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

Yaoqin Gong, 1, * Miikka Vikkula, 2 Laurence Boon, 2 Jin Liu, 1 Peter Beighton, 4 Raj Ramesar, 4 Leena Peltonen, 5 Hannu Somer, 6 Tatsuo Hirose, 3 Bruno Dallapiccola, 7 Anne De Paepe, 8 Walter Swoboda, 9 Bernhard Zabel, 10 Andrea Superti-Furgi, 11 Beat Steinmann, 11 Han G. Brunner, 12 Ab Jans, 13 Richard G. Boles, 14 William Adkins, 15 Marie-Jose van den Boogaard, 16 Bjorn R. Olsen, 2 and Matthew L. Warman 1

1 Department of Genetics, Case Western Reserve University School of Medicine, and Center for Human Genetics, University Hospitals of Cleveland, Cleveland; 2 Department of Cell Biology, Harvard Medical School, and 3 Retina Associates, Boston; 4 Department of Human Genetics and MRC Unit for Medical Genetics, University of Cape Town Medical School, Cape Town; 5 Department of Human Molecular Genetics, National Public Health Institute, and 6 Department of Neurology, University of Helsinki, Helsinki; 7 Center of Medical Genetics, Tor Vergata University, Rome; 8 Center for Medical Genetics, University of Ghent, Ghent; 9 Ludwig Boltzmann Institute for Pediatric Endocrinology and Immunology, Vienna; 10 Department of Genetics, University of Mainz, Mainz; 11 Division of Metabolic and Molecular Diseases, Department of Pediatrics, University of Zurich, Zurich; 12 Department of Human Genetics, University of Nijmegen, Nijmegen; 13 Institutes for the Mentally Handicapped, De Blauwe Kamer, Breda and de Hondsberg, Oisterwijk; 14 Division of Medical Genetics, Children's Hospital Los Angeles, Los Angeles; 15 Central Wisconsin Center for the Developmentally Disabled, Madison; and 16 Department of Human Genetics, University of Utrecht, Utrecht.

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.

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Address for correspondence and reprints: Dr. Matthew L. Warman, Department of Genetics, BRB 719, Case Western Reserve University School of Medicine, 2109 Adelbert Road, Cleveland, OH 44106. E-mail: mlw14@po.cwru.edu
*Present address: Department of Genetics, Shandong Medical University, Jinan, Shandong, China.
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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 (Brude 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-$\mu$l 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-$\mu$l 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 $10^{-5}$, 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 a 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-
Figure 2  Pedigree structure and genotypes in six additional families. Four nonconsanguinous, single-offspring families are not shown. Sex-averaged genetic distances spanning the two candidate intervals (on the assumption of homozygosity by descent in family 10) are indicated. Notations are the same as for figure 1. Affected individuals in family 4 are homozygous for most markers in the region, which is consistent with unsuspected consanguinity. Marker GSTP1 (asterisk [*]), appears recombinant in family 9. This is more likely to represent a de novo SSRP mutation than a double-recombinant event. A deduced genotype for the deceased mother in family 13 is in italics.

tinopathy (VRNI) and exudative vitreoretinopathy-1 (EVR1) (Li et al. 1992; Stone et al. 1992; Leppert et al. 1994; Nichols et al. 1994); however, none colocalize with OPS. It has been suggested that the abundance of nonallelic eye disorders on chromosome 11q may be due to a clustered gene family or a family of interacting proteins, having roles in ocular development (Nichols et al. 1994). This is particularly intriguing, since OPS shares several features with both VRNI and EVR1, and genes for interacting retinal proteins have previously been colocalized to 11q13 (Benovic et al. 1991; Calabrese et al. 1994). Autosomal recessive forms of isolated osteoporosis, as well as isolated congenital retinal detachment, have also been described (e.g., 259750 and 221900, OMIM 1995). This may reflect locus heterogeneity for these traits. However, certain of these patients may have had unrecognized OPS, since ocular and skeletal manifestations may not have been manifest at the time patients were evaluated. One may also speculate that OPS, which is quite rare, is the consequence of

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-
affected offspring, the coefficient of inbreeding, and the
approach (Lander and Botstein 1987). However, the
is increased, and homozygosity mapping is a powerful
utility of this approach is dependent on the number of
associated with significant risk.
in heterozygotes is coincidental, or truly associated with
mutations or polymorphisms within this gene could be
zygosity for the OPS mutation will not be a common
intriguing that osteoporosis is not progressive in every
roles for the OPS gene and its protein product. It is
characterized by hypercalcuria and metaphyseal rickets with
concurrent osteoporosis, not osteoporosis. Osteochondro-
dystrophy is characterized by abnormal growth plate
morphology and stunted growth. Although growth plate
morphology has not been specifically evaluated in pa-
ents with OPS, disproportionate growth is not a feature
of the disease. A second region of homology exists be-
tween human 11q12.13 and murine chromosome 7. Mu-
rine skeletal or ocular phenotypes have not been as-
signed to this region.

Until the precise pathophysiological processes ac-
counting 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 heterozy-
gotes for the OPS mutation have developed adult-onset
osteoporosis (Superti-Furga et al. 1986; authors' unpub-
ished 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, hetero-
yzosity 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-
ning, 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 ap-
proach 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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