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Extended HLA/complement allele haplotypes: Evidence for T/t-like complex in man

(major histocompatibility complex/linkage disequilibrium/t locus/transmission bias)

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ABSTRACT **The chromosomal distribution of alleles for HLA-A, -B, -C, and -DR and the serum complement protein alleles of factor B and C2 and C4 was studied in normal Caucasian families. Eight combinations of HLA-B, DR, BF, C2, C4A, and C4B markers were found to occur in haplotypes at frequencies significantly higher than expected. In these combinations, which were defined as extended major histocompatibility complex haplotypes, HLA-A showed limited variation. A possible mechanism for the maintenance of extended haplotypes are human analogs of murine *t* mutants which are characterized by crossover suppression and male transmission bias. One human 6p haplotype, *HLA-B8, DR3, SCO1, GLO 2*, was found to be transmitted from males to 83% of their offspring. The same haplotype with *GLO 1* had no transmission bias. It is suggested that this *GLO* 2-marked chromosome is a human analog of a murine *t* mutant.**

The structural genetic loci for three serum complement components, factor B (*BF*), C2 (*C2*), and C4 (*C4A* or Rodgers and *C4B* or Chido) are very closely linked to each other and to the loci of the major histocompatibility complex on the short arm of the human chromosome 6 (1–3). They are also very close to *HLA-D, DR* (4), perhaps located between *HLA-D, DR* and *HLA-B*. In family studies involving a large number of informative meioses, no crossovers among the serum complement loci were detected, suggesting that specific *BF, C2, C4A*, and *C4B* alleles form haplotypes (complotypes) that are inherited as gametic units. In normal Caucasians there are 14 complotypes that occur at a frequency of 0.01 or higher and a large number of rare ones (5). A genetic locus for the erythrocyte enzyme glyoxalase I (*GLO*) is also linked to the major histocompatibility complex. The position of these loci and the approximate relative distances between them are shown in Fig. 1.

In the present investigation, the nonrandom associations or linkage disequilibria between the complotypes and the other loci of the major histocompatibility complex have been studied in Caucasian families. Although in many instances there is a random distribution of complotypes among the different *HLA-A, -B*, and *-DR* alleles, in other cases there is strikingly restricted distribution. When this occurs, it generally involves the complotypes and both *HLA-B* and *HLA-DR* alleles, but often *HLA-A* as well. This results in the formation of "extended" major histocompatibility complex haplotypes that occur in frequencies significantly greater than those expected from random association. A brief report of some of these findings has been published (6).

Because we have postulated that some of such extended haplotypes might arise in man by mechanisms and features similar to those exhibited by the murine *T/t* complex (7), we have also studied transmission ratios from the male of one of these extended haplotypes. In the mouse, most *t*-bearing chromosomes are transmitted from the male to his offspring in a ratio greater than 0.5 (8, 9). Our findings suggest that at least one human extended haplotype has a transmission ratio different from the expected level of 0.5 and therefore may mark a human *t*-like mutant.

MATERIALS AND METHODS

Families. Typings were performed on 151 Caucasian families from the Boston area. In those cases in which a family member had a disease, only haplotypes not occurring in that patient were considered normal for use in analysis of HLA–complotype associations. Blood for the typing of BF, C2, and C4 was collected into EDTA at 1 mg/ml. Plasma was obtained by centrifugation and immediately frozen at −80°C until just before analysis. Blood for HLA and GLO typing was collected into heparin at a final concentration of 1.0 unit/ml. For the studies of transmission ratios, chromosomes were included for study whether they were "normal" or occurred in subjects with HLA-linked disease except that, in the latter case, propositi were not counted in the analysis of transmission ratio.

Factor B (BF) Typing. Plasma samples were subjected to electrophoresis in agarose gel in 0.05 M barbital buffer (pH 8.6) containing 1.8 mM calcium lactate as described (10). Patterns were developed by immunofixation with goat anti-human B (Atlantic Antibodies, Scarborough, ME).

C2 Typing. Plasma samples were subjected to isoelectric focusing in polyacrylamide gels as described (11).

C4 Typing. Aliquots (10 μl) of plasma were incubated separately with 0.1 unit of neuraminidase from *Clostridium perfringens* (type VI; Sigma) for 15 hr at 4°C while undergoing microdialysis (12) against 0.1 M phosphate, pH 6.8/5 mM EDTA. Desialated samples were subjected to crossed immunoelectrophoresis for detection of half-null haplotypes as described (13). For the detection of C4 structural variants, desialated plasma samples were subjected to immunofixation electrophoresis in 0.75% agarose (ICN) using a discontinuous Tris/glycine/barbital buffer and 5 mM EDTA in the gel. After completion of electrophoresis, C4 bands were visualized by immunofixation with goat anti-human C4 (Atlantic Antibodies, Scarborough, ME) (14).

Complement Genetic Nomenclature. The nomenclature for genetic polymorphism of *BF, C2*, and *C4* in this paper is that proposed earlier (14), which was designed to conform to the International System for Human Gene Nomenclature (1979) (15). Null alleles and null variants are designated "QO." For *C4*, the locus controlling the acidic Rodgers (Rg) positive variants is designated *C4A* and that controlling the basic Chido (Ch)

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Abbreviations: BF, factor B; GLO, glyoxalase.

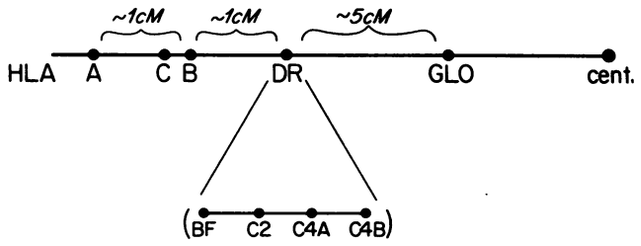

FIG. 1. Schematic representation of the short arm of human chromosome 6 (6p).

positive variants is *C4B*. Among unrelated Caucasians, six common structural variants and one null variant detected at the *A* locus are designated as C4A 1-6 and C4A QO respectively. At the *B* locus, there are three common structural variants and one null variant, C4B 1-3 and C4B QO. Complotypes are given (in arbitrary order) as BF-C2-C4A-C4B types. Thus, SC42 represents the complotype *BF*S, C2*C, C4A*4, C4B*2*. Extended haplotypes are given as *HLA-(A), B, DR*, complotype, *GLO*. Because there is some variability (see *Results*) at the *HLA-A* locus, parentheses are used to indicate the most frequent *HLA-A* allele.

HLA Typing. HLA-A, -B, -C, and -DR antigens were assigned by the microlymphocytotoxicity assay (16, 17).

GLO Typing. Hemolysates were used for GLO typing. Detection was by use of cellulose acetate electrophoresis and specific enzyme reagents (18).

Statistical Analyses. Linkage disequilibrium was analyzed as described by Piazza (19) assuming a Poisson distribution. For transmission bias analysis, the χ^2 test was used.

RESULTS

Family studies of HLA, complotype, and GLO markers yielded 350 *HLA-A, -B, -DR* and complotype haplotypes of which 295 had the GLO markers determined. The *HLA-A, -B, -DR* and complotype associations are shown graphically in Figs. 2 and 3. The graphs are constructed so that the widths of the columns and rows reflect the distribution of individual *HLA* alleles in this population of chromosomes. Rare *HLA* alleles and complotypes with frequency <0.01 are not shown. If complotypes were randomly distributed, an even distribution of each complotype would be expected. Clusters were taken to reflect nonrandom allelic associations or linkage disequilibria.

It is apparent from the graphs that the complotypes reveal a number of restricted distributions superimposed upon a random background. These restricted distribution clusters differ completely from complotype to complotype. For example, the distribution of *SCO1* is markedly restricted to *HLA-A1, -B8*, and *DR3* (Fig. 2), whereas *FCO1*, which differs from SCO1 only at the *BF* locus, shows neither definite clustering nor association with *HLA-B8* or *-DR3* (Fig. 3). Such differences are also striking for the complotype pairs *SC31* and *FC31*, as well as for *SC30* and *FC30*. In addition to the primary cluster for *SCO1*, involving the alleles *HLA-A1, -B8*, and *-DR3*, a secondary cluster representing *HLA-A3, -B8* (and *-DR3*) is also evident (Fig. 2*A*). Many of the remaining examples of *SCO1* have either *B8* or *DR3* and very few instances of *SCO1* have neither. In contrast to *SCO1*, the complotype *SC42* appears to be randomly distributed because no clusters could be discerned among the 10 independent instances in which it was found. Relative rarity itself, however, is not a problem when clustering is striking. All four examples of *SC22* are linked to *HLA-B14* and *-DR1*, and the *HLA-A* is equally divided between *HLA-A2* and *HLA-A3*. Eight *HLA-B, -DR*, complotype combinations show obvious clustering (Fig. 3). We refer to these haplotypes as "extended". The combined frequency of these extended haplotypes is 28% of the total normal haplotypes generated (Table 1). Extended haplotypes were analyzed for three-point (*HLA-B, -DR*, and complotype) linkage disequilibrium. As shown in Table 2, all of the eight extended haplotypes tested showed significant linkage disequilibrium ($P < 0.05$). Of the 350 major histocompatibility complex haplotypes analyzed in this study, 166 were generated

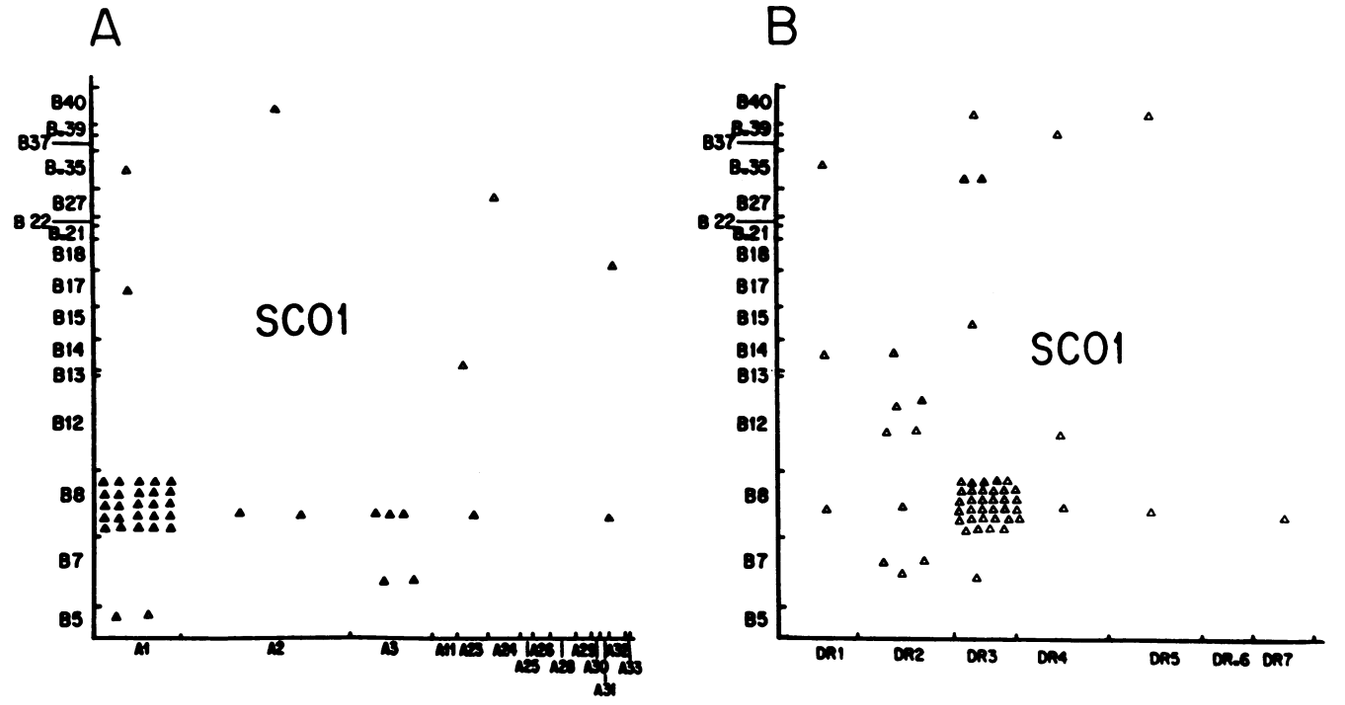

FIG. 2. Distribution of the two complotypes *BF*S, C2*C, C4A*QO, C4*B1* (*SCO1*), in relation to the *HLA-A, -B*, and *-DR* on the same chromosomes.

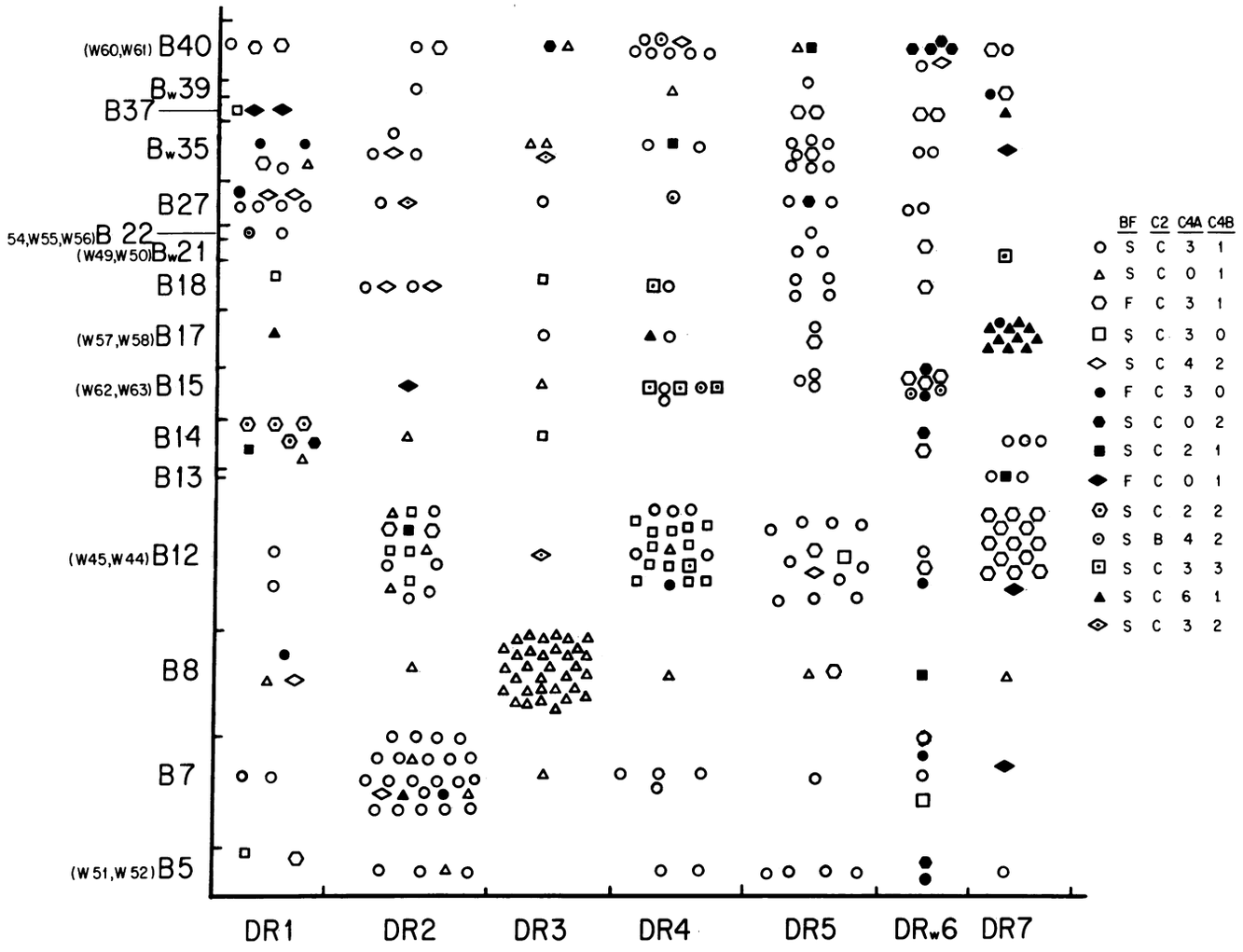

FIG. 3. Distribution of the common complotypes (frequency >0.01) in relation to *HLA-B* and *-DR*. This graph is constructed so that the widths of the rows and columns are proportional to the frequency of the *HLA-B* and *-DR* alleles in this population.

from apparently normal families and the remaining 184 were the "nondisease" haplotypes generated from families with one or more disease members but not occurring in any of the latter. When the frequencies of the extended haplotypes from both of these sources were compared, they were found to be very similar. For example, in normal and nondisease chromosome populations, the frequencies of *B8, DR3, SCO1* were 0.097 and 0.091; *B7, DR2, SC31* were 0.056 and 0.061; and *B12, DR7, FC31* were 0.042 and 0.034. This suggests that no bias was introduced by combining normal and nondisease haplotypes in this study.

It is seen from Table 1 that there are two extended haplotypes marked by *HLA-B12 (w44)*. They differ by both complotype and *HLA-DR*. Even more remarkably, they differ absolutely by *HLA-C* types.

In order to investigate the possibility that extended haplotypes may represent *t*-like mutant-bearing chromosomes, we analyzed the transmission ratios for the most common one, *HLA-(A1), B8, DR3, SCO1, GLO 1*, and *GLO 2* from both males and females. The most striking finding was that the extended haplotype *HLA-B8, DR3, SCO1, GLO 2* was transmitted from the male to 83% of his offspring (Table 3). No such bias was observed from the female, and no bias from either sex was seen for *HLA-B8, DR3, SCO1, GLO 1*. Insufficient data were available for other extended haplotypes to test for male transmission bias.

DISCUSSION

In an investigation of the association of complotypes with HLA alleles in Caucasian families, more than one-quarter of the major histocompatibility complex haplotypes occurred as linkage disequilibrium sets, here referred to as extended haplotypes. Previously, alleles in linkage disequilibrium have been regarded as pairs (20). Thus, *HLA-A1* and *B8* on the one hand and *HLA-B8* and *DR3* on the other were recognized as two such sets. It appears more reasonable to view the haplotype *HLA-A1, B8, DR3* together with SCO1 as the basic linkage disequilibrium set. Chromosomes in which this extended haplotype is combined with *GLO 1* have biologically different behavior with respect to transmission from the male compared to that with *GLO 2*. Thus it seems likely that the extent of this haplotype is at least 5–7 centimorgans and that it includes *GLO*. The limited variation at the *HLA-A* locus suggests that there are closely related "families" of extended haplotypes, with similar or identical characteristics in the *HLA-B, HLA-DR*, and complotype regions and differing to a limited extent in *HLA-A*.

Several mechanisms—including recent mutation, founder effects, inbreeding, random drift, and similar phenomena—have been suggested in explanation of the maintenance of linkage disequilibrium in populations. Due to crossover events, however, such effects are rapidly dissipated at chromosomal map distances of 2–7 centimorgans and thus are unlikely to ac-

Table 1. Extended haplotypes

*HLA-A**		*HLA-B*	Complotype	*HLA-DR*	Haplotype frequency	Fraction of *DR*	Fraction of *B, DR*
1	0.75						
3	0.11						
2	0.07						
other	0.07	*B8*	*SCO1*	*DR3*	0.093	0.71	1.00
3	0.46						
2	0.31						
other	0.23	*B7*	*SC31*	*DR2*	0.059	0.27	0.84
2	0.45						
29	0.27						
w23	0.18						
other	0.09	*B12(w44)**	*FC31*	*DR7*	0.037	0.31	0.93
2	0.60						
other	0.40	*B12(w44)*†*	*SC30*	*DR4*	0.034	0.22	0.63
1	0.50						
2	0.30						
other	0.20	*B17(w57)*	*SC61*	*DR7*	0.028	0.35	0.91
2	0.75						
3	0.25	*B40(w61)*	*SCO2*	*DRw6*	0.011	0.09	0.67
2	0.5						
3	0.5	*B14*	*SC22*	*DR1*	0.011	0.10	0.57
1	0.33						
2	0.33						
9	0.33	*B15(w62)*	*SC33*	*DR4*	0.009	0.10	0.50

* In all four instances in which *HLA-C* types could be ascertained, they were *HLA-Cw4*.
† In all three instances in which *HLA-C* types could be ascertained, they were *HLA-Cw5*.

count for most of the observed extended haplotypes. Two other possibilities more likely to result in sustained linkage disequilibrium over relatively long stretches of chromosome are selection for specific genes within this region (21) and phenomena associated with possible human analogs of the murine *T/t* complex (7). The murine *T/t* region extends throughout the major histocompatibility or *H2* complex and is associated with crossover suppression and male transmission bias (22). The latter effect constitutes a strong selective advantage for genes within the area of crossover suppression. Specific *t* mutants vary from one wild mouse population to another but may have frequencies of around 0.25. Clearly, if human *t*-like mutants occur, they would be manifested as extended major histocompatibility complex haplotypes.

It is evident that the extended haplotype *HLA-(A1), -B8, -DR3, SCO1, GLO 2* has marked male transmission bias in excess of 80% whereas the same haplotype with *GLO 1* does not. Cudworth *et al.* (23) noted that *HLA-A1, -B8* was transmitted from the male in a ratio of about 63–65% and suggested that this might represent a *t*-like mutation. However, subsequent reports did not consistently confirm these findings (24–26). The absence of *HLA-D, DR* and complotype markers and the failure to take *GLO* into account certainly contributed to the conflicting results. In any case, the present findings clearly show that *HLA-(A1), B8, DR3, SCO1, GLO 2* exhibits marked male transmission bias and therefore seems likely to bear a *t*-like mutation. The same may also be true of other extended haplotypes, but more data are needed to be certain.

Table 2. Three-point *HLA-B, -DR*, and complotype linkage disequilibria

Haplotype	Δ	*P*
B8,DR3,SCO1	0.058	1.4×10^{-5}
B7,DR2,SC31	0.019	4.8×10^{-2}
B12,DR7,FC31	0.025	2.0×10^{-3}
B17,DR7,SC61	0.024	2.8×10^{-4}
B12,DR4,SC30	0.019	1.2×10^{-2}
B14,DR1,SC22	0.010	6.3×10^{-3}
B40,DRw6,SCO2	0.009	2.6×10^{-2}
B15,DR4,SC33	0.005	1.5×10^{-2}

Another feature of the murine *T/t* complex is crossover suppression between *t* and wild chromosomes but not between different *t*-bearing chromosomes (27). If some extended haplotypes are the human equivalent of mouse *t*-bearing chromosomes, suppression would be expected in meioses between these extended and other, nonextended, chromosomes but not in meioses involving two extended or nonextended haplotypes. Although the number of recombinant events observed in this study was insufficient to test this possibility, previously published data suggest that this may be the case. The 1980 Histocompatibility Workshop (28) included study of 21 crossover events involving the haplotype *HLA- B8, Dw3/DR3*. (In the present study, this haplotype was always found with the complotype *SCO1*, revealing a high male transmission bias when accompanied by *GLO 2*.) Of these 21 events, 12 occurred in meioses with other recognizable extended haplotypes. The expected rate of recombination between this and any other extended haplotype is less than 25% (i.e., the frequency of all other extended haplotypes). Thus, there may be preferential crossing over between extended haplotypes, with suppression of crossover between extended and nonextended haplotypes. Such an effect may explain the presence of recombinants involving extended haplotypes even though it does not explain why the chromosomes involved remain intact and do not randomize. In the mouse, different *t*-bearing chromosomes have little chance of recombination because each deme has a single *t* mutant exhibiting crossover suppression with all the wild chromosomes of that specific deme (29). In man, this is not the case at present, especially in the North American population where intermixture of previously isolated populations is common. Situations more analogous to mouse demes may have existed in the recent human past and may still hold for some isolated populations. This would suggest that single extended chromosomes

Table 3. Extended haplotype transmission ratios

Haplotype	Transmission from	Families, no.	Children, no.	Children with haplotype, no.	%	*P*
B8,DR3,SCO1,GLO 1	Female	10	33	14	42	NS
B8,DR3,SCO1,GLO 1	Male	6	22	10	46	NS
B8,DR3,SCO1,GLO 2	Female	12	34	12	35	NS
B8,DR3,SCO1,GLO 2	Male	15	41	34	83	<0.0001

originally present in isolated populations may have remained "frozen" over many generations as a result of crossover suppression with nonextended chromosomes. The recent admixture of populations, however, may be bringing a number of different extended haplotypes into the same genetic pool, thus producing the observed recombinants involving extended haplotypes.

Clearly, this study has not identified all extended haplotypes of normal Caucasians, particularly those that occur in low frequency in the study population. Many of the extended haplotypes we have identified have previously been partially noted as linkage disequilibrium pairs. Even some of the complement associations have been noticed in partial form. For example, linkage disequilibrium between Rodgers negativity and *HLA-B8* was found (30) and later this was shown to hold for what in our nomenclature is called the *C4* haplotype *C4 AQO,B1*. Given the concept of the complete complotype, it could be shown that *SCO1* (not *FCO1*) was involved in the entire extended haplotype which encompasses *HLA-A1, B8, DR3*. Previous attempts to characterize extended haplotypes were hampered by the lack of availability of full complotypes. Nevertheless, Dausset *et al.* (31) pointed out that, for some haplotypes, linkage disequilibrium extended the whole distance from *HLA-A* to *GLO*.

It has generally been considered that linkage disequilibria noted for *HLA-B* and *HLA-C* alleles arise because of the very close chromosomal location of these loci. The present study suggests that the situation is more complex because the *HLA-C* alleles in the two extended haplotypes marked by *HLA-B12(w44)* are completely different.

The finding that about one in four Caucasian chromosomes 6 carry extended major histocompatibility complex haplotypes, together with the possibility that some of these carry *T/t* complex-like mutations, has additional implications. If a significant proportion of chromosomes 6 have altered crossover rates, reevaluation of distances between loci of the major histocompatibility complex is in order. If recent observations suggesting multiple DNA inversions in the mouse *t* region (32) apply to the human counterparts, even gene locus order may need to be reexamined for the appropriate chromosomes. From a more general point of view, however, the existence of relatively fixed extended haplotypes provides an important tool for investigating human evolution and population migration.

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