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Human C4 Haplotypes with Duplicated C4A or C4B

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SUMMARY

In the course of study of families for the sixth chromosome markers HLA-A, C, B, D/DR, BF, and C2, the two loci for C4, *C4A*, and *C4B*, and glyoxalase I, we encountered five examples of probable duplication of one or the other of the two loci for C4. In one of these, both parents and one sib expressed two different structural genes for C4B, one sib expressed one, and one sib expressed none, suggesting that two C4B alleles were carried on a single haplotype: *HLA-A2, B7, DR3, BFS1, C2C, C4A2, C4B1, C4B2, GLO1*. In a second case, two siblings inherited *C4B*1* and *C4B*2* from one parent and *C4B*Q0* from the other. This duplication appeared on the chromosome as *HLA-AW33, B14, DR1, BFS, C2C, C4A2, C4B1, C4B2, GLO2*. In a third, very large family with 3 generations, a duplication of the *C4B* locus occurred which was followed in 2 generations. In one individual, there were three *C4B* alleles and two *C4A* alleles. One of the *C4B* alleles had a hemolytically active product with electrophoretic mobility near C4B2 and was designated *C4B*22*. It segregated with *C4B1* in the family studied. The complete haplotype was *HLA-A11, CW1, BW56, DR5, BFS, C2C, C4A3, C4B22, C4B1, GLO2*. In another family with 12 siblings, one parent and eight children expressed two *C4A* alleles on the haplotype *HLA-AW30, BW38, DR1, BFF, C2C, C4A3, C4A2, C4BQ0, GLO1*. In a fifth family, we found a duplication of the *C4A* locus on the haplotype *HLA-A3, CW4, BW35, DR1, BFF, C2C, C4A3, C4A2, C4BQ0, GLO2*. Thus, we found duplications of *C4A* and *C4B* loci on several different haplotypes. These

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are postulated to have arisen by unequal crossing over between the tandemly duplicated loci of *C4*.

INTRODUCTION

Definition of the polymorphism of C4 in humans was, until recently, elusive. Early attempts employed crossed immunoelectrophoresis of EDTA plasma with anti-C4. This gave different patterns among individuals that were constant for any one person over long periods [1]. However, various attempts failed to resolve these patterns or patterns produced by agarose gel electrophoresis of plasma and immunofixation with anti-C4 in a way consistent with Mendelian inheritance of alleles at a single locus [2–8]. An important step forward was made when it was observed that C4 immunofixation patterns in general had four fast, four slow, or all eight bands [9]. It had also been recognized that Chido and Rodgers blood groups are distinct antigenic components of C4 [10]. It was proposed that there are two closely linked loci for C4, the product of one marked by Chido and of the other by Rodgers. In this system, the Chido reactivity resided in the four slow bands and the Rodgers reactivity in the four fast bands. Since there were some individuals who had only one set of bands (fast or slow), it was postulated that there are common blank alleles at each locus. However, in the system used, it was not possible to detect heterozygotes for blank alleles with accuracy.

By desialyzation prior to electrophoresis and subsequent crossed immunoelectrophoresis, it was possible to type all individuals [11, 12] in a manner predicted by the two-locus model and to detect null alleles. Since the frequency of C4 deficiency appears to be very low, it was proposed that the alleles at these loci are in linkage disequilibrium so that there are few double null haplotypes. We initially proposed that these frequent null alleles arose by unequal crossing over and predicted chromosomes with duplications of one or another of the two *C4* loci, termed *C4A* and *C4B*.

Following desialyzation, the product of each locus forms three bands on electrophoresis. Hence, serum or plasma from a single individual with four structural genes may produce 12 bands [13]. There are six common variants produced by alleles at the *C4A* locus and three common variants at the *C4B* locus. These are also coded close to the loci for *BF*, *C2*, and *HLA-D/DR*. The alleles of the loci for *C2*, *BF*, *C4A*, and *C4B* are inherited as common groups much as alleles of the *Rh* or *MNS* system and are termed complotypes [14]. A number of common complotypes have been described and are notable for linkage disequilibrium between alleles of complotypes and between complotypes and alleles of the HLA system [15]. We report here studies of families interpreted as showing duplications of either *C4A* or *C4B*.

MATERIALS AND METHODS

The five families studied were ascertained because the propositus had aplastic anemia (no. 234), juvenile-onset insulin-dependent diabetes mellitus (no. 128), juvenile rheumatoid

arthritis (no. 237), had no disease (no. 235), or was a worker in our laboratory (no. 236). All families except one were Caucasian. No. 234 was Saudi-Arabian, no. 128 was of Italian origin, no. 237 was English-German, no. 235 was of Mexican-Indian origin, and no. 236 was of Italian-Ashkenazi origin.

BF, C2, and C4 Typing

Fresh or freshly frozen (at −70°C) serum or EDTA plasma samples were subjected to agarose gel electrophoresis at pH 8.6 and immunofixation with goat antiserum to human factor B (Atlantic Antibodies, Scarborough, Me.) for BF typing [16]. For C2 types, the same samples were analyzed by isoelectric focusing in polyacrylamide gels [17] and an overlay of agarose gel containing EA and human serum diluted 1:90 in isotonic Veronal-buffered saline, pH 7.4, containing 0.1% gelatin, 10^{-3} M Mg^{++}, and 1.5×10^{-4} M Ca^{++}. Portions of the same samples were treated with *Clostridium perfringens* neuraminidase (Sigma, St. Louis, Mo.) at 10 U/ml for 15 hrs at 4°C prior to agarose gel electrophoresis at pH 8.6 and crossed immunoelectrophoresis for detection of half-null haplotypes [11] or to agarose gel electrophoresis at pH 8.6 and immunofixation with anti-C4 (Atlantic Antibodies) for detection of structural variants of C4A and C4B [13].

GLO Typing

Red cell lysates were spotted on cellulose acetate, and electrophoresis was performed in a 0.03 M Tris, 0.03 M barbituric acid, 0.2 mM mercaptoethanol, 0.4 mM $MgCl_2$ buffer, pH 8.0, at 200 V for 1 hr or until albumin had run 10 cm. Plates were stained first with 0.02 M glutathione and 0.34 M methylglyoxal in a 0.1 M phosphate buffer, pH 6.5, and then with 0.1 mM 3-[4,5-dimethylthiazolyl-2]-2,5-diphenyltetrazolium bromide (MTT), 0.69 mM 2,4-dichlorophenol-indophenol in a 0.1 M Tris hydrochloride buffer, at pH 7.8 [18].

HLA, B, C, and DR Typing

Routine typing was performed using a modification of the NIH Standard Microlymphocytotoxicity Test procedure. One hundred forty antisera were used for defining 19 A-locus, 28 B-locus, and six C-locus antigens. HLA-DR typing was performed using the technique of the Oxford (7th International Histocompatibility) Workshop [19]. Seventy antisera (40 reference and 30 local) were used for defining seven HLA-D/DR antigens.

Functional Assay

Functional assay was performed as follows: C4 electrophoresis was carried out as previously described. The plates were overlaid with 7.5 ml EA cells (prewashed twice with veronal-buffered saline with Mg^{++} and Ca^{++}) in 100 ml veronal-buffered saline with Mg^{++} and Ca^{++}, 0.6% agarose, and 0.4 ml C4-deficient guinea pig serum at 37°C [13].

RESULTS

In family 234 (table 1), one parent and one sib expressed two structural genes for *C4B*, while one parent and one sib expressed one and one sib expressed none, suggesting that two *C4B* alleles were carried on a single haplotype *HLA-A2, B7, DR3, BFS1, C2C, C4A2, C4B1, C4B2, GLO1*. In family 128 (table 1), one parent and one sib appeared to have a double dose of *C4B1* along with *C4B2*, suggesting a duplication of *C4B* on the chromosome *HLA-AW33, B14, DR1, BFS, C2C, C4A2, C4B1, C4B2, GLO2*. In family 235 (table 1) with 12 siblings, one parent and eight children expressed three *C4A* alleles and shared the haplotype with duplicated *C4A*: *HLA-AW30, BW38, DR1, BFF, C2C, C4A3, C4A2, C4BQ0,*

TABLE 1

C4 TYPES IN FAMILIES WITH DUPLICATION

	Genotypes	Phenotypes
FN 234:		
Parents	C4A3BQ0/C4A3B1	C4A3A3B1BQ0
	C4A3BQ0/*C4A2B1B2*	C4A3A2B1B2
Offspring	C4A3B1/C4A3BQ0	C4A3A3B1BQ0
	C4A3BQ0/C4A3BQ0	C4A3BQ0
	C4A3BQ0/*C4A2B1B2*	C4A3A2B1B2
FN 128:		
Parents	C4A3BQ0/C4A3B1	C4A3A3B1BQ0
	C4A2C4B1B2/C4A3B1	C4A3A2B1B1B2
Offspring	C4A3BQ0/*C4A2B1B2*	C4A3A2B1B2
	C4A3B1/*C4A2B1B2*	C4A3A2B1B1B2
FN 235:		
Parents	*C4A3A2BQ0*/C4A3B1	C4A3A3A2B1BQ0
	C4A3B1/C4AQ0B1	C4A3AQ0B1B1
Offspring	*C4A3A2C4BQ0*/C4A3B1	C4A3A3A2B1BQ0
	C4A3A2BQ0/C4AQ0B1	C4A3A2B1BQ0
	C4A3B1/C4AQ0B1	C4A3AQ0B1B1
	C4A3B1/C4A3B1	C4A3A3B1B1
FN 236:		
Parents	C4A6B1/C4A4B2	C4A6A4B2B1
	C4A3A2BQ0/C4A3BQ0	*C4A3A3A2BQ0*
Offspring	C4A6B1/*C4A3A2BQ0*	C4A6A3A2B1BQ0
	C4A6B1/C4A3BQ0	C4A6A3B1BQ0
FN 237 Subfamily—RoS × LS:		
Parents	C4A3B3/*C4A3B1B22*	C4A3A3B1B3B22
	C4A3B1/C4A3BQ0	C4A3A3B1BQ0
Offspring	C4A3B3/A3B1	C4A3A3B3B1
	C4A3B1B22/C4A3B1	C4A3A3B1B1B22

GLO1. Figure 1 demonstrates the inheritance of the duplication *C4A3, C4A2* in family 236, shows C4 patterns of four members of this family including that of individual D with three *C4A* alleles, and compares these patterns to those produced by the heterozygous combination C4A3, A2 and a homozygous C4A2, A2 person ET. In large family 237 shown in figure 2, a duplication of the *C4B* locus occurred that can be followed in 2 generations. Individual RoS had three *C4B* alleles and two *C4A* alleles (table 1). The product of the duplicated locus had C4 hemolytic function, further suggesting that it was a *C4B* product, and it is termed B22 with mobility slightly acidic to C4B2. Figure 3 shows the pattern of individual MS who was C4A3, C4AQ0, C4B3, C4B1 compared to that of his sister RoS who was C4A3, C4A3, C4B22, C4B3, C4B1; her husband LS who was C4A3, C4A3, C4B1, C4BQ0; and his children: MaS who was C4A3, C4A3, C4B22, C4B1, C4B1; and RaS who was C4A3, C4A3, C4B3, C4B1. This clearly demonstrates segregation of the duplicated allele from *C4B*3* and with *C4A*3* and *C4B*1*. These patterns were confirmed in lateral family members (fig. 2). Table 2 shows the haplotypes to which the duplications were found to be linked.

DISCUSSION

There is ample evidence that there are two separate closely linked loci for human C4, *C4A* and *C4B*, the products of which differ antigenically [10], func-

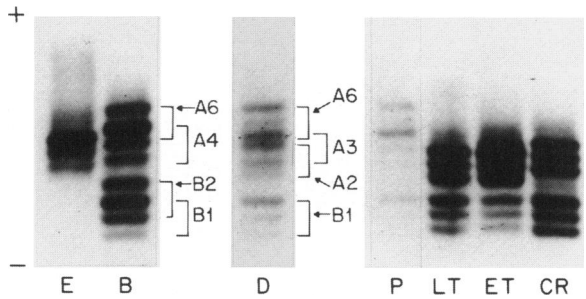
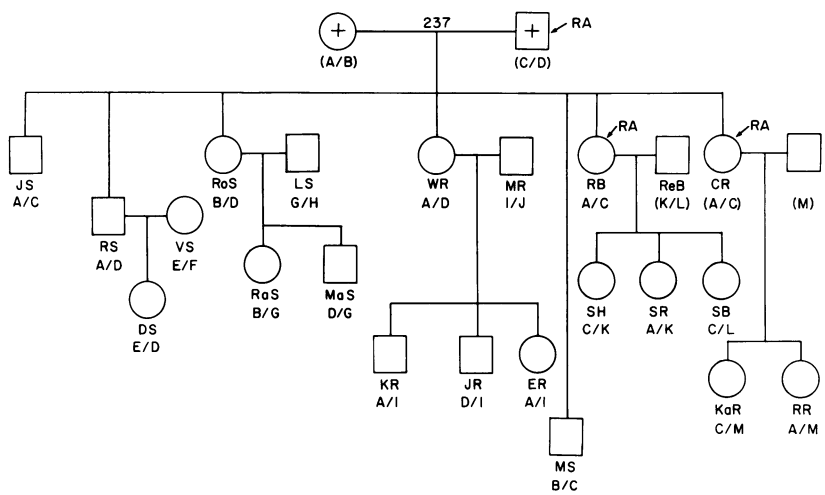


FIG. 1.—C4 patterns in family 236. C4A patterns are anodal (acid) to the C4B (basic) patterns. The sample *CR* is a control phenotype C4A3, A3, B1, B1; *E* is the mother with phenotype C4A3, A3, A2, BQ0, BQ0; *B* is the father with phenotype C4A6, A4, B2, B1; *D* is a daughter with phenotype C4A6, A3, A2, B1, BQ0; and *P* is a daughter with phenotype C4A6, A3, B1, BQ0. These are compared to individual *LT* with phenotype C4A2, A2, B1, BQ0 and individual *ET* with phenotype C4A3, A2, B1, BQ0.

tionally [13], in average net charge [9, 13], and in subunit molecular size [20]. Since this duplication has apparently occurred in the mouse with generation of *C4* (Ss) and a closely linked locus producing a molecule very similar to C4 but without C4 hemolytic complement function (Slp) [21] and in the chimpanzee with generation of *C4A* and *C4B* loci similar to those in man [22], it is likely to

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A=HLA-A2,     BW38,DR2,BFS,C2C,C4AQ0,B1 ,GLO1     H=HLA-A2 ,    BW44,DR4,BFS,C2C,C4A3 ,BQ0
B=HLA-A2, CW3,B15 ,DR1,BFS,C2C,C4A3 ,B3 ,GLO2     I=HLA-A1 ,    B17 ,DR7,BFS,C2C,C4A6 ,B1 ,GLO2
C=HLA-A1,     B8  ,DR3,BFS,C2C,C4AQ0,B1 ,GLO2     J=HLA-A2 ,CW3,B15 ,DR4,BFS,C2C,C4AQ0,B3 ,GLO1
D=HLA-A11,CW1,B22,DR5,BFS,C2C,C4A3,B1,22,GLO2     K=HLA-A2 ,CW3,B40 ,DR6,BFS,C2C,C4AQ0,B2 ,GLO2
E=HLA-A32,    B44 ,DR2,BFS,C2C,C4A3 ,B1 ,GLO1     L=HLA-A33,    BW50,    BFS,C2C,C4AQ0,B1 ,GLO2
F=HLA-A25,CW1,B27 ,DR6,BFS,C2C,C4A3 ,B1 ,GLO1     M=HLA-A24,    B27 ,    BFS,C2C,C4A3 ,B1 ,GLO1
G=HLA-A11,    B7  ,DR2,BFS,C2C,C4A3 ,B1 ,GLO2
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FIG. 2.—Pedigree of a large family (237) studied for sixth chromosome markers that demonstrates a duplication of C4B and its inheritance on the D haplotype. Haplotypes have been deduced and *are printed under each individual's initials*. The 13 haplotypes deduced in this family are printed below the pedigree. Individuals who were not studied but for whom it was possible to deduce haplotypes *are indicated by haplotypes in parentheses*.

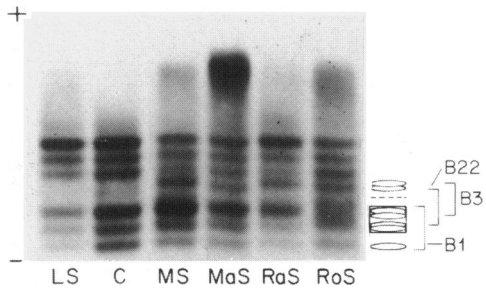

FIG. 3.—C4 immunofixation patterns comparing types of the subfamily of *RoS* of FN 237 with sibling *MS* and known control, *C*, who is C4A3, A3, B1, B1. Phenotypes are *MS*: C4A3, AQ0, B3, B1; *MaS*: C4A3, A3, B22, B1, B1; *RaS*: C4A3, A3, B3, B1; *RoS*: C4A3, A3, B22, B1, B3; and *LS*: C4A3, A3, B1, BQ0. *MaS* and *RaS* are children of *RoS*. *RoS* has three C4B alleles. *MaS* clearly inherited C4B3 from his mother. *RaS* inherited both C4B1 and B22.

be ancient. In man [9, 11] and, probably, in the chimpanzee [22], half-null C4 haplotypes are common in which no product for *C4A* or *C4B* is detected. The double null haplotype occurs but is much rarer than predicted by the frequencies of the individual null alleles [23].

It has been suggested [13] that the half-null haplotypes arose by gene deletion as a result of unequal crossing over due to mispairing at meiosis. A similar mechanism had previously been postulated as the basis of the form of α-thalassemia common in African populations [24, 25], since there are two juxtaposed α-globin genes. This mechanism, of course, would produce chromosomes with duplications at either *C4A* or *C4B*.

Our report presents evidence that there are haplotypes in Caucasians with such duplications. This evidence is of two types. In three families, quantitative increases are seen on one allele while in two families individuals with three *C4A* or three *C4B* alleles have been seen. Five forms of duplication at either *C4A* or *C4B* and their inheritance are described. Similar findings in one family have been reported by Hauptmann et al. [26]. In that family, there was duplication of *C4A* on the haplotype *HLA-A3, CW3, B7, DR5, BFF, C4A3, C4A2, C4B1*. Duplications involving *C4B* and *C4A* have been described by Bruun-Petersen et al. [27]. The *C4A* duplication is probably the same as that seen in FN 236, and a duplication of *C4A* has been described by Nordhagen et al. [28].

TABLE 2

COMPLETE HAPLOTYPES LINKED TO C4 DUPLICATION

	Haplotypes
FN 234	HLA-A2,B7,DR3,BFS1,C2C,C4A2,*C4B1,B2*, GLO1
FN 128	HLA-AW33,B14,DR1,BFS,C2C,C4A2,*C4B1,B2*, GLO2
FN 235	HLA-AW30,BW38,DR1,BFF,C2C,*C4A3,A2*,C4BQ0, GLO1
FN 236	HLA-A3,CW4,BW35,DR1,BFF,C2C,*C4A3,A2*,C4BQ0, GLO2
FN 237	HLA-A11,CW1,B22,DR5,BFS,C2C,C4A3,*C4B1,B22*, GLO2

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