Hereditary inclusion body myopathy maps to chromosome 9p1-q1

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Hereditary inclusion body myopathy (HIBM) is a unique disorder of unknown etiology that typically occurs in individuals of Persian Jewish descent. Distinguishing features of the disorder from other limb girdle myopathies include elderly age of onset, ethnic predisposition, and sparing of the quadriceps despite severe involvement of all other proximal leg muscles. Involved muscles demonstrate fibers with rimmed vacuoles and filamentous cytoplasmic and nuclear inclusions. Additional histological features are accumulations of β-amyloid protein and the absence of inflammatory cells. To identify the chromosomal location of the gene responsible for HIBM, nine Persian Jewish families with HIBM were evaluated. Genome-wide linkage analyses identified the recessive IBM locus on chromosome 9 band p1-q1 (maximum lod score at D9S166 = 5.32, θ = 0.0). This region contains the Friedreich’s Ataxia gene, raising the possibility that HIBM may be a related neurogenic disorder.

INTRODUCTION

Hereditary inclusion body myopathy (HIBM) is a poorly understood adult-onset disorder characterized by severe progressive muscular weakness and typical pathologic findings (1–3). The unique histologic features include the presence of rimmed vacuoles that contain concentric membranous bodies and typical inclusion bodies in the cytoplasm and nucleus. These inclusions are composed of double twisted filaments with a diameter of 15–18 nm (4). In contrast to the sporadic inclusion body myositis, HIBM lacks inflammatory infiltrates. The most common form of HIBM was described by Argov and Yarom in Jews of Persian origin (3). The onset of this disorder usually occurs after the age of 20 years but before the middle of the fourth decade of life. Proximal and distal muscle weakness and wasting of the upper and lower limbs is progressive and results in severe incapacitation within 10 to 20 years. Despite this, there is typically sparing of the quadriceps muscles even in advanced stages of the disease (5–7), which is unique to this form of HIBM.

To date, it is not clear whether HIBM is primarily a neurogenic or a myopathic disorder. Electromyography of affected muscles have been interpreted to indicate chronic denervation/reinervation, implying a neurogenic etiology (5), as well as a primary myopathic process (5,6). The degenerating muscle fibers contain besides the filaments abnormal accumulations of β-amyloid protein (3,8), ubiquitin (3), normal prion protein (3), and other pathological markers found in brain specimens from neurodegenerative disorders such as Alzheimer’s disease. However, even the older HIBM patients do not show central nervous system (CNS) disease.

To investigate the cause of HIBM, we have performed genetic linkage analyses in nine Persian Jewish families. Clinical studies provided evidence for autosomal recessive inheritance. A genome-wide analysis demonstrated linkage to chromosome 9 band p1-q1, which contains the Friedreich’s ataxia gene (9). A recently identified candidate gene for Friedreich’s ataxia has homology to yeast genes involved in intracellular protein trafficking (10). Based on genomic location and putative function, this gene is also a candidate for mutations which cause hereditary IBM.

RESULTS

Nine families of Persian Jewish descent were selected for study because at least one member was previously diagnosed with hereditary IBM based on criteria (Materials and Methods) developed by the IBM workshop (11). History and clinical evaluations performed by one of us (Z.A.) identified 25 affected and 54 unaffected family members (Fig. 1 and Materials and Methods). Nineteen of 24 affected individuals exhibited their disease before age 35.

The distribution pattern of muscle involvement was similar to that described previously (5,6). Upper limb weakness and

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atrophy was most marked in the shoulder girdle muscles; the humeral muscles and deltoids were generally less affected. Lower limb involvement was most marked in the hip flexors and extensors, hamstrings, and peroneal muscles. Quadriceps strength was preserved in all affected individuals except for two patients who had quadriceps sparing at the initial phase of the disease but developed involvement of this muscle group with disease progression, as has been recently reported (12).

Two modes of inheritance of IBM could be postulated based on pedigree analyses of these families. In two kindreds, (Fig. 1, Families 3 and 6) dominant transmission was plausible. However, because of documented consanguinity in Family 2, and the recognized high incidence of intrafamilial marriages in this community (even among younger individuals living in modern states), recessive transmission of IBM could account for disease inheritance in all families. Based on the finding of disease by age 35 in 19 of 24 affected individuals, we assumed a disease gene penetrance of 80% for linkage analyses purposes. Unaffected individuals below age 35 were not included in these linkage studies.

Genetic linkage analyses were performed using standard methods for mapping autosomal recessive disease loci (13). Highly polymorphic loci containing short tandem repeats sequences (STRs) were selected for study based on their location throughout the genome and their polymorphism information content (14). Lod scores (Materials and Methods) were calculated to be less than –2 in analyses of 74 loci, thereby excluding approximately 40% of the human genome. In contrast analyses with D9S165 yielded a lod score = 4.93 (θ= 0.01) providing odds of greater than 85,000:1 that the disease gene maps to chromosome 9p1.

To further define the location of the disease locus, genotypes were determined at 27 additional loci in this region. Lod scores indicative of linkage (Table 1) were found in analyses of loci that...
spanned a 20 cM region of chromosome 9p. A maximum lod score of 5.32 was achieved at locus D9S166 (θ = 0.00; Table 1). Multipoint linkage analysis did not refine this map location because these loci were almost fully informative in these study families. Haplotypes were therefore analyzed for recombination events. Genotype data from five individuals provided further information (Fig. 2) and refined the most likely disease interval to reside between D9S165 and D9S273. However, because individual F5 III-2 is clinically unaffected at age 36, the disease interval may span a 13 cM interval between D9S165 and D9S175. Haplotype analyses also demonstrated two recombinant events between D9S166 and D9S175. This finding is more consistent with earlier estimates of the genetic distance between these loci (15) than those recently reported (16).

**DISCUSSION**

Our data demonstrate that the hereditary IBM found in Persian Jews is an autosomal recessive disorder with an age-related penetrance that maps to chromosome 9p1-q1. This chromosome location is a region that also contains the gene defect for Friedreich’s ataxia (chromosome 9q12–21.1) (9). Although EMG studies have suggested a neurogenic component (lower motor neuron involvement) for HIBM, both motor and sensory nerve conductions are normal (5,6). In Friedreich’s ataxia there is preponderance of sensory impairment, abnormal CNS signs, cardiac involvement and slowed nerve conductions. The lack of these characteristics in HIBM makes it unlikely that these are allelic disorders. Recently a novel human gene MSS4 has been identified within the Friedreich’s ataxia critical region (10), although to date, mutations have not been identified in affected individuals. MSS4 has significant amino acid homology to the *Saccharomyces cerevisiae* genes FAB1 and STT4 which appear to function in the yeast endocytic vacuolar pathway which trafficks intracellular proteins and metabolites. Because rimmed vacuoles are the pathologic hallmark of HIBM and these collective mapping data, we hypothesize that MSS4 is a candidate gene for HIBM.

The number of adult Jews of Persian ancestry is estimated to be approximately 150 000 worldwide (17) and the number of affected individuals identified by us (Z.A., unpublished results) or reported by others (3,5–7) is at least 100. Based on these statistics we estimate the prevalence of IBM to equal 1:1500, and the disease gene carrier frequency to approximate 1–5% in this ethnic group.

**Table 1.** Pairwise lod scores reflecting linkage between chromosome 9 loci and hereditary IBM

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<th>Locus</th>
<th>Recombination fraction (θ)</th>
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<tr>
<td>D9S319</td>
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<td>D9S43</td>
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<tr>
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<tr>
<td>D9S165</td>
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<tr>
<td>D9S276</td>
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<td>D9S15</td>
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<td>4.84</td>
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<tr>
<td>D9S166</td>
<td>5.32</td>
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<tr>
<td>D9S175</td>
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Hereditary IBM has been reported in other ethnic groups too. In some there seems to be clinical and histological homology to the Persian Jewish HIBM (18). In others there are somewhat different features (19–22) to those described here. Age of onset, mode of inheritance, ethnic background, involvement of quadriceps muscles and the presence or absence of inflammatory cells clinically appear to define distinct subtypes of HIBM. The genetic relationship, if any, of these disease phenocopies can now be assessed. Because of the history of migration of Persian Jews, we suspect that HIBM in the Iranian, Afghanistan (6), Iraqi-Kurdish (6,7), and possibly Egyptian communities (6) may result from a common mutation. At present we have not yet demonstrated linkage disequilibrium with any DNA loci (data not shown), but we anticipate that this powerful technique will help further localization of the disease gene.

Degenerating muscle fibers in both hereditary and sporadic forms of IBM have been shown to accumulate twisted tubofilaments (4), amyloid (3,8) and β-amyloid precursor protein (3), normal prion protein (3) and ubiquitin (3), proteins that previously have been found in degenerating neurons of Alzheimer’s disease and Creutzfeldt-Jakob’s disease (3). Our mapping data support previous hypotheses that the similar composition of intracellular accumulations in IBM and in neurodegenerative disorders probably reflects a non-specific cellular degenerative process.

Although HIBM is a rare genetic disease, myopathies that exhibit similar inclusion bodies or rimmed vacuoles are more common and can be sporadic or inherited (both as a recessive and as a dominant trait). Localization of hereditary IBM to chromosome 9p1-q1 will permit studies to determine the genetic relationship between HIBM and clinically-related heritable myopathies. Isolation and characterization of the disease gene should also provide insights into the more frequent, acquired, sporadic inclusion body myositis.

MATERIALS AND METHODS

Clinical studies

At least one affected individual from each family was studied using accepted diagnostic criteria, recently refined by the IBM workshop (11). These evaluations included muscle power testing, conventional EMG and nerve conduction studies, serum creatine kinase determination, serum biochemical profile, thyroid function tests, and hematological profiles. A muscle biopsy was obtained from the tibialis anterior or biceps and evaluated by routine H&E, Gomori, NADH and ATPase stains and electron microscopy.

Hereditary IBM was diagnosed in the proband based on the finding of:

(i) marked proximal muscle weakness with quadriceps sparing that began during adult life; (ii) mild elevation of serum creatine kinase (less than four times the normal level); mixed electromyogram pattern of neurogenic and myopathic changes in the absence of other identifiable causes of myopathy, normal nerve conduction velocities; and (iii) biopsy histology demonstrating rimmed vacuoles in several fibers without inflammation with typical filamentous inclusions in the cytoplasm (and usually but not always in the nucleus).

Genotype analyses

Twenty milliliters of blood were obtained from all affected and unaffected family members who consented to the study. Genomic DNA was prepared either from whole blood using the SDS-proteinase K method, or further purified by phenol extraction (23). Polymorphic loci were amplified using radiolabeled PCR as described (13). All PCR products were resolved according to size by denaturing gel electrophoresis and visualized by autoradiography as described (13).

Linkage analyses

Two point analyses were performed using the MLINK computer program (24). Logarithm of odds (lod) scores were calculated assuming a recessive mode of inheritance. Allele frequencies were adjusted according to their incidence in the population studied.

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REFERENCES


