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Localization of a Gene for Autosomal Dominant Osteopetrosis (Albers-Schönberg Disease) to Chromosome 1p21

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Summary

Albers-Schönberg disease, the classical form of osteopetrosis, is an autosomal dominant condition with generalized increased skeletal density due to reduced bone resorption. Characteristic radiological findings are generalized osteosclerosis, with, most typically, endplate sandwichlike thickening of the vertebrae (Rugger-Jersey spine) and the bone-within-bone (endobones) phenomenon. We studied an extended kindred with Albers-Schönberg disease and found linkage with several markers from chromosome 1p21. The Albers-Schönberg gene is located in a candidate region of ~8.5 cM flanked by markers D1S486 and D1S2792. A maximum LOD score (Z_{max}) of 4.09 was obtained in multipoint analysis at loci D1S239/D1S248. Possible linkage of osteopetrosis to this chromosomal region was analyzed because the CSF-1 gene, which is mutated in the *op/op* mouse model for osteopetrosis, is located in 1p21. However, SSCP and mutation analysis in patients did not reveal any abnormality, which excludes the CSF-1 gene as the disease-causing gene. This was confirmed by refined physical mapping of the CSF-1 gene outside the candidate region for the Albers-Schönberg gene. The identification of the molecular defect underlying Albers-Schönberg disease will therefore be dependent on the isolation of other genes from an 8.5-cM candidate region on chromosome 1p21.

Introduction

In 1904 Albers-Schönberg described a patient suffering from several fractures due to generalized sclerosis of the skeleton (Albers-Schönberg 1904). Later on, the terms "osteopetrosis" and "marble bone disease" were introduced (Karschner 1926). At present, osteopetrosis comprises a clinically and genetically heterogeneous group of disorders sharing a defective mechanism of bone resorption, which results in an increased bone density (Beighton and Horan 1977). The terminology of the different types of osteopetrosis, however, is confusing (table 1). In general, osteopetrosis is divided into three forms (Shapiro 1993). One form (MIM 259700) is a malignant osteopetrosis with a recessive mode of inheritance affecting children from the neonatal period on. The patients have different neurological symptoms (blindness, deafness, and/or facial palsy), hematological abnormalities (anemia, thrombocytopenia, and/or leucopenia) and usually die of bleeding or infection within the first years of life (Loria-Cortés et al. 1977; Gerritsen et al. 1994). A second form (MIM 166600), now referred to as "Albers-Schönberg disease" or "marble bone disease," is more benign and has an autosomal dominant form of inheritance (Johnston et al. 1968; Bollerslev 1989). A third form (MIM 259710) is intermediate, with an autosomal recessive inheritance but a much milder course (Horton et al. 1980; Kahler et al. 1984). A particular subtype (MIM 259730) of the latter type is associated with renal tubular acidosis and cerebral calcification (Whyte et al. 1980), because of a deficiency of carbonic anhydrase II (Sly et al. 1983, 1985) (table 1).

Patients with the autosomal dominant form of osteopetrosis suffer from periodic back pain, bone pain, headache, and recurrent fractures (table 1). Other symptoms may include osteomyelitis and compression of cranial nerves, leading to deafness, loss of vision, and facial nerve palsy. The frequency of this condition has been estimated at 1:100,000–1:500,000 (Johnston et al. 1968), but an extended and detailed epidemiological study in Denmark revealed a frequency >1:20,000 (Bollerslev 1987). The underestimation of the disease frequency in the first study can be explained partly by the fact that approximately half of the cases are asymptomatic and are often detected only by incidental radiography. A radiological study of 34 patients from the Danish epidemiological study revealed two phenotypic subtypes (Bollerslev and Andersen 1988). In autosomal dominant osteopetrosis type I, sclerosis is most striking in the cra-

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Table 1

Different Types of Osteopetrosis

Type	Inheritance	Typical Symptom(s)	Primary Defect	MIM Number
Malignant	Recessive	Neurological symptoms, bleeding, infection, early death	Unknown	259700
Benign:				
Type I	Dominant	Cranial vault sclerosis	Unknown	166600
Type II (Albers-Schönberg)	Dominant	Rugger-jersey spine, endobones	Unknown	166600
Intermediate	Recessive	Short stature, anemia, fractures	Unknown	259710
	Recessive	Renal tubular acidosis, cerebral calcification	Carbonic anhydrase II	259730

nial vault, whereas, in autosomal dominant osteopetrosis type II (Albers-Schönberg disease), only the base of the skull is dense. Albers-Schönberg disease is further characterized by the sandwichlike appearance of the spine, which is due to end-plate thinkening of the vertebrae (Rugger-Jersey spine), and by endobones ("bone within a bone") in the pelvis (fig. 1). Albers-Schönberg patients show increased serum values of acid phosphatase, increased frequency of fractures, and, sometimes, nonpenetrance. None of the latter findings is present in type I patients, and both types of osteopetrosis seem to breed in different families. This indicates that the autosomal dominant form of osteopetrosis consists of two different entities (Bollerslev and Andersen 1988).

The diversity of gene mutations leading to osteopetrosis is illustrated by the fact that ≥ 10 distinct animal models have already been reported (Marks 1989), including four mouse mutants (*gl/gl, mi/mi, oc/oc,* and *op/op*) (Grünberg 1935, 1948; Marks and Lane 1976; Marks et al. 1985), three rat mutants (*ia/ia, op/op,* and *tl/tl*) (Marks 1973, 1977, 1989), and undefined osteopetrosis in rabbit (Pearce and Brown 1948), cow (Thomson 1966), and dog (Riser and Frankhauser 1970). For two of these models, the underlying mutation has been identified: in the *op/op* mouse, a mutation has been found in the coding region of the macrophage-colony-stimulating–factor gene (*Csfm*) (Yoshida et al. 1990), whereas the *mi/mi* (microphthalmia) mouse is associated with mutations in the *microphthalmia* gene (Hodgkinson et al. 1993). Besides these naturally occurring autosomal recessive animal mutants, it has been shown that disruption of the proto-oncogenes c-*src* and c-*fos* in mice also results in osteopetrosis (Soriano et al. 1991; Wang et al. 1992). More recently, it has been shown that the disruption of the Acp 5 gene (Hayman et al. 1996) and of the hematopoietic transcription factor PU.1 also results in osteopetrosis (Tondravi et al. 1995). We now report the results of a linkage study in an extended kindred with Albers-Schönberg disease.

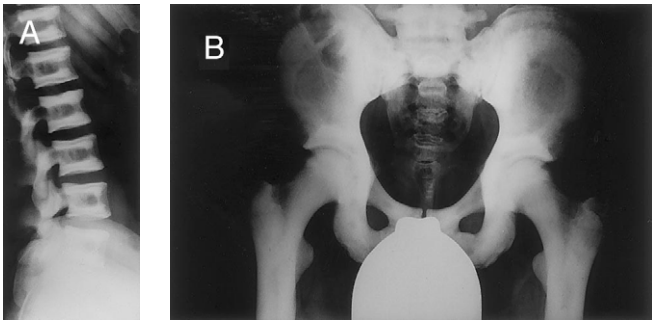

Figure 1 Typical radiological features in Albers-Schönberg disease. *A,* Lateral view of spine in a young man with Albers-Schönberg disease, showing the characteristic "Rugger-Jersey spine" with sandwichlike vertebrae that are due to sclerosis of the end plates. *B,* Frontal view of the pelvis in an Albers-Schönberg patient, showing the "bone-within-bone" appearance in the iliac wings.

Subjects and Methods

Ascertainment of the Family

Blood samples were taken from 31 family members from an extended Danish kindred with Albers-Schönberg disease, already described elsewhere (Bollerslev 1987). Patients were diagnosed on the basis of *x*-rays taken from the skull and the thoracolumbal spine. In comparison with the pedigree described in the original study, two individuals (II-16 and III-26) have meanwhile been diagnosed as affected. Genotyping of individuals II-8, III-9, and III-12 showed three different paternal haplotypes, indicating that at least one of them originates from a different biological father.

Genotyping

Genomic DNA was isolated from whole blood by standard techniques. Microsatellite markers were typed by radiolabeled PCR, with separation according to size, on polyacrylamide gels and were vizualized by autoradiography. For linkage analysis with candidate genes, microsatellite markers used were selected on the basis of the available mapping of these genes.

The human SRC gene was assigned by FISH to chro-

mosome band 20q11.2 (Morris et al. 1989), and the availability of an intragenic dinucleotide repeat (Xiang et al. 1991) allowed detailed genetic mapping between D20S106 (proximal side) and D20S107 (distal side) (Gyapay et al. 1994). The human FOS gene is located within chromosomal region 14q21-q31 (Barker et al. 1984). No highly polymorphic marker is available within this gene, but physical mapping data indicate that microsatellite markers D14S284 and D14S273 are on opposite sites of the FOS gene, at a distance $\leqslant$100 kb (Cruts et al. 1995). This allowed us to use these two markers to study linkage with the FOS gene. The MITF gene has been assigned by FISH to chromosome 3p14.1-p12.3 (Tichibana et al. 1994), but no intragenic polymorphisms in the MITF gene have been described. Mutations in the MITF gene lead to Waardenburg syndrome type 2 (Tassabehji et al. 1994), and, since no recombinations between this disease and D3S1261 have been reported (Hughes et al. 1994), D3S1261 was used in our linkage analysis. The CSF-1 gene has been assigned by FISH to chromosome band 1p21 (Landegent et al. 1992). No detailed genetic mapping information of the CSF-1 gene is currently available, since only one RFLP with low information content has been reported for this gene (Heinzmann et al. 1989). However, the murine homologue, *csfm,* maps to chromosome 3 in the mouse, close to the cluster of *Amy* genes (Gisselbrecht et al. 1989). The *Amy* genes are part of a syntenic group that is conserved between mouse chromosome 3 and human chromosome 1 (Moseley and Seldin 1989). Therefore, a CA repeat in the AMY2B gene (Dracopoli and Meisler (1990) was used to test linkage with the CSF-1 gene.

Linkage Analysis

Two-point LOD scores were calculated by use of MLINK (version 5.1) in the FASTLINK implementation (version 2.2) (Lathrop and Lalouel 1984). Multipoint analysis was performed by use of the VITESSE algorithm (O'Connell and Weeks 1995). The disease frequency was set at 1/100,000. An autosomal dominant mode of inheritance was assumed, with a disease penetrance of .60. Allele frequencies for the microsatellite markers were set at $1/n$ (n = number of alleles).

Mutation Analysis

To detect large rearrangements, DNA from patients II-9, II-11, and II-22 was digested with several restriction enzymes, separated by gelelectrophoresis, and transferred to Hybond N+ membranes by Southern blotting, and hybridized with radiolabeled probes covering exons 2, 4, and 6 of the CSF-1 gene. To detect small mutations, SSCP analysis was performed by use of HydroLink MDE gel (J.T. Baker) according to standard protocol. Intronic primers for the eight coding exons of the CSF-1 gene (Ladner et al. 1987) were synthesized on the basis of available intron sequences (M. Ladner, personal communication). The results obtained on three patients (II-10, II-11, and III-25) were compared with those on several control samples. Finally, the exons and intron-exon boundaries of the CSF-1 gene of patients II-9 and II-10 were sequenced by direct sequencing of the PCR products, on an automatic sequencer, ABI-373, using dye terminator-sequencing protocols.

Screening of YACs

DNA from CEPH YACs was prepared by use of standard protocols and was tested for the presence of loci, by standard PCR conditions.

Results

Since the gene implicated in Albers-Schönberg disease has not yet been mapped on the human genome, we performed linkage analysis in a large Danish family with this disease. The clinical, radiological, and biochemical features of this family have been reported elsewhere (Bollerslev 1987, 1995). We performed linkage analysis in this family, with DNA markers in or around genes mutated in animal models for osteopetrosis—including genes SRC, FOS, MITF, and CSF-1—even though these are recessive mutants.

In the initial phase of our linkage study, eight affected family members were typed for markers in or close to candidate genes. This excluded SRC and FOS as candidate genes (data not shown). However, no exclusion for MITF and CSF-1 could be obtained. Therefore, 23 additional family members were included in the linkage analysis (fig. 2). One additional marker, D3S3551, located 1.6 cM proximal of D3S1261, was also analyzed, to study MITF as a candidate gene. However, pairwise LOD scores for linkage with Albers-Schönberg disease were negative, both for D3S1261 and for D3S3551. Since multipoint analysis resulted in LOD scores <-2 for a chromosomal region $>$20 cM around the MITF gene (data not shown), the latter is excluded as a candidate gene for Albers-Schönberg disease. To study CSF-1 as a possible candidate gene, linkage analysis with AMY2B was performed. A positive LOD score of 3.07 was obtained at zero recombination (table 2). To confirm linkage of Albers-Schönberg disease to the CSF-1 region, nine additional polymorphic markers in and around the CSF-1 gene were analyzed. First, the only intragenic CSF-1 polymorphism (a *Kpn*I RFLP) was analyzed but proved to be uninformative in this family. Second, eight additional markers from the CSF-1 region on chromosome 1p21 were selected from the Généthon human linkage map (Gyapay et al. 1994; Dib et al. 1996). Two-point linkage analysis resulted in positive LOD scores for all eight markers, and no recombination

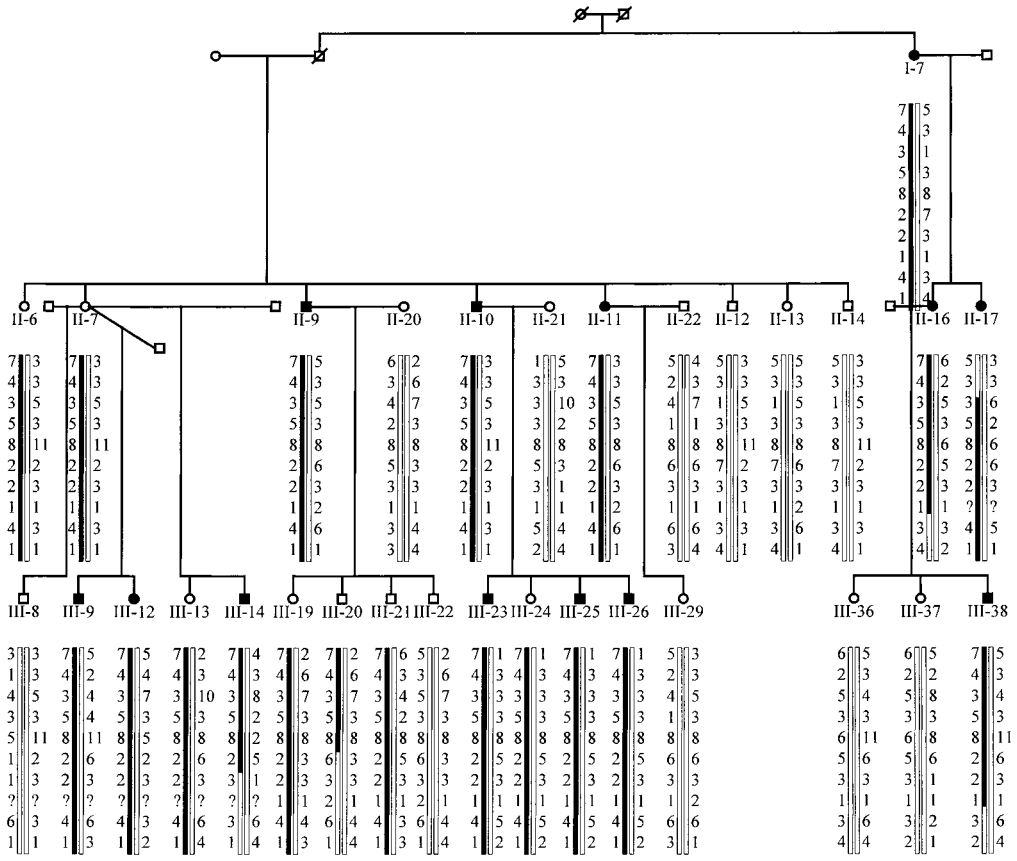

Figure 2 Pedigree of Danish family with Albers-Schönberg disease. Male family members are represented by squares, and females are represented by circles; blackened squares and circles denote affected individuals. Haplotypes for the chromosome 1 markers are shown below the symbols. The haplotype consists of alleles from nine marker loci. The order, from telomere (top) to centromere (bottom), is D1S497, D1S486, D1S495, AMY2B, D1SD248, D2S239, D1S2792, D1S221, and D1S457. The blackened bars represent the haplotype that cosegregates with the disease. On the basis of the presence of the disease-associated haplotype, 19 individuals carry the disease gene, but only 13 show the phenotype. This implies a penetrance of ~68% in this family, which is slightly higher than the 56% previously estimated (Bollerslev 1989).

was found with markers D1S495, AMY2B, D1S248, and D1S239 (table 2). Conclusive LOD scores >3 were obtained for two markers, D1S495 (Z_{max} = 3.65) and AMY2B (Z_{max} = 3.07) (table 2). Multipoint analysis with markers D1S495, D1S248, D1S239, and D1S2792 revealed Z_{max} = 4.09 at loci D1S248/D1S239, confirming linkage between this region and Albers-Schönberg disease. Key recombinants, represented in figure 3*B*, delineate a candidate region of 8.5 cM between D1S486 and D1S2792. On the distal side the disease recombines with D1S486 in individual II-17, whereas on the proximal side a recombination is detected both with D1S221 and D1S457 in individual II-16 and with D1S2792, D1S221, and D1S457 in individual III-14. The recombinational event between D1S495 and D1S239 in individual III-20 does not narrow the candidate region for the disease, since individual III-20 is not clinically affected and nonpenetrance is present in this pedigree, as illustrated in individual II-7.

To test the possible involvement of the CSF-1 gene in the disease, mutation analysis was performed. Southern blot, SSCP, and sequencing analysis of both the complete coding sequence and the exon-intron boundaries of the CSF-1 gene did not reveal any abnormality in the CSF-1 gene, indicating that CSF-1 is not involved in the disease. Additionally, refined physical mapping of the CSF-1 gene relative to the genetic candidate region was performed. As shown in figure 3*C*, the CSF-1 gene is located within seven YACs that are positive for D1S221 but not for the distal locus D1S2792. Since D1S2792 forms the proximal boundary of the genetic candidate region, the mapping of the CSF-1 gene proximal to this marker implies that the CSF-1 gene is not located in the genetic candidate region of the Albers-Schönberg disease gene.

Discussion

Animal models for genetic diseases can provide obvious candidate genes for the disease. In the mouse, naturally occurring mutations in *mitf* and *csf*-1, as well as

targeted disruption (knockout mutation) of c-*src* and c-*fos,* result in autosomal recessive osteopetrosis (Yoshida et al. 1990; Soriano et al. 1991; Wang et al. 1992; Hodgkinson et al. 1993). To test the involvement of the human homologues of these genes in Albers-Schönberg disease, linkage analysis with polymorphisms located either within these genes or in the flanking regions was performed. The significance of the obtained results mostly depends on both the accuracy of the available mapping data of the human gene and the availability of closely linked microsatellite markers. The current availability of genetic maps saturated with highly polymorphic markers and of detailed physical maps makes such candidate-gene strategy to identify disease genes a valuable alternative for a random genome search.

After exclusion of SRC, FOS, and MITF as candidate genes for Albers-Schönberg disease, positive LOD scores were found with markers around CSF-1 on chromosome 1p21. Key recombinants in family members II-17 and III-14 indicated a candidate region of 8.5 cM between D1S486 (distal side) and D1S2792 (proximal side). A recombinational event between D1S495 and D1S239 in individual III-20 did not narrow the candidate region, since individual III-20 is not clinically affected and nonpenetrance is present in this pedigree (Bollerslev 1989), as illustrated in individual II-7. On the basis of the presence of the disease-associated haplotype, 19 individuals carry the disease gene, but only 13 show the phenotype. This suggests a penetrance of ~68% in this family, which is slightly higher than the 56% estimated by a previous study (Bollerslev 1989).

The selection of the 1p21 region for linkage analysis with Albers-Schönberg disease was based on both the regional assignment of the CSF-1 gene to this region and the observation that the murine homologue of CSF-1 is mutated in the osteopetrotic *op/op* mouse model. To further analyze the possible involvement of the CSF-1 gene, a mutation analysis in this gene was performed, but this did not reveal a mutation. This makes it unlikely that the CSF-1 gene harbors the disease-causing mutation in this family. This was further confirmed by the physical mapping of the CSF-1 gene outside the candidate region. It is therefore likely that different pathological mechanisms underly the osteopetrotic *op/op* mouse model and Albers-Schönberg disease. Since CSF-1 is a hematopoietic growth factor playing a role in the maturation of osteoclast and macrophages from progenitor

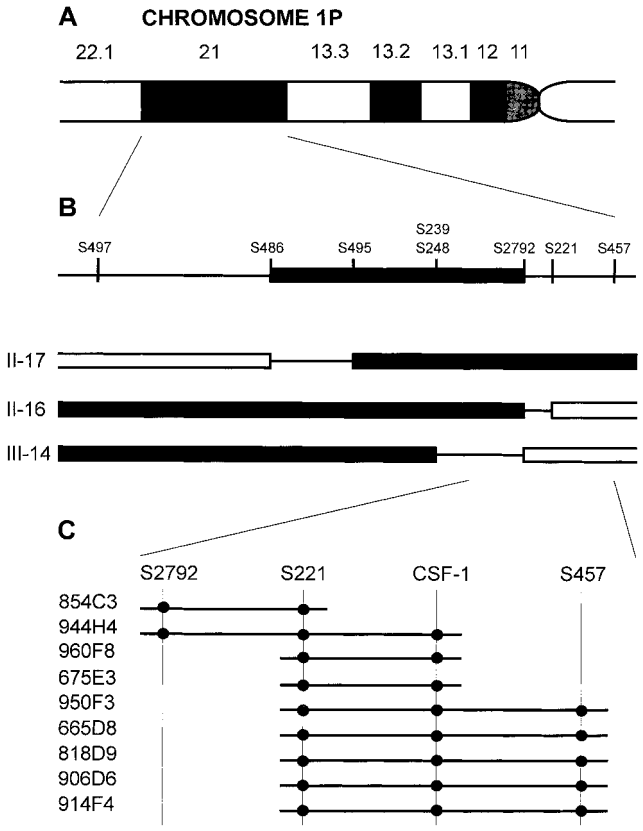

Figure 3 Delineation of the candidate region for the Albers-Schönberg disease gene. *A,* Idiogram of the proximal chromosome 1p region. *B,* Key recombinants in the family, which delineate the candidate region for the Albers-Schönberg disease gene. The blackened bars represent the chromosomal regions that contain the disease gene, and the unblackened bars represent regions that do not contain the disease gene; the horizontal lines represent noninformative regions. The recombination, in individual II-17, between D1S486 and the Albers-Schönberg disease gene localizes the disease gene proximal to D1S486. The recombinational event with D1S221 in patient II-16 and that with D1S2792 in patient III-14 indicate that the Albers-Schönberg disease gene must be located distal to D1S2792. *C,* Detailed physical mapping of the CSF-1 gene, by use of YACs from the chromosome 1p21 region. The CSF-1 gene is not present in YAC 854C3, which is positive for D1S2792, the proximal border of the genetic candidate region of the Albers-Schönberg gene. Furthermore, the CSF-1 gene is found in seven YACs that are located proximal to the candidate region; therefore, the CSF-1 gene maps outside the candidate region of the Albers-Schönberg disease gene.

Table 2

Pairwise LOD Scores between Albers-Schönberg Disease and Markers from Chromosome 1p21

	LOD Score at Recombination Fraction of						
Marker	.00	.01	.05	.10	.20	.30	.40
D1S497	−1.76	1.53	2.05	2.10	1.81	1.29	.62
D1S486	−2.09	1.20	1.75	1.83	1.59	1.11	.51
D1S495	3.65	3.60	3.40	3.12	2.48	1.71	.81
AMY2B	3.07	3.03	2.83	2.55	1.93	1.22	.47
D1S248	1.88	1.85	1.74	1.59	1.25	.87	.45
D1S239	2.49	2.46	2.29	2.07	1.58	1.02	.42
D1S2792	−2.00	1.32	1.79	1.78	1.41	.86	.29
D1S221	−7.60	−.99	.27	.67	.82	.63	.29
D1S457	−3.30	−1.91	−.64	−.19	.09	.10	.01

cells (Kawasaki et al. 1985), impaired osteoclastic differentiation can be expected after inactivation of CSF-1. In the *op/op* osteopetrotic mouse, a mutation in CSF-1 (Yoshida et al. 1990) leads to a decreased number of abnormally small osteoclasts (Marks and Lane 1976). In Albers-Schönberg disease, however, osteoclasts are large and even increased in number (Bollerslev et al. 1993). Consequently, also on pathological grounds decreased activity of CSF-1 is not very likely to be the mechanism involved in Albers-Schönberg disease.

In conclusion, the Albers-Schönberg gene has been located within an 8.5-cM candidate region on chromosome 1p21. The mapping of this gene will facilitate its identification, which might result in new insights into the regulation of osteoclastic bone resorption and might contribute to an understanding of other clinical entities, such as osteoporosis, that are associated with abnormal bone density.

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