Human autosomal recessive osteopetrosis maps to 11q13, a position predicted by comparative mapping of the murine osteosclerosis (oc) mutation

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Autosomal recessive osteopetrosis is a rare congenital disorder characterized by the development of abnormally dense bones, acrocephaly, severe anemia, hepatosplenomegaly and progressive deafness and blindness. The clinical course is rapidly progressive and is lethal at a very young age in the absence of a bone marrow transplant. The failure to remodel developing bone that is the basis of the disease process is most likely due to a dysfunction of the bone resorptive cell, the osteoclast. This phenotype is similar to that of the murine mutation osteosclerosis (oc), which is localized to proximal mouse chromosome 19. Given the similarity between the human and murine phenotypes, we tested whether human osteopetrosis maps to a region of conserved synteny. Microsatellite markers in the region of 11q12–13 were found to be linked to osteopetrosis in two consanguineous Bedouin kindreds. Recombination events were used to define the disease interval to an ∼14 cM region between D11S1983 and D11S2371. A maximum LOD score of 7.94 was obtained with D11S449 at θ = 0.

INTRODUCTION

The growth and remodeling of bone that occurs during vertebrate development requires the carefully balanced activities of bone-forming osteoblast cells and bone-resorbing osteoclast cells. Disruption of this dynamic equilibrium can lead to a variety of pathological states. Human osteopetrosis, also known as marble bone disease and Albers–Schonberg disease, describes a group of hereditary disorders characterized by abnormal bone resorption. There are three clinical variants of this disease. Autosomal recessive osteopetrosis, also known as infantile or malignant osteopetrosis, is a congenital disorder that is lethal at an early age in the absence of a bone marrow transplant (1). Autosomal dominant osteopetrosis is a more benign disorder, generally presenting in early adolescence and characterized by multiple fractures (2). Osteopetrosis with renal tubular acidosis is a distinct entity, caused by mutations in the carbonic anhydrase gene (3).

Autosomal recessive osteopetrosis presents as a congenital disorder with a particularly devastating phenotype. Clinically, affected patients have abnormally dense bones, as well as acrocephaly, progressive deafness and blindness, hepatosplenomegaly and severe anemia (1). The various clinical features found in autosomal recessive osteopetrosis are secondary to a functional defect in osteoclasts. The anemia is a consequence of the inadequate bone marrow space, which then results in extra-medullary hematopoiesis, causing hepatosplenomegaly. The deafness and blindness likely represent effects of encroachment of bone on the small foramina which house the optic and auditory nerves. Iliac bone biopsy demonstrates that osteoclasts are usually present in osteopetrosis; however, the ruffled border (the highly convoluted cell membrane on the bone-opposed osteoclast surface) that is characteristic of active cells is absent (4). The only curative therapy for osteopetrosis is a bone marrow transplant, which further supports the presumption that this is a functional disorder of osteoclasts, which are bone marrow derived. In one large study, recipients of a genotypically HLA-identical bone marrow transplant had an actuarial probability of 79% for 5 year survival with osteoclast function (5).

The exact incidence of autosomal recessive osteopetrosis is not known, but is estimated to be 1 in 200 000 (2). The distribution of reported cases is widespread (1,2) and consanguineous sibships with multiple affected patients have been described in Costa Rica (6), Saudi Arabia (7) and Israel (8). While a dominantly inherited form of osteopetrosis has recently been localized to chromosome 1p21 (9), a map position for the recessive disease has not been reported. There exist a number of rodent models in which osteopetrosis is a prominent feature of the mutant phenotype. Mutations in genes such as Csfl, Fox, and Sfpi1 (also called PU.1) affect the differentiation of hematopoietic progenitor cells into osteoclasts (10–12). In contrast, in mice with mutations at Src or at the osteosclerosis (oc) locus, osteoclasts are histologically present in normal to elevated numbers, but lack the ruffled membrane and are non-functional (13,14). The oc mutation was first described in 1966 and was characterized in more detail and mapped to the proximal region of chromosome 19 by Marks et al. (14,15). Mice homozygous for this recessive mutation are easily distinguishable.

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Figure 1. Pedigrees of two Bedouin sibships segregating a mutation causing infantile osteopetrosis. Affected members are shown as filled symbols. Marker positions are based on physical mapping using the Genebridge 4 radiation hybrid panel (www.genome.wi.mit.edu and www.well.ox.ac.uk).

from their sibs prior to weaning due to delayed eruption of incisors and generalized growth deficiency. Histological analysis of these mice reveals absent marrow spaces and other changes evident of a marked deficiency in bone resorption. Changes consistent with rickets are also evident: specifically, thick epiphyseal plates and abundant unmineralized bone matrix. These findings are often also found in human osteopetrosis and are possibly secondary to hypocalcemia and hypophosphatemia, which have been documented in the oc mouse (16). These deficiency states are likely due to the fact that the defect in resorption in this disorder impairs the ability to mobilize calcium and phosphate from bone.

The proximal region of mouse chromosome 19 has conserved synteny with human 11q12–13. We report here that human autosomal recessive osteopetrosis maps to this region, suggesting the likelihood that the gene mutated in this disease is the same as that affected in the mouse oc mutation.

RESULTS

Patient evaluation

Ten patients (two male and eight female) with infantile osteopetrosis were ascertained from Bedouin families living in the Negev region of Israel. The affected patients were part of two extended kindreds with the same surname (Fig. 1). The exact relationship between the two kindreds could not be traced. All parents of the affected individuals were consanguineous. At the time of the study, four patients (aged 2 months to 7 years) were still living. The affected probands in these families presented with anemia, hypocalcemia, hypophosphatemia, hepatosplenomegaly and, in some cases, impaired vision. Plasma lactate dehydrogenase, bone alkaline phosphatase and parathyroid hormone were markedly elevated and urine calcium excretion was markedly reduced. Additional affected individuals were identified at earlier ages based on presenting symptoms in infancy of bulging fontanels and hepatosplenomegaly. Two cases were diagnosed at birth by radiological demonstration of increased bone density. Seven patients had bone marrow transplants; four did not survive the procedure. Of the three survivors, two (aged 5 and 6 years old) have short stature and impaired vision and one (aged 4 years) has impaired vision but is growing normally. In two cases, no compatible donor could be found and the patients died at age 5–6 months of respiratory failure.

Genetic mapping

The similarity of the phenotype of oc mice to human osteopetrosis suggests that these may be analogous disorders. This hypothesis was tested by analyzing two Bedouin Arab kindreds affected with osteopetrosis showing an autosomal recessive pattern of inheritance (Fig. 1) for genetic linkage to the region of conserved synteny with proximal mouse chromosome 19. At least 18 genes which map to this region in the mouse have human homologs in 11q12–13 (17). Polymorphic markers mapping to human chromosome 11q12–13 were selected and used to genotype all available members of the two kindreds. Three markers (D11S449, D11S4933 and D11S913) were shown to give highly significant LOD scores at θ = 0 (Table 1). The highest LOD score was 7.94 for the fully informative marker D11S449 at θ = 0. The two pedigrees shared a common disease haplotype, confirming an ancestral relationship and a likely common founder mutation event.
Table 1. Linkage analysis of human osteopetrosis to loci on 11q12–13

<table>
<thead>
<tr>
<th>Marker</th>
<th>LOD score at recombination fraction of</th>
<th>Max LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>D11S4953</td>
<td>–∞</td>
<td>4.75</td>
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<tr>
<td>D11S1983</td>
<td>–∞</td>
<td>5.10</td>
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<tr>
<td>D11S449</td>
<td>7.94</td>
<td>7.76</td>
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<tr>
<td>D11S4933</td>
<td>6.23</td>
<td>6.07</td>
</tr>
<tr>
<td>D11S913</td>
<td>7.40</td>
<td>7.23</td>
</tr>
<tr>
<td>D11S2371</td>
<td>0.44</td>
<td>3.58</td>
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Recombination events in two unaffected individuals (484 and 472) were used to localize the disease to the interval between D11S1983 and D11S2371. Individual 472 shares the genotype of her affected sibling proximal to D11S480 and is recombinant for markers distal to D11S480 on the maternal chromosome (470). Individual 470 is homozygous for this marker and the recombination event is thus uninformative at this locus. The proximal disease flank has been assigned to D11S1983, the closest informative marker proximal to D11S480 on the maternal chromosome.

Individual 484 has only one affected sibling, for which no DNA sample was available. The haplotype of the affected paternal (471) chromosome can be deduced from the affected children of a second marriage. The affected maternal chromosome (475) can be inferred from the two affected children of her niece (489). Individual 484 inherits the affected maternal chromosome and is recombinant for the paternal chromosome proximal to D11S2371, thus defining our distal flank for the disease locus. This defines the disease interval to a 68 cM interval between D11S1983 and D11S2371, which corresponds to a genetic interval of ~14 cM based on the physical map proximity of these markers to genetically mapped markers.

DISCUSSION

Human osteopetrosis describes disorders characterized by abnormal bone resorption. The elucidation of the pathophysiology of the human disease process will lead to a better understanding of the events which control the cellular processes of bone resorption central to the events of bone homeostasis. Many mouse models which share the osteopetrotic phenotype have already been described and have been invaluable in elucidating bone resorptive mechanisms (10–14). However, no mutation in the mouse has yet been correlated with the human disease. The pathology of the human autosomal recessive disorder osteopetrosis is very similar to that of oc mutant mice, in that osteoclasts are present but histologically lack a ruffled membrane and are physiologically non-functional, and the disease is usually lethal in the absence of a bone marrow transplant (15,16). This suggests the possibility that the gene mutated in the oc mouse corresponds to that affected in the human disease. This hypothesis would be supported by evidence that the human disorder maps to 11q13, which is the region that has conserved synteny with the position on chromosome 19 to which oc has been mapped. Linkage analysis demonstrates that loci in this region are linked with the disease in two Bedouin kindreds, with a maximum LOD likelihood of 7.94 for the marker D11S449.

Both in vivo and in vitro studies suggest that the phenotype of oc mutant mice is due to a defect in a cellular component of hematopoietic origin. Experiments in which bone marrow transplants were used to correct the oc defect first demonstrated this (18). Additional experiments which support this hypothesis are in vitro studies which demonstrated that hematopoietic cells grown on a bone marrow-derived stromal line under conditions in which osteoclasts differentiate could generate cells with an osteoclast-like appearance but which lacked bone-resorbing function (19). Stromal cells obtained from oc mutant mice, however, function to support differentiation of osteoclasts derived from wild-type bone marrow.

The evidence that the defect in oc is due to a defect in a cell of hematopoietic origin suggests the possibility that osteopetrosis is a cell-autonomous defect of osteoclasts. Two genes known to map to human 11q13 are of interest as candidate loci for this disorder. The fos-related antigen 1 (Fra1 or Fosl1), a protein identified as being homologous to Fos (20), is of note because a targeted disruption of Fos causes a defect in osteoclast maturation which results in osteopetrosis (21). However, a complementation test between a targeted disruption of the Fra1 gene and oc, and linkage studies using an intragenic marker suggest that Fra1 and oc are not allelic (21).

Another candidate for the gene mutated in osteopetrosis is a novel presumptive transporter expressed in kidney and developing bone and mapped to 11q13 by analysis of a radiation hybrid panel. The expression of this gene, which has similarity to a family of transporters involved in organic anion and cation transport, has been demonstrated to be markedly decreased in kidneys from oc homozygous mice (D.R.Beier, manuscript submitted; GenBank accession no. AF078869). However, the causal role of this gene, provisionally called Rodt (for reduced in oc transporter) in the oc mutation has not been conclusively established, since no sequence changes in the coding region, splice junctions or putative promoter region have been identified.

Interestingly, this region on chromosome 11 also corresponds to that in which a dominantly inherited trait of increased bone density has been localized (22). One possibility is that this trait corresponds to the heterozygous state for a mutation causing osteopetrosis. Bone density studies have generally not been reported for obligate heterozygote parents of patients with recessive osteopetrosis. However, there are two reports of kindreds in which both the mild adult and severe infantile forms of osteopetrosis were found (23,24). Even in the case that heterozygote carriers for osteopetrosis have normal bone density, this does not preclude the possibility that the high bone mass trait is due to a mutation at the osteopetrosis locus that functions in a dominant negative fashion.
Osteoporosis is the most common disorder of bone growth, affecting ~15 000 000 individuals in the USA alone. The reduction in bone mass that occurs in this disease appears to be due to insufficient osteoblast-mediated synthesis relative to osteoclast-mediated resorption, although the specific defects in this disorder remain unknown. The identification of a gene responsible for the rarer disorder of increased skeletal mass, osteopetrosis, will likely suggest novel areas of therapeutic intervention against the more common disease of decreased skeletal mass.

MATERIALS AND METHODS

Blood samples were obtained from all available pedigree members (seven affected and 30 unaffected individuals) in EDTA-containing vials. Genomic DNA was isolated from whole blood using a non-organic extraction procedure (25). Short tandem repeat polymorphic markers developed by the Cooperative Human Linkage Center (26) were amplified by PCR in 8.35 μl reactions containing 20 ng genomic DNA template, 1.25 mM MgCl₂, 0.5 μM each primer and 0.25 U Taq polymerase.

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REFERENCES


