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Osteoporosis-Pseudoglioma Syndrome, a Disorder Affecting Skeletal Strength and Vision, Is Assigned to Chromosome Region 11q12-13

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Summary

Osteoporosis-pseudoglioma syndrome (OPS) is an autosomal recessive disorder characterized by severe juvenile-onset osteoporosis and congenital or juvenile-onset blindness. The pathogenic mechanism is not known. Clinical, biochemical, and microscopic analyses suggest that OPS may be a disorder of matrix homeostasis rather than a disorder of matrix structure. Consequently, identification of the OPS gene and its protein product could provide insights regarding common osteoporotic conditions, such as postmenopausal and senile osteoporosis. As a first step toward determining the cause of OPS, we utilized a combination of traditional linkage analysis and homozygosity mapping to assign the OPS locus to chromosome region 11q12-13. Mapping was accomplished by analyzing 16 DNA samples (seven affected individuals) from three different consanguineous kindreds. Studies in 10 additional families narrowed the candidate region, supported locus homogeneity, and did not detect founder effects. The OPS locus maps to a 13-cM interval between D11S1298 and D11S971 and most likely lies in a 3-cM region between GSTP1 and D11S1296. At present, no strong candidate genes colocalize with OPS.

Introduction

Osteoporosis is a common medical problem with major morbidity and societal cost (Melton 1993). Diminished bone strength, a consequence of low bone mineral content, is the significant complication of the disease (Smith and Smith 1976). Heritable factors have been identified as major contributors to bone mineral content, accounting for most of the observed variance in bone density (Peacock 1995). Although biological analyses indicate the complexity of this process (Manolagas and Jilka 1995), the precise genetic factors involved are not known (Morrison et al. 1994; Peacock 1995). Osteoporosis-pseudoglioma syndrome (OPS) (259770, OMIM 1995) provides an opportunity to study genetic factors involved in skeletal homeostasis. This autosomal recessive disorder is characterized by severe juvenile-onset osteoporosis and congenital or juvenile-onset blindness (Frontali et al. 1985). Collagen I biosynthesis, osteoid, osteoblasts, osteocytes, and osteoclasts appear normal in patients with OPS (Brudev and Stoss 1986; Somer et al. 1988; Swoboda and Grill 1988), and visual loss in patients has been associated with aberrant vitreo-retinal vascular growth (Saraux et al. 1969; Sauvegrain et al. 1981). These observations suggest a regulatory role, rather than a structural role, for the OPS gene product. We report the assignment of the OPS locus to chromosome region 11q12-13, using a combination of traditional linkage analysis and homozygosity mapping (Lander and Botstein 1987).

Subjects and Methods

Patient Ascertainment

All families in this study were diagnosed with OPS on the basis of a constellation of clinical, ophthalmologic,
and radiographic findings (Frontali et al. 1985). After informed consent was obtained, blood was obtained for DNA extraction. Descriptions of several families participating in this study have been previously published (Beighton et al. 1985; Frontali et al. 1985; Superti-Furga et al. 1986; Somer et al. 1988; Swoboda and Grill 1988; De Paepe et al. 1993).

Genotyping
DNA extraction and linkage analysis were performed as described by Boon et al. (1994). Primers flanking simple-sequence repeat polymorphisms (SSRPs) were used to PCR amplify genomic DNA in 10-μl volumes containing 37.5 ng of DNA and 2 pmol of each primer. Alleles were detected by end-labeling the forward primer. Primer sequences were obtained from published databases, and primers were purchased from Research Genetics.

Typical conditions for PCR included an initial denaturation at 95°C for 4 min, followed by 30 cycles of 94°C for 40 s, 55°C for 50 s, and 72°C for 50 s, with a final extension of 72°C for 7 min. PCR products were denatured in the presence of 40% formamide. Then, 1.5-μl aliquots were separated on denaturing polyacrylamide gels and alleles were detected by autoradiography.

Linkage Analysis
Two-point lod scores were calculated using the program MLINK (Lathrop et al. 1985) with consanguinity loops, where known, as indicated in figure 1. Linkage calculations assumed autosomal recessive inheritance with complete penetrance of the mutant phenotype, a phenocopy frequency of $10^{-3}$, and a mutant gene frequency of $10^{-3}$. Linkage calculations initially assumed a 4-allele system with equal allele frequencies. Following the identification of linkage, >20 ethnically/geographically matched controls for families 1–6 and 9 were used to determine specific allele frequencies for each kindred.

SSC Analysis and Cycle Sequence Analysis to Exclude PPP1CA, ROM1, FKBP13, and CNTF
Previously published primer pairs or sequences (Hendrickson et al. 1993; Mochizuki and Prochaza 1994; Nichols et al. 1994; Takahashi et al. 1994) were used to amplify portions of the above genes for evaluation by either SSC (Orita et al. 1989) or cycle sequence (Murray 1989) analysis.

Results
Sixteen DNA samples from three different consanguineous kindreds (from Finland, South Africa, and Italy) were used in the initial mapping (fig. 1). If OPS exhibited locus heterogeneity, only family 2 would have provided significant statistical strength to achieve a lod score >3. Conversely, if there were locus homogeneity, homozygosity for a tightly linked informative marker would have yielded a combined lod score >5. Since there were no a priori candidate genes, we initiated a genome-wide scan using SSRPs spaced at 20-cM intervals. For the preliminary scan, regions having lod scores <−1 were not evaluated further, while intervals with combined lod scores >1 in all three families, or >0 in family 2 alone, were tested with additional nearby SSRPs.

One-hundred sixty SSRPs distributed across 20 autosomes were tested before marker D11S905 yielded a combined lod score of 1.6 at $\theta = .1$. Testing additional nearby markers suggested identity by descent in affected patients (fig. 1). D11S987 yielded the highest combined lod score (5.99 at $\theta = 0$), when ethnically/geographically matched control haplotype frequencies were used for each kindred.

On the assumption of homozygosity by descent for the OPS mutation in each consanguineous kindred, the OPS locus can be placed within an 13-cM genetic interval bounded by D11S1298 and D11S971 on chromosome region 11q12-13 (Leppert et al. 1994). However, results in family 2 indicate that the homozygous markers within this 13-cM region comprise two noncontiguous, although physically close, intervals (fig. 1) (Leppert et al. 1994; van Heyningen and Little 1995). One interval is bounded by D11S1298 and D11S1368, the other by PYGM and FGF3. The occurrence of heterozygosity between these two intervals in family 2 may reflect either a double recombinant event occurring in an intermediate ancestor, or reintroduction of ancestral markers through additional consanguineous unions (Beighton et al. 1985). An alternative, less likely, explanation is that affected individuals in family 2 are compound heterozygotes for allelic OPS mutations and that markers are homozygous by chance rather than by descent.

Ten additional kindreds with OPS, including two consanguineous kindreds, were studied to refine the candidate region and to test for locus homogeneity and founder effects. Data from families 4 and 9–13 were consistent with a single OPS locus on chromosome 11q but did not exclude either interval (fig. 2). However, results in family 10 potentially narrow both intervals (fig. 2). Families 5–8 were too small to provide linkage data but could be used to look for shared haplotypes. Analysis of markers compatible with identity by descent did not suggest a common founder mutation, even among families of similar geographic background (table 1). However, shared founder haplotypes may emerge as the OPS locus is further refined.

Discussion
We have mapped the OPS locus to chromosome 11q12-13 by initially analyzing 16 DNA samples (seven
affected individuals) from three different consanguineous kindreds. Studies in 10 additional families confirmed the assignment, supported locus homogeneity, and did not detect founder effects. On the assumption of homozygosity by descent in all affected patients from consanguineous unions, the most likely site of the OPS locus is between GSTP1 and D11S1296. This interval has a genetic distance of 3 cm and a physical distance of >3 Mb (van Heyningen and Little 1995). Within the interval, a highly polymorphic SSRP, D11S987, was fully informative in every kindred. In contrast, the other homozygous interval, between D11S1298 and D11S1335, contained SSRPs that were not fully informative. Consequently, in some families, homozygosity by chance, rather than homozygosity by descent, may have occurred. The low heterozygosity content of several other markers (e.g., D11S913, D11S970, D11S1917) also probably results in homozygosity by chance for these loci in several patients (table 1). On the assumption of homozygosity by descent, family 10 requires at least two recombinant events closely flanking the OPS locus to have occurred in only six meioses. Locus heterogeneity or compound heterozygosity are alternative explanations for this family’s results; additional DNA samples from intermediate relatives, and additional highly polymorphic markers within the candidate region, may resolve this issue.

At present, no likely candidate genes have been mapped within either OPS candidate interval. On the assumption of homozygosity by descent for mutations at the OPS locus in all consanguineous kindreds, several genes (CNTF, FKBP13, ROM1, PYGM, PPP1CA, GSTP1, and FGF3) can be excluded as candidates on the basis of the finding of heterozygosity for intragenic SSRPs or sequence polymorphisms in one or more affected individuals (data not shown). Ophthalmologic disorders have been previously assigned to chromosome 11q12-13, including neovascular inflammatory vitreore-
Family 4  Family 9  Family 10  Family 11  Family 12  Family 13

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**Table 1**

**OPS-Linked Marker Alleles in Affected Patients**

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**Note.**—Several homozygous marker alleles (e.g., D11S913, D11S970, D11S1917) also have high homozygosity rates in ethnically/geographically matched controls (control frequencies were available for families 1–6 and 9).

^a Family with known consanguinity.
AmHum, Genet

suggest initially the OPS region and then saturate this
ping, we were able to use a less-dense marker screen to
traditional linkage mapping with homozygosity map­
is increased, and homozygosity mapping is a powerful
the occurrence of consanguinity within affected kindreds
utility of this approach is dependent on the number of
mutations or polymorphisms within this gene could be
zygosity for the OPS mutation will not be a common
in heterozygotes is coincidental, or truly associated with
the skeletal and ocular manifestations of
considered to this region.

Until the precise pathophysiological processes account­
for the skeletal and ocular manifestations of OPS are
known, it remains difficult to predict specific
roles for the OPS gene and its protein product. It is
intriguing that osteoporosis is not progressive in every
affected individual and that several obligate heterozyg­
gotes for the OPS mutation have developed adult-onset
osteoporosis (Superti-Furga et al. 1986; authors' unpub­
lished observations); whether the osteoporosis observed
in heterozygotes is coincidental, or truly associated with
the OPS mutation, is not known. We speculate that the
OPS gene serves a regulatory function, which is itself
modulated by other factors. Even in the absence of clon­
ing the OPS gene, the locus can now be tested as a
 genetic determinant for other osteoporotic conditions
(Spotila et al. 1993; OMIM 1995). These conditions
may include common forms of osteoporosis, such as
senile and postmenopausal osteoporosis, which can be
tested using approaches similar to those applied to the
vitamin D–receptor locus (Morrison et al. 1994; Pea­
cock 1995). OPS is a rare disorder; consequently, heterozygosity for the OPS mutation will not be a common
risk factor for developing osteoporosis. However, other
mutations or polymorphisms within this gene could be
associated with significant risk.

For rare autosomal recessive disorders, such as OPS, the occurrence of consanguinity within affected kindreds
is increased, and homozygosity mapping is a powerful
approach (Lander and Botstein 1987). However, the
utility of this approach is dependent on the number of
affected offspring, the coefficient of inbreeding, and the
map density at which markers are tested. By coupling
traditional linkage mapping with homozygosity map­
ing, we were able to use a less-dense marker screen to
suggest initially the OPS region and then saturate this
region with additional markers to reveal homozygosity,
which is presumed to be by descent. This coupled
approach can facilitate the mapping of any rare autosomal
recessive disorder in which consanguinity or a founder
mutation is present, because it need not rely on a dense
primary mapping screen or on the analysis of a large
number of DNA samples.

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Stafford, A. Monaco, R. McInnes, M. Higgins, J. Lu, T. Shows,
M. Prochazka, A. Beggs, D. Beier, P. Byers, and G. Wallis
and the Japanese Cancer Research Resources Bank for sharing
resources and unpublished data.

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