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DNA linkage analysis of X chromosome-linked chronic granulomatous disease

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ABSTRACT **Chronic granulomatous disease (CGD) is a disorder of phagocytes that is usually inherited as an X chromosome-linked trait. Previous family studies suggested that the *CGD* locus resides on the distal short arm (Xp22–Xpter). Using cloned, polymorphic DNA probes we have performed a linkage analysis within CGD families that suggests a more proximal location (Xp21). In addition, the *CGD* locus is proximal to the Duchenne muscular dystrophy locus and lies within a broad region of Xp in which recombination appears to be greater than anticipated on the basis of physical distance between markers. Regional localization of the X chromosome *CGD* locus should facilitate molecular cloning of the *CGD* gene and molecular dissection of the phagocyte oxidase system.**

Chronic granulomatous disease (CGD) is an inherited disorder of polymorphonuclear leukocytes and monocytes. Affected patients suffer chronic and recurrent bacterial and fungal infections due to an inability to kill catalase-positive microorganisms. In the majority of families the disease is transmitted as an X chromosome-linked trait (1). Carrier females and affected males may be identified by the nitroblue tetrazolium test (2, 3), which measures the capacity of phagocytes to activate an NADPH oxidase system involved in the reduction of molecular oxygen to superoxide and hydrogen peroxide (4). Phagocytes of CGD patients have an unknown lesion in the oxidative system and fail to reduce nitroblue tetrazolium. A membrane-associated *b*-type cytochrome, one component of the NADPH oxidase system, is absent in granulocytes of boys affected with the X-linked form of the disease (5).

Previous studies have reported potential linkage of *CGD* with the blood group antigen Xg and with absence of a surface antigen K_x, which is normally found on polymorphonuclear leukocytes and erythrocytes (6–8). Whereas the locus for Xg resides on the distal segment of the short arm of the X chromosome in the region Xp22.3–Xpter and escapes inactivation in somatic cells of females (6), the *CGD* locus is susceptible to lyonization since approximately half of the leukocytes of carrier females fail to reduce nitroblue tetrazolium (9). The recent identification of a boy with Duchenne muscular dystrophy (DMD), CGD, and retinitis pigmentosa and the absence of K_x antigen on erythrocytes (known as the McLeod phenotype) and an interstitial deletion within Xp21 suggested that the *CGD* locus might reside in Xp21 rather than more distally at Xp22.3–Xpter (10).

We have initiated molecular studies of X-linked CGD in an attempt to more clearly define the location of the disease gene on the X chromosome and its position relative to other markers and *DMD*. Using a collection of cloned Xp DNA probes that recognize restriction fragment length polymorphisms (RFLPs), we have performed a formal genetic linkage analysis. Based on close linkage of *CGD* with two Xp21 DNA markers we conclude that the *CGD* locus is proximal rather than distal to the *DMD* locus on the short arm of the X chromosome. In addition, our data are consistent with a higher degree of recombination within this chromosomal region than anticipated by the physical distance between the Xp markers.

MATERIALS AND METHODS

Cloned DNA probes encompassing proximal Xp21.1 to Xp22.2–Xpter have been used in this study (see Table 1). Some of the probes define DNA segments of Xp21 deleted in the patient of Francke *et al.* (10–18). Peripheral blood DNA samples were prepared from CGD patients and their families, digested with the appropriate restriction endonucleases, and analyzed by Southern blot hybridization (19) with selected radiolabeled DNA probes (20). Chromosomes for cytogenetics were prepared as in refs. 21–23. Linkage analysis was performed with the computer program package LINKAGE (24).

RESULTS

Xp21 Deletion in a CGD–DMD Patient. We first studied patient N.F., a 9-year-old boy affected with CGD and DMD, whose clinical history has been described (25). Cytogenetic analysis revealed a small interstitial deletion within Xp21 (Fig. 1). As was also observed in the case of Francke *et al.* (10), no hybridization of cellular DNA with probes *754* and *PERT 55, 84, 87,* and *145* (17) was observed in Southern blot analyses (data not shown). This deletion case tends to support assignment of the *CGD* locus to the Xp21 region. It is difficult, however, in such unusual instances to exclude other chromosomal rearrangements or position effects. Therefore, assignment of the position of the locus in this manner cannot be considered definitive.

Linkage of the *CGD* Locus to Xp21.1 Markers. To establish the position of the *CGD* locus relative to other Xp markers independent of these rare deletion cases we analyzed families of classical X-linked CGD patients with DNA probes that detect RFLPs. DNAs of carrier females were digested with the enzymes appropriate for each probe to identify those heterozygous for a marker. Additional family members were

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Abbreviations: CGD, chronic granulomatous disease; DMD, Duchenne muscular dystrophy; RFLP, restriction fragment length polymorphism; lod, logarithm of odds.

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Table 1. lod scores between *CGD* and X chromosome markers

Marker	Dx no.	Location	Polymorphism	θ male = θ female							(θ) Peak	lod
				0.00	0.05	0.10	0.15	0.20	0.30	0.40		
Xg		Xp22.3–pter		—	−0.09	+0.12	+0.20	+0.22	+0.19	+0.10	0.21	+0.22
Dic-56	DXS143	Xp22–pter	*Bcl* I	—	−3.88	−2.48	−1.72	−1.21	−0.59	−0.22	0.50	0.00
D2	DXS43	Xp21.3–Xp22	*Pvu* II	—	−1.02	−0.51	−0.26	−0.11	+0.03	+0.06	0.38	+0.06
99-6	DXS41	Xp21.3–Xp22	*Pst* I	—	−1.41	−0.63	−0.18	+0.00	+0.20	+0.16	0.33	+0.21
C7	DXS28	Xp21.3	*Eco*RV	—	−6.02	−3.81	−2.57	−1.76	−0.75	−0.22	0.50	0.00
PERT 87	DXS164	Xp21.2	*Bst*XI *Taq* I	—	−6.62	−3.79	−2.34	−1.45	−0.52	−0.15	0.50	0.00
PERT 84		Xp21.1	*Taq* I	+2.11	+1.91	+1.70	+1.48	+1.26	+0.78	+0.30	0.00	+2.11
754	DXS84	Xp21.1	*Pst* I	—	+3.63	+3.67	+3.43	+3.06	+2.10	+0.99	0.08	+3.70
OTC	OTC	Xp21.1	*Msp* I *Bam*HI	—	−5.07	−2.76	−1.57	−0.86	−0.18	−0.02	0.50	0.00

The number of informative meioses versus *CGD* were as follows: *Xg*, 7; *Dic-56*, 10; *D2*, 7; *99-6*, 11; *C7*, 17; *PERT 87*, 33; *PERT 84*, 9; *754*, 25; *OTC*, 26. The designated DNA markers are described in refs. 12–18.

then examined to assess linkage between each DNA marker and *CGD*. Thirteen families were informative for one or more of the eight polymorphic DNA probes (see Table 1). The data of 1 particularly informative family are presented in Fig. 2. In this instance, the *CGD* gene cosegregates with *754*, *PERT 84*, and *OTC* alleles and recombines with more distal markers *PERT 87*, *99-6*, and *Xg*. Table 1 presents the logarithm of odds scores (lod scores) calculated in the 13 families for each probe with *CGD*.

Several conclusions are derived from the data of Table 1.

(*i*) As suggested by the case of Francke *et al.* (10) and our own (patient N.F.), the *CGD* locus does not lie in the distal portion of Xp (Xp22.3–Xpter). Specifically, the DNA marker *Dic-56*, which was mapped previously to Xp22–Xpter (26), displays no appreciable linkage with *CGD*. In addition, we have not demonstrated linkage of *Xg* with *CGD* in the small number of informative meioses (7) we have studied. Our data conflict with prior and less extensive linkage studies of *CGD* (6–8).

(*ii*) Recombination is infrequent between *CGD* and two Xp21.1 loci, *PERT 84* and *754*, the latter of which was first shown to be deleted in the Francke case (10). The lod scores of 2.11 ($\theta = 0.00$) and 3.70 ($\theta = 0.08$), respectively, strongly suggest close linkage of *CGD* to these markers.

(*iii*) Recombination overall is surprisingly extensive between markers within the entire Xp21 region. For example, recombination between *PERT 87* and *CGD* was frequently observed [8 recombinants in 33 informative meioses], even though the physical distance in which these markers reside is estimated to be only 2–5000 kilobases, or 0.1% of the haploid genome (10, 17). Since *PERT 87* is very tightly linked to *DMD* (18), our data suggest that the *CGD* locus lies a considerable

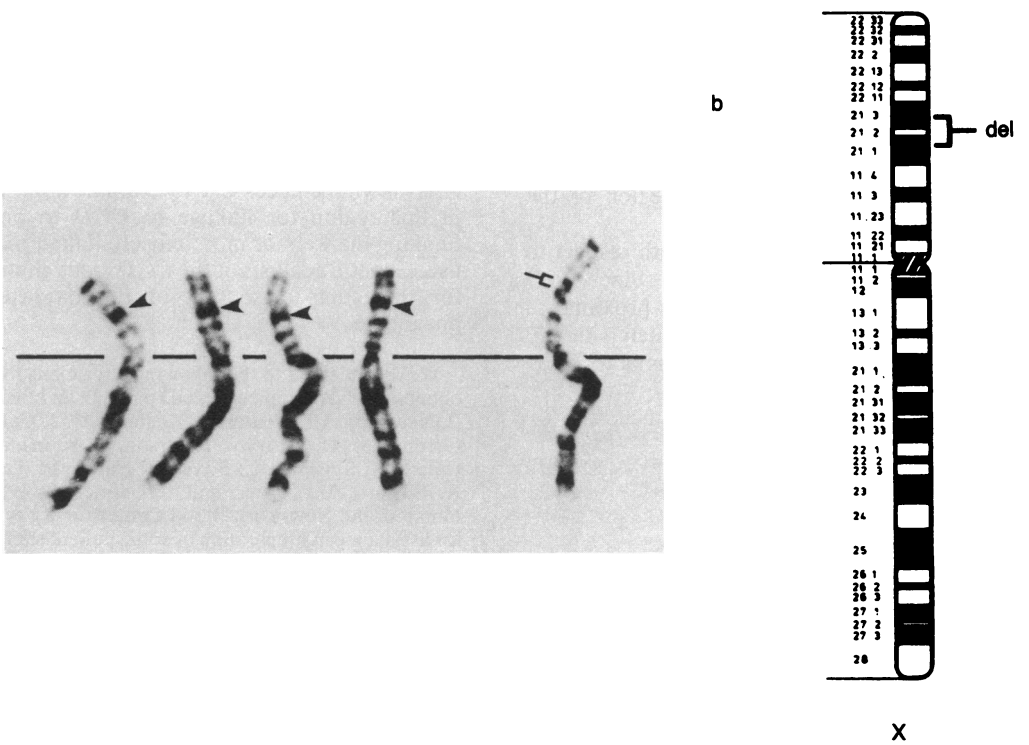

FIG. 1. Interstitial chromosomal deletion in patient N.F. (*a*) Left four chromosomes are the X chromosomes taken from different metaphases of the patient. The arrowheads indicate the site of an Xp21 deletion. Band Xp21.1 is missing and therefore Xp21 does not split even at band levels of greater than 550. The remaining dark G band is smaller than in normal X chromosomes, so part of Xp21.1 and/or Xp21.3 is also deleted. The chromosome at the far right is a normal X at approximately the same band level as the first and third deleted X chromosomes. (*b*) Ideogram of normal X chromosome with the site of the deletion indicated.

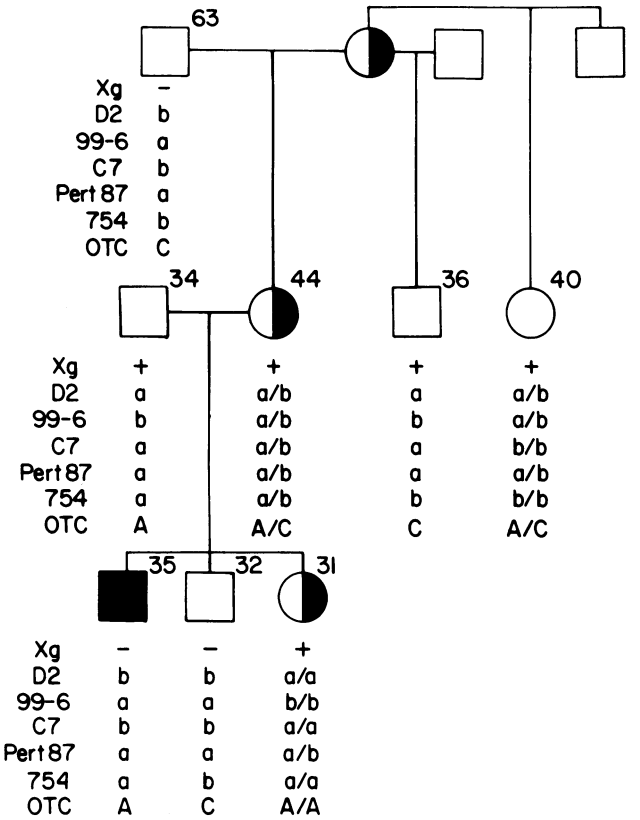

FIG. 2. Markers in a family carrying *CGD*. Alleles determined by DNA polymorphism analysis are arbitrarily assigned a or b for the larger and smaller restriction fragments, respectively. The data for an *Msp* I polymorphism at the ornithine transcarbamylase locus (*OTC*) are presented by haplotype designation as given by Rozen *et al.* (16). Blackened squares represent boys affected with CGD. Half-blackened circles are carrier females. The numbers above the symbols refer to the patient identification number in our panel.

distance from the *DMD* locus. These findings provide additional support for the concept of an increased rate of genetic exchange within the Xp21 region, suggested previously by Davies and colleagues from analysis of DMD families (11). Genetic and physical distances within this region of the genome appear discordant.

(*iv*) These data permit us to localize *CGD* with respect to other major Xp disease loci as shown in Fig. 3. Since *CGD* is linked to *754* and *PERT 84*, which mapped proximal to *DMD* (17), and less well linked to *PERT 87*, which is tightly linked to *DMD* (18), we infer that *CGD* is proximal to *DMD*

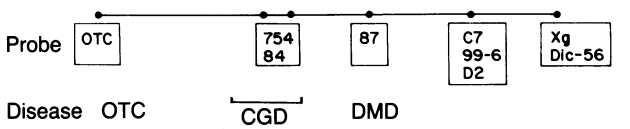

FIG. 3. Relative position of the *CGD* locus and other Xp21 markers. The region of Xp from its terminus (at the right) to the *OTC* locus (at the left) is diagrammed schematically. Markers are positioned according to their estimated distances from one another. Recombination fractions were first calculated for two-point crosses to maximize the amount of information contained in the linkage studies. The order of loci was determined by using the most likely three-point order. Because the data were not sufficient to define the order between *754* and *PERT 84* and among *C7*, *99-6*, and *D2*, markers have been enclosed in boxes in these regions. Based on the linkage data *CGD* appears to reside proximal to *DMD* but closer to probes *754* and *PERT 84* than to the *OTC* locus. The general region in which the *CGD* locus could possibly reside is indicated by the bracketed line.

rather than distal as believed previously (7). The linkage data suggest that *PERT 84* lies proximal to *754* (Table 1 and Fig. 3). Physical mapping based on the study of patients with various forms of DMD and glycerol kinase deficiency in which probe *754* and more distal Xp21 segments are deleted, however, reveals the converse (unpublished results). The relatively few informative meioses obtained with *PERT 84* (9 in total) lead to a higher uncertainty in the lod score relative to that obtained with probe *754*. Similarly, although the recombination map suggests an order of *PERT 87–D2–(C7, 99-6)*, the order *PERT 87–(C7, 99-6)–D2* was only slightly less favored. In fact, the latter is consistent with previous physical mapping based on chromosomal translocations (refs. 12–14 and 17; unpublished data).

DISCUSSION

CGD is a classical inherited disorder of phagocytic function. Determining the position of the *CGD* locus within Xp21 should assist in the identification of the gene product mutated in this disorder and in the molecular characterization of the oxidase system.

At present, a *b*-type cytochrome is the most likely primary gene product based on the absence of its spectrum in nearly all X-linked cases of CGD (5). Given the potential complexity of the granulocyte oxidase system, there remains the possibility that this is an associated and not a causative finding. For example, specific, as yet unknown, protein components may be required for proper cytochrome *b* activation and function. Our more precise assignment of the *CGD* locus within Xp21 should facilitate characterization of putative clones for the CGD gene product as the relevant sequences must map to this subregion of the X chromosome. Second, careful attention may now be directed to genomic segments lying within the proximal portion of the Xp21 segment deleted in the Francke *et al.* (10) and N.F. CGD–DMD patients in an effort to identify the *CGD* gene. A search for RNA transcripts derived from this region may yield the desired sequences independent of any assumptions regarding the nature of the critical protein(s). Third, probes such as *754* and *PERT 84*, which detect RFLPs in this chromosomal region, may aid in the development of DNA-based prenatal diagnosis for CGD. In view of the apparent increased genetic exchange within Xp21 it will be necessary to examine additional DNA probes in this region for linkage to *CGD* to obtain neighboring flanking markers or more closely linked markers for clinical use. Genetic analysis of the *CGD* locus should provide a basis for molecular dissection of the oxidase system of the phagocyte.

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