alpha-chain was seen, thus confirming the phenotypic absence of C4B in the proband (Fig. 2).

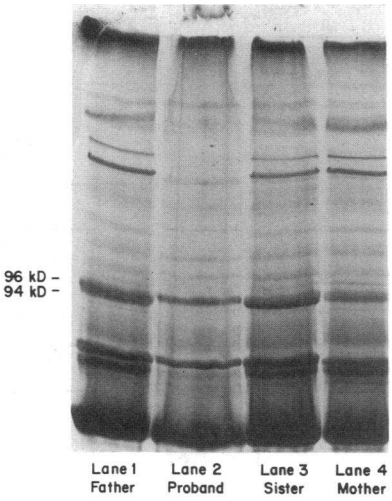

Figure 2. SDS-PAGE of immunoprecipitated serum samples from the proband and family members. No 94-kD band representative of the C4B alpha-chain was present in the proband.

The proband's mother demonstrated a C4A3,Q0,B1,1 phenotype, while both the father and an older sister demonstrated a C4A3,2,B1,2 phenotype (Fig. 1). The sister had an increased intensity of the C4B1 band, a finding compatible with a previously described extended haplotype, *C4A*2,B*(1,2),* which contains a duplicated C4B locus (29). The presence of this extended haplotype was confirmed by Southern blot analysis (see below). Analysis of the pedigree (Fig. 3) demonstrated that the father must also carry this haplotype containing the duplicated C4B locus. However, he did not demonstrate the expected increased intensity of his C4B band (Fig. 1), a finding consistent with the presence of a C4BQ0 allotype on his other chromosome. Although one of the proband's *C4BQ0* alleles was donated by the father, his second *C4BQ0* was not present on either maternal haplotype. Parental C4 haplotype analysis,

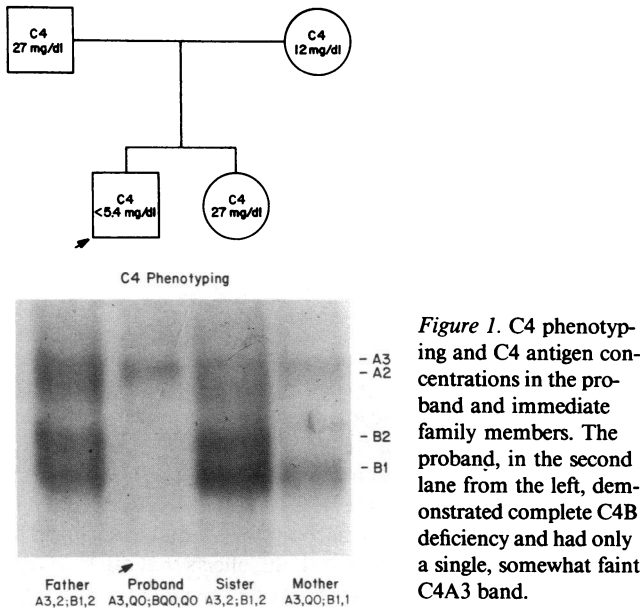

Figure 1. C4 phenotyping and C4 antigen concentrations in the proband and immediate family members. The proband, in the second lane from the left, demonstrated complete C4B deficiency and had only a single, somewhat faint C4A3 band.

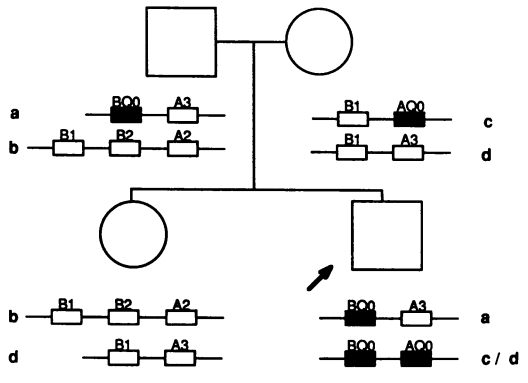

Figure 3. Family pedigree and C4 phenotyping. The proband (*bottom right*) has no detectable C4B protein as indicated by the two BQ0 notations.

Table I. HLA Haplotypes

		DRW	DQ	DR	B	C	A
Father	(a)	—	1	2	7	7	3
	(b)	52	4	8	14	8	33
Mother	(c)	—	1	2	8	7	1
	(d)	53	3	4	62	3	2
Proband	(a)	—	1	2	7	7	3
	(c/d)	53	3	4	8	7	1
Sister	(b)	52	4	8	14	8	33
	(d)	53	3	4	62	3	2

HLA typing was performed on the proband and immediate family members. The proband has inherited a haplotype ("c/d,") which was the consequence of a maternal recombination event. The recombination event occurred between HLA-B and HLA-DR, which is precisely the region in which the C4 loci are found on chromosome 6.

therefore, was not compatible with the complete C4B deficiency seen in the proband. This supports the conclusion that a de novo *C4BQ0* allele has appeared in the proband.

HLA phenotyping. Analysis of HLA phenotyping suggested that the proband had inherited a haplotype that was the consequence of a maternal recombination event occurring between HLA-B and HLA-DR (Table I). Given that the recombination event occurred in the region on chromosome 6 in which the *C4* loci are found, we considered the possibility that this could be responsible for the appearance of the new *C4B* null allele seen in the proband.

Southern blot analysis. To further localize the recombination event, Southern analysis using *C4* and 21-hydroxylase specific cDNA probes was performed. Probes for 21-hydroxylase genes have been useful in studying C4 polymorphisms, since the functional gene encoding adrenal 21-hydroxylase B (*CYP21B*) is < 2 kb downstream from *C4B* and 21-hydroxylase A (CYP21A), a pseudogene, is 2 kb downstream from *C4A* (30, 31). Fig. 4 *A* represents a Southern blot probed for *C4* TaqI restriction fragment length polymorphisms (RFLP)[1] (32). The *C4A* locus is represented by a 7.0-kb fragment. The *C4B* locus is represented by either a 6.0-kb (long gene) or a 5.4-kb (short gene) fragment depending on the presence or absence of an intron (31). In addition, a specific 6.4-kb TaqI fragment has been described, which represents a single short *C4B* gene on a haplotype with a deletion of both the *C4A* and *CYP21A* genes (32).

The proband has two gene fragments: a 7.0-kb fragment and the unique 6.4-kb fragment (Fig. 4 *A*). This 6.4-kb fragment is present in the mother, confirming the presence of her C4AQ0 allele demonstrated by C4 phenotyping (Fig. 1). The 6.4-kb fragment is also present in the proband in spite of the fact that he has no detectable C4B protein by phenotype analysis (Fig. 1). The proband's 7.0-kb *C4A* gene, therefore, must have been inherited from the father. The absence of a second *C4B* gene fragment in the proband indicates that the 7.0-kb

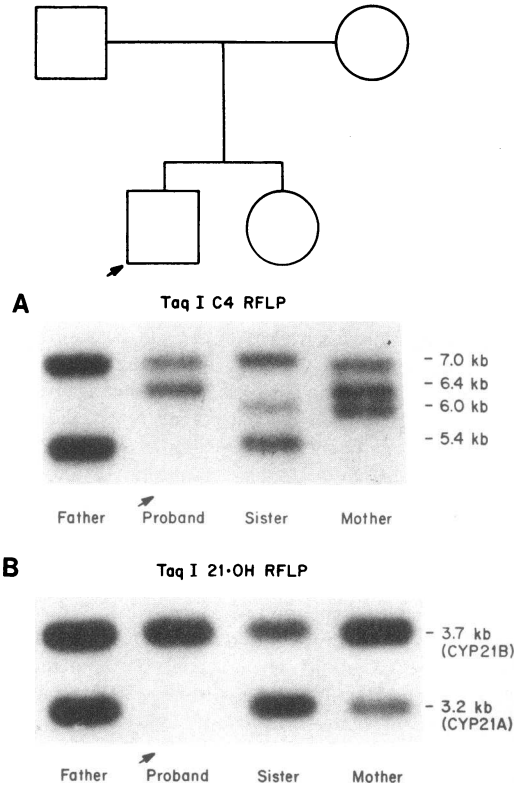

Figure 4. Southern blot of DNA which had been digested with TaqI and probed for C4 (*A*) and 21-hydroxylase (*CYP21A* and *CYP21B*) (*B*).

fragment inherited from the father is part of the haplotype (*C4A3,BQ0*) carrying a *C4B* gene deletion (Fig. 3), and confirms the results of the C4 phenotyping (see Figs. 1 and 2).

The Southern blot shown in Fig. 4 *B* was also probed for 21-hydroxylase (*CYP21A* and *CYP21B*) TaqI fragments. A 3.7-kb fragment, representing a 21-hydroxylase B gene, is found in all immediate family members, including the proband. The 3.2-kb fragment, representing a 21-hydroxylase A gene, although present in other family members, is absent in the proband. The equal intensity of the *CYP21A* and *CYP21B* bands in the father (Fig. 4 *B*) confirms the finding in him of a recognized *C4B* deletion that is associated with a *CYP21A* gene deletion (33). The mother's *CYP21A* band is less intense than her *CYP21B* band, confirming the presence of another recognized *CYP21A* deletion associated with a deletion of the C4A gene (32). The absence of a 3.2-kb *CYP21A* fragment in the proband is consistent with his inheritance of paternal haplotype "a," and that portion of the maternal haplotype "c," which carries the *C4A*Q0* (Fig. 3).

Discussion

Null alleles at the two C4 loci are relatively common (6) and have been attributed to several mechanisms. Approximately 60% of C4 null alleles are attributed to deletions of a C4 gene, together with the flanking *CYP21* gene (34). Unequal crossing-over may be the mechanism responsible for such deletions, as has been described for the steroid 21-hydroxylase gene (35). Gene conversions may also account for *C4* null loci (36). This mechanism involves nondeleted null alleles that are transcriptionally active but have been converted to code for a product

1. *Abbreviation used in this paper:* RFLP, restriction fragment length polymorphism.

similar to that of the adjacent *C4* locus. Both loci, therefore, encode proteins of the same isotype. Finally, nonexpressed genes resulting from a point mutation or small deletion may also result in a null allele (37). The presence of the double null haplotype (*C4A*Q0,C4B*Q0*) occurs less frequently than would be expected, based on the frequency of *C4A*Q0* and *C4B*Q0* in normal populations (6). The mechanisms responsible for its occurrence have not been previously identified. The present report describes an informative family in which the *C4A*Q0,C4B*Q0* double null haplotype was found to be the result of a maternal recombination event within the *C4B* locus.

The proband expressed a single C4A3 allotype in low concentration and had no expressed C4B protein (Figs. 1 and 2). Inspection of the pedigree (Fig. 3), however, would not have predicted his homozygous C4B deficiency. Only one C4B null allele (inherited from the father) would have been expected. Inspection of the segregation of HLA antigens (Table I) demonstrated that a recombination event between maternal haplotypes "c" and "d" occurred in the proband's maternal haplotype between the *HLA-B* and *HLA-DR* region. This is precisely where the two C4 genes map, suggesting a possible mechanism to explain the proband's second C4B null gene. The recombination event appeared to occur between the "c" and "d" haplotypes within the *C4B* gene. This is supported by Southern blot analysis (Fig. 4 *A*) which showed that the proband possessed a *C4B* 6.4-kb TaqI RFLP inherited from the mother. As depicted in Fig. 5, the recombination event occurred within the *C4B* gene, but centromeric to the 5′ end of the 6.4-kb TaqI fragment. This recombination event has produced the second null *C4B* gene and the *C4A*Q0,C4B*Q0* double null haplotype seen in the proband.

The C4 deficient patient reported here presented with a history of recurrent infections and a family history of autoimmune disease. Increased susceptibility to infection has been described in patients with complete C4 deficiency, as well as in patients with isolated C4B deficiency (7, 10, 11). It is likely that the increased susceptibility to infection seen in patients with complete C4 deficiency relates to their inability to activate C3 and C5–C9 via the classical pathway and generate complement-dependent opsonic, bactericidal, and chemotactic activities. Although the mechanism by which isolated homozygous C4B deficiency predisposes individuals to infection is less clear, it may relate to the greater efficiency of C4B to transesterify with carbohydrates present in bacterial capsules (3). The patient presented here was homozygous C4B deficient, yet had no history of systemic bacterial infections. What role, if any, his complement deficiency played in his increased susceptibility to sinopulmonary infections is unclear.

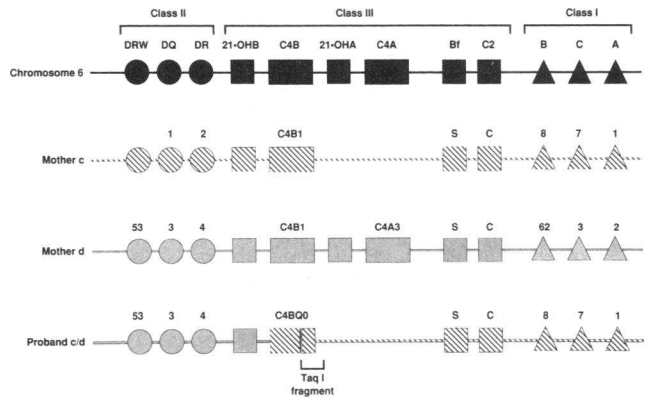

Figure 5. Schematic representation of chromosome 6, with the centromere to the left. The second line represents maternal haplotype "c," which carries the unique 6.4-kb fragment located at the 5′ end of the *C4B* gene. Note its association with gene deletions of 21-hydroxylase A (*CYP21A*) and *C4A*. The third line represents maternal haplotype "d," where both a *C4B* and *C4A* gene are present. The bottom line depicts the proband's "c/d" recombinant haplotype inherited from the mother. Knowing that he has the 6.4 TaqI fragment places the recombination event within the *C4B* locus, to the left, or centromeric to where the TaqI enzyme cuts the *C4B* gene.

The autoimmune disease seen in this family, however, has also been reported in association with both complete C4 deficiency and heterozygous as well as homozygous C4A deficiency (7, 8, 9). The mechanisms by which C4 deficiency predisposes to rheumatic disorders is unclear. Recent evidence suggests that activation of C3 via the classical pathway plays an important role in the clearance of immune complexes (38). Therefore, complete C4 deficiency may result in impaired processing of immune complexes and increased propensity for the development of rheumatic disorders. In addition, because C4A is known to transamidate with proteins more efficiently than C4B, it is likely to be more efficient than C4B in interacting with protein-containing immune complexes (39). Thus, patients with homozygous C4A deficiency lack the C4 isotype, which is most efficient in the processing of immune complexes.

The present case represents the first demonstration of a recombination event within *C4*, producing a C4 double null haplotype. Furthermore, this recombination event documents one possible mechanism by which the rare *C4A*Q0,C4B*Q0* double null haplotype can occur. Additional studies in individuals with the double null haplotype, *C4A*Q0,C4B*Q0*, should reveal other molecular events responsible for its occurrence.

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