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Homozygosity Mapping of the Gene for Chediak-Higashi Syndrome to Chromosome 1q42-q44 in a Segment of Conserved Synteny That Includes the Mouse *beige* Locus (*bg*)

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Summary

Chediak-Higashi syndrome (CHS) is an autosomal recessive disorder characterized by hypopigmentation or oculocutaneous albinism and severe immunologic deficiency with neutropenia and lack of natural killer (NK) cell function. Most patients die in childhood from pyogenic infections or an unusual lymphoma-like condition. A hallmark of the disorder is giant inclusion bodies seen in all granule-containing cells, including granulocytes, lymphocytes, melanocytes, mast cells, and neurons. Similar ultrastructural abnormalities occur in the *beige* mouse, which thus has been suggested to be homologous to human CHS. High-resolution genetic mapping has indicated that the *bg* gene region of mouse chromosome 13 is likely homologous to the distal portion of human chromosome 1q. Accordingly, we carried out homozygosity mapping using markers derived from distal human chromosome 1q in four inbred families or probands with CHS. Our results indicate that the human *CHS* gene maps to an 18.8-cM interval in chromosome segment 1q42-q44 and that human CHS therefore is very likely homologous to mouse *bg*.

Introduction

Chediak-Higashi syndrome (CHS) is a rare, autosomal recessive disorder characterized by hypopigmentation and severe immunologic deficiency. First reported by Beguez-Cesar (1943), CHS was further described by Steinbrinck (1948), Chediak (1952), and Higashi (1954), the latter two of whose names then came to be associated with the disorder (Sato 1955; Donohue and Bain 1957). Dermatological manifestations of CHS range from moderate hypopigmentation of the skin and hair to tyrosinase-positive oculocutaneous albinism (OCA) with low vision, nystagmus, and photophobia. Immunologic manifestations include neutropenia and lack of natural killer (NK) cell function, resulting in recurrent pyogenic infections, hepatosplenomegaly and pancytopenia, and a strange lymphohistiocytic proliferative syndrome that resembles lymphoma (Argyle et al. 1982; Rubin et al. 1985). There may also be neurological manifestations, including seizures, mental retardation, cranial nerve palsies, clumsiness, abnormal gait, and progressive peripheral neuropathy. Death usually results in mid childhood, from overwhelming infection, hemorrhage, or the lymphoma-like phase of the disorder, although some patients appear to have a clinically milder, "adult" form of the disorder. There is no specific treatment for CHS, although bone-marrow transplantation has been beneficial in some cases (Kazmierowski et al. 1976; Filipovich et al. 1992).

The hallmark of CHS is the occurrence of giant inclusion bodies in virtually all granulated cells, including granulocytes, histiocytes, mast cells, platelets, melanocytes, Schwann cells, neurons, renal tubular epithelium, and fibroblasts. Histochemical and electron micrographic studies have suggested that the giant inclusions of many cell types are derived from lysosomes (White 1966; Windhorst et al. 1966; Lockman et al. 1967; Burkhardt et al. 1993), whereas in others they may be derived from secretory granules. CHS melanocytes are characterized by the presence of giant melanosomes (Windhorst et al. 1966; Zelickson et al. 1967). Recent evidence suggests that lysosomes and melanosomes may originate via a shared intracellular pathway (Orlow 1995), and it thus seems likely that CHS results from a defect of a protein required in common for the normal genesis, structure, or function of a variety of intracellular organelles: melanosomes, lysosomes, melanosomes, and intracellular secretory granules (Windhorst et al. 1966). In this regard CHS is similar to Hermansky-Pudlak syndrome in humans (Fukai et al. 1995) and to a number of different abnormalities of mice (Silvers 1979; Lyon and Searle 1989; Bennett 1991).

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Disorders similar to human CHS occur in many species of animals, including mink (Leader et al. 1963; Padgett et al. 1964), cattle (Padgett et al. 1964), cats (Kramer et al. 1977), foxes (Nes et al. 1983, 1985), killer whales (Taylor and Farrell 1973), rats (Nishimura et al. 1989), and mice (Lutzner et al. 1966). The *beige* mouse, in particular, has been proposed as a likely homologue to human CHS (Lutzner et al. 1966; Windhorst and Padgett 1973), and fusion of *CHS* and *bg* fibroblasts apparently fails to complement the lysosomal structural defects (Perou and Kaplan 1993). The murine *bg* locus is on mouse chromosome 13, in close proximity to the *Tcrg, Hist1, Prf, Fim-1* (Holcombe et al. 1987, 1991), and *Nid* (Jenkins et al. 1991) genes. The human *TCRG* gene maps to chromosome segment 7p15 and is not linked to *CHS* (Murre et al. 1985), whereas the *NID* gene maps to 1q43 (Olsen et al. 1989). Thus, this region has undergone considerable genomic rearrangement during mammalian evolution.

We have carried out homozygosity mapping in four inbred probands with classic childhood CHS, using markers derived from human chromosome segment 1q42-q44 as a candidate region. Our results indicate that the *CHS* gene is indeed located within an 18.8-cM interval in 1q42-q44 and that human CHS thus is homologous to mouse *bg*. However, at least some cases of "adult" CHS do not map to 1q, suggesting the likelihood of locus heterogeneity.

Subjects, Material, and Methods

Subjects

Four unrelated families or probands with typical childhood CHS were studied; in each case the parents were first cousins. Family 1 consisted of 19 members of a complex inbred Kuwaiti Bedouin kindred (Kandil et al. 1993), although not all were genotyped completely. Proband 2 consisted of a frozen skin-biopsy specimen from an inbred Japanese patient (Fukai et al. 1993); DNAs of her parents were prepared from blood. Proband 3 consisted solely of an autopsy liver specimen from another inbred Japanese patient (H. Ito, unpublished data); the parents were not available. Proband 4 was an inbred Turkish patient; his parents were not available.

Genotype and LOD-Score Analyses

High-molecular-weight DNA was prepared and genotyped by standard procedures (Dracopoli et al. 1994). Simple-sequence-length polymorphism (SSLP) markers in the 1q42-q44 region were identified from public databases (Murray et al. 1994; J. Weber, personal communication), and corresponding PCR primer pairs were purchased from Research Genetics. One primer of each pair was end-radiolabeled with ^{32}[P], and the PCR products were analyzed by denaturing gel electrophoresis and autoradiography. Scoring was by visual inspection, and allele sizes were assigned by comparison to ^{32}[P]-radiolabeled DNA molecular-size standards also on the gel and by parallel analyses of CEPH pedigree members 1331-01 and 1331-02 as standards. For each marker, DNAs of eight unrelated normal Arabs and nine unrelated Japanese were genotyped in parallel, to estimate population-specific allele frequencies. LOD scores were calculated as described by Lander and Botstein (1987).

Results

A total of 15 SSLP markers distributed across distal chromosome 1q were tested in some or all of the four inbred CHS probands (fig. 1). All four were homozygous for a number of these markers, although for different alleles in each case, several of which are quite uncommon in the subject populations (data not shown). Since the parents in each of these families were first cousins, for each proband the probability of homozygosity at a highly polymorphic marker locus, given homozygosity by descent for CHS, is 1/16. The combined odds in favor of linkage for these three probands is thus $(16)^4$, yielding a LOD score of 4.82 (Lander and Botstein 1987), indicative of genetic linkage between these markers on distal 1q and the *CHS* locus.

As shown in figure 2, three markers located in the middle of the interval studied—D1S235, D1S1594, and D1S204—were homozygous in all four of the inbred CHS probands. D1S235 is located 12.5 cM proximal to D1S1594, and D1S1594 is located 2.2 cM proximal to D1S204 (Murray et al. 1994; J. Weber, personal communication). However, proband 2 was not homozygous at either D1S446, located 4.1 cM proximal to D1S235, or D1S184, located 2.2 cM distal to D1S1594. The genetic interval between D1S446 and D1S184 that thus delimits the location of the *CHS* gene spans ~18.8 cM.

Discussion

We have mapped the gene for the classic form of CHS to an 18.8-cM interval flanked by D1S446 and D1S184. Three of the markers tested in this study have been cytogenetically mapped (Bray-Ward et al. 1996). D1S446, which defines the centromeric margin of the CHS genetic interval, has been mapped to 1q42-q43. D1S235, located within the CHS genetic interval, has also been mapped to 1q42-q43. And D1S304, located at the telomeric margin of the CHS genetic interval, has been mapped to 1q44. Together, these assignments delimit the cytogenetic interval for CHS to 1q42-q44. A number of genes have been mapped to this cytogenetic interval, but only one, *ACTN2*, has been precisely mapped within the CHS genetic interval. *ACTN2* maps to 1q42-q43 (Beggs et al. 1992) and to the genetic interval D1S446-

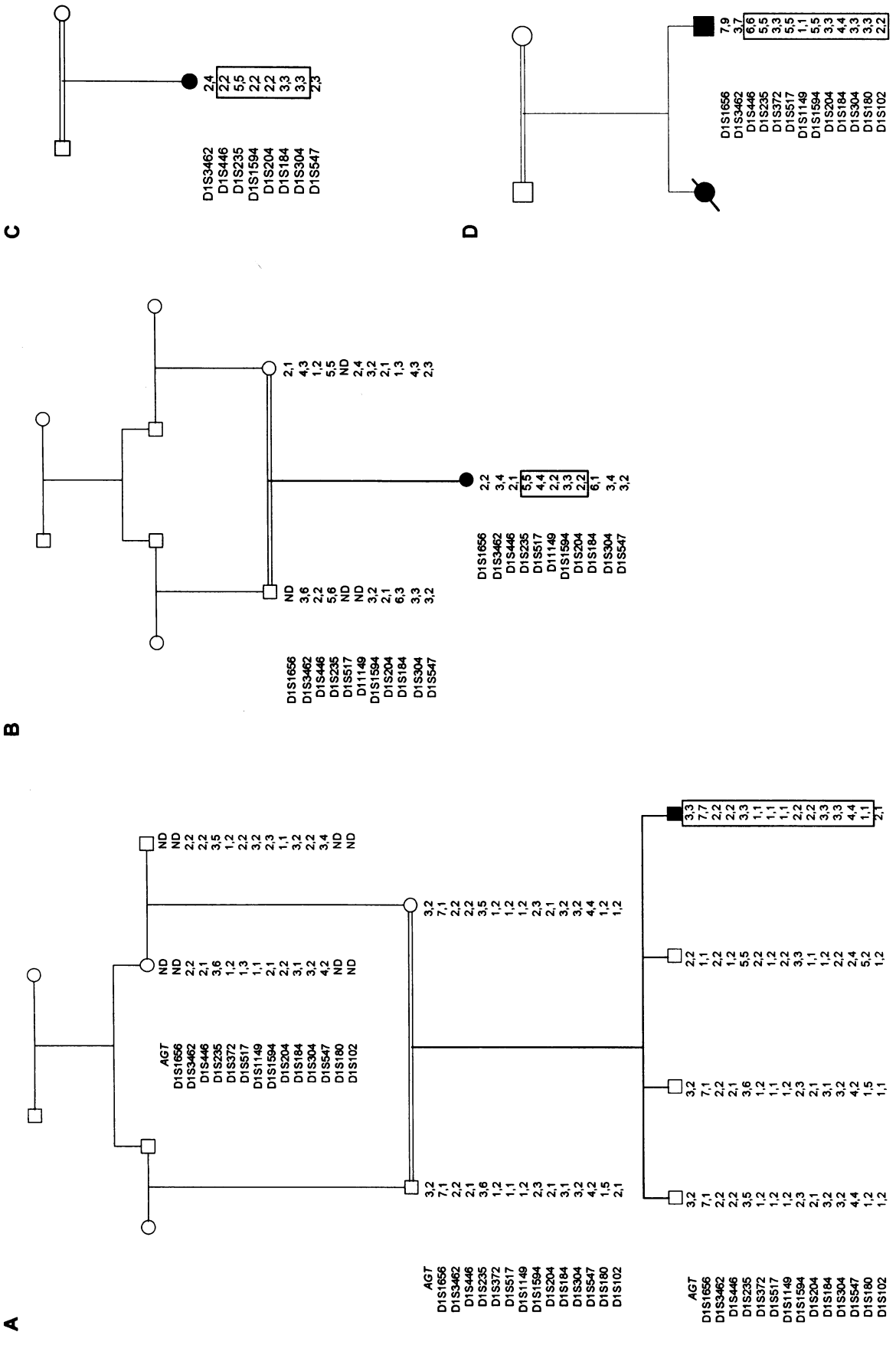

Figure 1 Chromosome 1q marker genotypes in the four CHS families or probands. *A*, Family 1. *B*, Family 2. *C*, Proband 3. *D*, Proband 4. The boxes indicate regions of homozygosity. ND = genotype not determined.

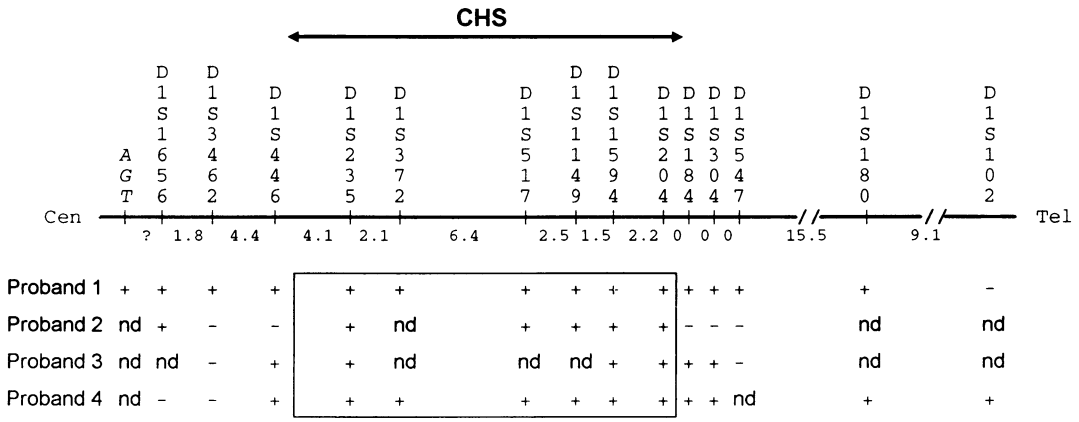

Figure 2 Marker zygosity in CHS probands. A plus sign (+) denotes that the individual is homozygous; and a minus sign (−) denotes that the individual is heterozygous. The box indicates a region of marker homozygosity common to the four probands, and the arrow indicates the maximum genetic interval for CHS. Genetic distances are from J. Weber (personal communication).

D1S594 (Beggs et al. 1992; Murray et al. 1994). The mouse *bg* gene has very recently been identified (Perou et al., 1996), but its human homologue has not yet been mapped either physically or genetically.

Our findings thus support the long-held belief that classic childhood CHS may be homologous to mouse *bg*. Furthermore, a series of loci that include at least *ACTN2, NID, RYR2,* and *CHS* appear to constitute a conserved linkage group with synteny between human chromosome 1q42-q44 and mouse chromosome 13. *bg* and *CHS* thus provide yet another example of the importance of coordinated analyses of homologous genetic disorders in man and in mouse. The very recent cloning of the mouse *bg* gene (Perou et al. 1996) will facilitate the isolation of the homologous human gene, thereby providing the opportunity for mutation analysis in patients with CHS. Furthermore, *beige* mice will provide a ready animal model of human CHS for biological studies and for eventual development of novel pharmacological or genetic therapies for this disorder.

We have also studied several inbred patients with the atypical, "adult" form of CHS. None of these individuals were homozygous for markers in distal 1q (data not shown), suggesting that at least some cases of this clinically distinct form of CHS may represent a distinct genetic entity located elsewhere in the genome.

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