Assignment of the Locus for PLO-SL, a Frontal-Lobe Dementia with Bone Cysts, to 19q13

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Summary

PLO-SL (polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy) is a recessively inherited disorder characterized by systemic bone cysts and progressive presenile frontal-lobe dementia, resulting in death at <50 years of age. Since the 1960s, ~160 cases have been reported, mainly in Japan and Finland. The pathogenesis of the disease is unknown. In this article, we report the assignment of the locus for PLO-SL, by random genome screening using a modification of the haplotype-sharing method, in patients from a genetically isolated population. By screening five patient samples from 2 Finnish families, followed by linkage analysis of 12 Finnish families, 3 Swedish families, and 1 Norwegian family, we were able to assign the PLO-SL locus to a 9-cM interval between markers D19S191 and D19S420 on chromosome 19q13. The critical region was further restricted, to ~1.8 Mb, by linkage-disequilibrium analysis of the Finnish families. According to the haplotype analysis, one Swedish and one Norwegian PLO-SL family are not linked to the chromosome 19 locus, suggesting that PLO-SL is a heterogeneous disease. In this chromosomal region, one potential candidate gene for PLO-SL, the gene encoding amyloid precursor–like protein 1, was analyzed, but no mutations were detected in the coding region.

Introduction

PLO-SL (polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy; OMIM 221770 [http://www3.ncbi.nih.gov:80/htbin-post/Omim/disp mim?221770])—also referred to by different combinations of the eponym "Järvi-Hakola-Nasu disease" or as "membranous lipodystrophy" or "brain-bone-fat disease"—is a rare recessively inherited disorder characterized by systemic bone cysts and progressive presenile frontal-lobe dementia (Hakola 1972, 1990). The first patients were described in the 1960s, in Japan (Terayama 1961; Nasu et al. 1973) and in Finland (Järvi et al. 1968, 1995; Hakola 1972). Subsequently, ~160 cases have been reported, mainly in Japan (98 cases) and Finland (32 cases) but also in Sweden (Adolfsson et al. 1978; Nylander et al. 1996), Norway (Edvardsen et al. 1983), the United States (Wood 1978; Bird et al. 1983), Italy (Pazzaglia et al. 1986; Iannaccone et al. 1992; Preziuso et al. 1992; Malandrini et al. 1996), South Africa (Stübgen and Lotz 1992), Austria (Deisenhammer et al. 1993), Turkey (Koçer et al. 1994), and Belgium (Deldinne et al. 1996).

PLO-SL is characterized by a unique combination of neuropsychiatric and skeletal symptoms (table 1) (Hakola 1972, 1990). Clinical course is typical, with first symptoms appearing in adolescence as pains and pathological fractures of bones of the extremities, followed by progressive neurological deterioration culminating in severe dementia and epileptiform seizures (Hakola 1972, 1990). Death occurs at <50 years of age. Radiological examination of the skeleton is diagnostic for symmetrical cystlike lesions in the small bones of the extremities and trabecular loss in the distal ends of the long tubular bones (Mäkelä et al. 1982; Virtama et al. 1990). Cerebral magnetic-resonance-imaging and computed-tomography scans reveal general diffuse brain atrophy, which is accentuated in the frontal lobes; reduced white-

Table 1

Typical Symptoms of PLO-SL

Age (Years)	Phase	Major Symptoms
≥13	Skeletal	Pains in the ankles and wrists; fractures in the bones of the extremities, after a minor trauma
≥25	Neuropsychiatric	Frontal-lobe dementia; agnostic-apractic-aphasic symptoms; lesion of the upper motor neuron; gait disturbance; myoclonic twitches; epileptic seizures; atrophy of the optic discs and retinae; urinary incontinence; impotence or frigidity

matter volume, with deep sulci; and bilateral calcifications in the basal ganglia (Iivanainen et al. 1984; Hakola and Puranen 1993). Microscopically, bone cavities are filled with characteristic convoluted membranocystic structures, which are also observed in local adipose tissue (Järvi et al. 1968; Nasu et al. 1973; Kitajima et al. 1989). Neuropathological symptoms, which are scattered throughout the nervous system, include demyelination, axonal degeneration with spheroids, fibrillary gliosis, calcospherites in basal ganglia, and thickening of the walls of small venules and capillaries (Matsushita et al. 1981; Sourander et al. 1981; Kalimo et al. 1994). The pathogenesis of PLO-SL is unknown, but an impairment in systemic lipid metabolism (Nasu et al. 1973) and disturbance of glycolipid or glycoprotein metabolism (Kitajima et al. 1989), as well as a defect in the basement membranes of blood vessels (Järvi et al. 1968; Kalimo et al. 1994), have been proposed.

The prevalence of PLO-SL is highest (1×10^{-6} to 2×10^{-6}) in Finland (Hakola 1990), and the disease is considered to belong to the so-called Finnish disease heritage, a group of ~30 rare monogenic diseases that are more common in Finland than elsewhere in the world (Norio et al. 1973). This disease group has its origins in the special population history of Finland, which is characterized by a small number of original settlers, relative isolation from the neighboring countries, and subisolates formed within the remote parts of the country. In all the Finnish diseases for which a gene defect has been identified, one major mutation accounts for 90%–98% of the disease chromosomes (de la Chapelle 1993; Peltonen et al. 1995). These features make linkage-disequilibrium–based methods highly useful for localization of disease genes in this population (Nikali et

al. 1995). In this study, we applied a modification of the haplotype-sharing method to mapping of the PLO-SL locus in the Finnish families, resulting in assignment of the PLO-SL locus to chromosome 19q13 as well as exclusion of this locus in one Norwegian and one Swedish PLO-SL family.

Subjects and Methods

PLO-SL Families and DNA Samples

Blood and tissue samples from 12 Finnish families segregating PLO-SL were collected for the genome screen. Families B, D, F, G, H, K1, K2, M, and P are described elsewhere, in the report by Hakola (1990), and families Q, R, and S have not been described before (fig. 1). None of these families is known to have common ancestors, but the majority of them originated in the eastern central region of Finland (Hakola 1972, 1990). In addition, samples from three Swedish PLO-SL families (U, V, and X in fig. 1), reported by Adolfsson et al. (1978) and Nylander et al. (1996), and from one Norwegian family (T in fig. 1), reported by Edvardsen et al. (1983), were obtained. DNA was extracted from blood samples, by use of a method modified from that described by Blin and Stafford (1976) to adapt Phase Lock Gel tubes (5 Prime→3 Prime). DNA from paraffin-embedded tissue samples was extracted as described by Isola et al. (1994).

Blood or tissue samples were collected from the patients (or from the spouse and children if the patient was deceased), their parents, and their siblings. Diagnosis of PLO-SL was made on the basis of radiographic examination of limb bones, combined with analysis of typical neuropsychiatric symptoms. Healthy siblings <40 years of age were examined radiologically, to exclude undiagnosed cases. No new PLO-SL cases were found at this phase. One asymptomatic sibling had a single bone lesion in her wrist. During the 5-year follow-up time, at the end of which she was 40 years of age, she showed no progression of bone changes and no symptoms of frontal-lobe dementia. Therefore, she was scored as healthy in the linkage analysis.

Samples were taken and clinical examinations were performed in accordance with the Helsinki Declaration. Participation was voluntary. Family members were told in advance the possible outcomes of the clinical examination, and they could decide whether they wanted to know the results. Samples from deceased individuals were used by permission from the next of kin.

Marker Genotyping

Primers for the DNA markers used in the genome screen were selected from the collection of primers from Généthon (Dib et al. 1996) or from the Cooperative
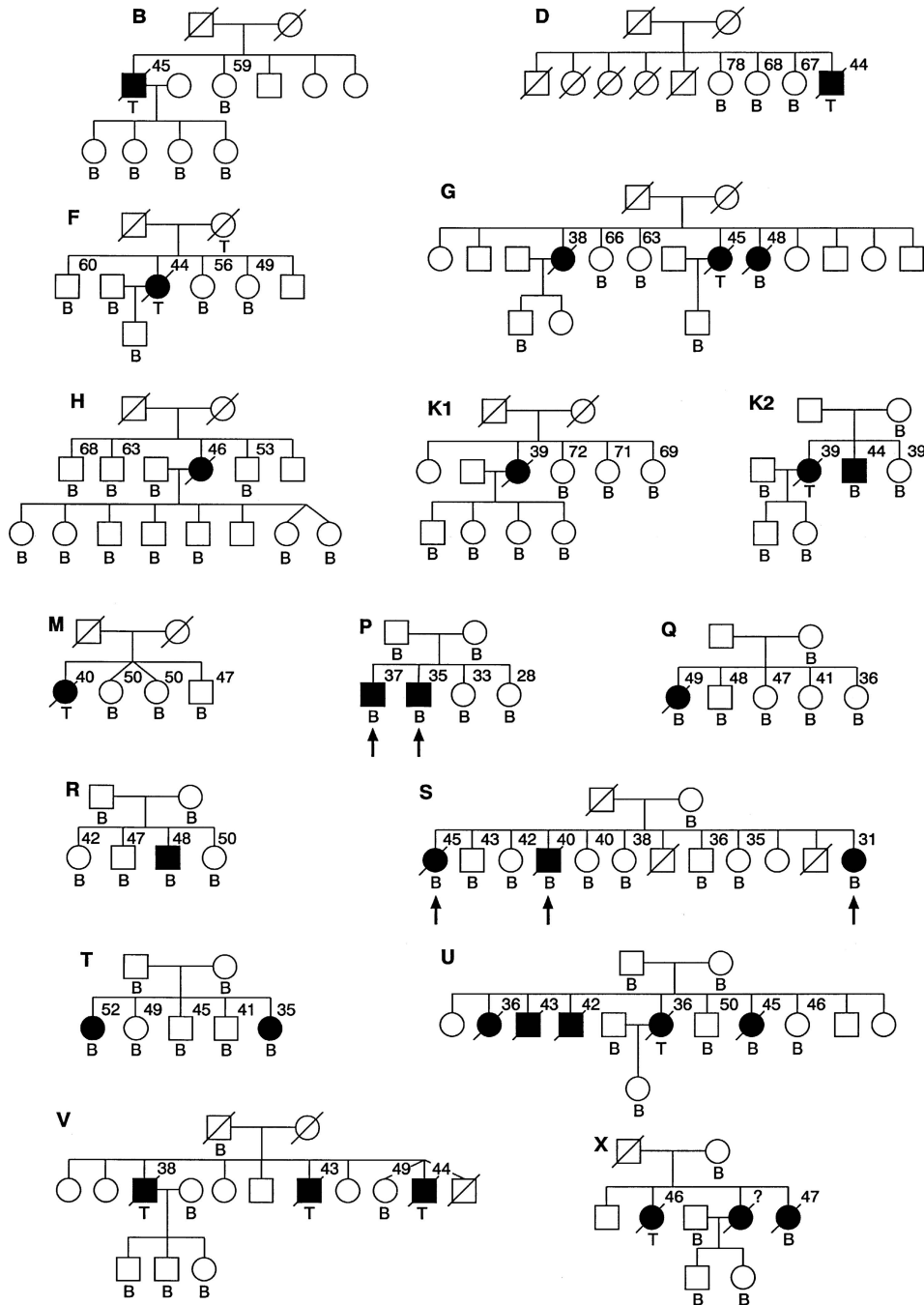
Figure 1 PLO-SL pedigrees. Families B–S are Finnish in origin, and families B–P were described earlier by Hakola (1990). Family T is from Norway and was reported by Edvardsen et al. (1983). Families U, V, and X originated in Sweden (Adolfsson et al. 1978; Nylander et al. 1996). Blackened symbols indicate PLO-SL patients. A "B" or a "T" beneath a symbol indicates an individual for whom blood or tissue samples, respectively, were available. A number above a symbol indicates age at time of study or at death, for affected individuals, or age at time of study, for unaffected siblings. Arrows indicate those PLO-SL patients whose samples were used in the genome screen using the haplotype-sharing method.

Human Linkage Center (CHLC) (Sheffield et al. 1995). Chromosome 19 markers D19S609, D19S610, D19S608, MK4, and MK5 had been developed by us (Männikkö et al. 1995; Kestilä et al., in press), and marker D19S191 was selected from the Genome Database (accession number GDB:181997 [http://www.gdb.org/]). Primers for the CHLC markers were purchased from the Nordic Human Genome Initiative, and one of the primers was labeled with cy5, during synthesis. Primers for other markers either were purchased from Généthon or were synthesized at the National Public Health Institute, by use of the 394 DNA/RNA Synthesizer (Applied Biosystems). If none of the primers was cy5 labeled, one of them was end labeled with ^{32}P. The PCR reactions were performed in a 15-μl reaction volume containing 20 ng genomic DNA, 4–5 pmol both primers, 0.2 mM dNTP, 1.5 mM MgCl$_2$, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, and 0.15 U DynaZyme (Finnzymes Oy). PCR was performed for 30–35 cycles of annealing for 30 s at a specific temperature for each primer pair, extension for 30 s at 72°C, and denaturation for 30 s at 95°C, after an initial denaturation for 3 min at 95°C, in multiwell microtiter plates in programmable thermal controllers (MJ Research or Hybaid OmniGene). PCR products were size separated, by PAGE, on either conventional sequencing apparatus (^{32}P label) or on an ALF express (cy5 label; Pharmacia). The radioactive gels were vacuum dried and autoradiographed overnight by use of Kodak X-OMATS film. Allele data from the ALF express were printed as fluorescence curves. The alleles were scored by eye and were entered into a database file during the initial stage of the genome screen and into a linkage file during the second stage.

SSCP Analysis of the APLP1 Gene

The sequence for chromosome 19 cosmid R28051, which contains the gene for amyloid precursor–like protein 1 (APLP1; Wasco et al. 1992, 1993), has been reported in the Genome Sequence Database (GSDB:S:1117815 [http://ncgr.org/gsdb/gsdb.html]). The exonic sequences of APLP1 were screened for mutations in the PLO-SL patients, by use of the SSCP method (Orita et al. 1989; Glavac and Dean 1993). Intronic primers for the 17 exons of APLP1 were designed (Lenkkeri et al., in press). Exons were amplified by PCR in a 50-μl reaction volume containing 50 ng genomic DNA, 50 pmol primers, 0.2 mM dNTP, 0.2 μl α-[^{32}P]-ATP, 1.5 mM MgCl$_2$, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 10% glycerol, and 0.5 U DynaZyme (Finnzymes Oy). PCR was performed for 30–35 cycles of annealing for 1 min at a specific temperature for each primer pair, extension for 1 min at 72°C, and denaturation for 1 min at 95°C, after an initial denaturation for 5 min at 95°C,

in a thermal controller (MJ Research). PCR products were run on nondenaturing 5% polyacrylamide gels, at 30-W constant power for 5 h in a cold room and at 10-W constant power, with 10% glycerol, for 15 h at room temperature (Glavac and Dean 1993). The gels were vacuum dried and autoradiographed. Migration of samples from two Finnish patients and from controls were compared.

Statistical Analyses

Linkage analysis was performed by use of the LINK-AGE package (Lathrop and Lalouel 1984; Lathrop et al. 1984, 1986), and heterogeneity analysis was performed by use of the program HOMOG (Ott 1986). Analysis was performed by use of a recessive-inheritance model (Hakola 1972). The disease-gene frequency was set to .001 for the Finnish population. Age-dependent penetrance classes were constructed on the basis of age at diagnosis of the Finnish patients (Ott 1991). The median age at diagnosis was 34 years. Penetrance was set to 0 for individuals <21 years of age, to .30 for individuals 21–34 years of age, to .80 for individuals 35–49 years of age, and to 1.00 for individuals ⩾50 years of age. No phenocopies were allowed. Since children of affected individuals had not been radiologically studied, their disease status was set as unknown, for the linkage analysis, and marker data were used only to reconstruct the genotype of their affected parent. Allele frequencies for DNA markers were calculated from analysis of the family material. The order and genetic and physical distances between chromosome 19q13 markers were taken from the Généthon map (Dib et al. 1996), the published physical map for chromosome 19 (Ashworth et al. 1995), and our previous studies (Männikkö et al. 1995; Kestilä et al., in press).

Linkage-disequilibrium analyses were performed by use of the programs HRRLAMB and HRRMULT (Nikali et al. 1995; Terwilliger 1995, 1996). These programs apply a likelihood-ratio test for linkage disequilibrium (Terwilliger 1995, 1996) to the haplotype-based "haplotype relative-risk" approach (Falk and Rubinstein 1987; Terwilliger and Ott 1992). The advantage of this statistic is that it has only 1 df, irrespective of the number of markers or the number of alleles of the marker. As a result, when multiallelic markers or multiple markers are analyzed jointly, this statistic becomes more powerful than that of conventional χ^2 analysis. In multipoint association analysis, the recombination fraction (θ) between marker loci is kept fixed, and the likelihood is maximized over α (the proportion of disease alleles originally associated with a certain allele) and n (the number of generations since introduction of the founder disease allele into the population).

Table 2

Chromosome 19 Genotypes for the First Stage of the Genome Screening

| | GENETIC DISTANCE TO NEXT MARKER | GENOTYPE | | | | |
| | | Family P | | Family S | | |
MARKER	(cM)	Patient 1	Patient 2	Patient 1	Patient 2	Patient 3
D19S215	3	4-5	4-5	4-7	3-4	4-7
D19S882	5	3-4	3-4	3-3	3-3	3-3
D19S225	7	3-5	3-4	3-3	3-3	3-3
D19S422[a]	4	2-5	2-5	5-5	5-5	5-5
D19S420	2	1-5	1-5	2-2	2-2	2-2
D19S217	2	2-2	2-2	6-6	6-6	6-6
D19S412	5	3-7	3-7	7-7	7-7	7-7
D19S907	1	2-3	2-3	2-2	2-2	2-2
D19S246	1	2-3	2-3	1-2	1-2	1-2
KLK1		4-4	4-4	3-4	4-4	4-4

[a] Localizes to the PLO-SL critical region bordered by recombinations in the markers D19S191 and D19S420.

Results

Genome Screen

The genome screen to locate the defective gene for PLO-SL was performed in two stages. In the first stage, DNA samples from five affected siblings (indicated by arrows in fig. 1), from Finnish families P and S, were analyzed by use of polymorphic DNA markers, to identify genomic regions showing haplotype sharing. In the second stage of the screening, the markers that were in regions where the initial screening showed haplotype sharing among siblings, in both families, were genotyped in a panel of 10 PLO-SL families (D, F, G, H, K1, K2, P, Q, R, and S in fig. 1).

Altogether, 592 DNA markers where genotyped in the sibships P and S, resulting in an approximate map density of 6 cM. For six genomic regions, both sibships showed haplotype sharing within the family, at two or more adjacent markers. For chromosome 2q37, siblings shared haplotypes of eight markers in an 18-cM region. On chromosome 19q13, sharing extended over six markers in a 14-cM region (table 2). In four regions on chromosomes 1, 4, 5, and 21, haplotype sharing extended to a maximum of 10 cM.

At the second stage, markers in these and some additional regions were genotyped in a panel of 10 PLO-SL families. A total of 156 markers were analyzed by linkage analysis of these families. One marker on chromosome 2q37 gave a significant LOD score >3, in the linkage analysis. Six markers, on chromosomes 2q37 and 19q13, gave LOD scores >2, and an additional 11 markers, in six chromosomal regions (on chromosomes 1, 2q37, 4, 10, 19q13, and 21), gave LOD scores >1. The promising regions on chromosomes 2q37 and 19q13 were selected for further study with the complete family sample and additional markers, after which only markers on 19q13 continued to provide significant evidence for linkage.

Assignment of the PLO-SL Locus to 19q13

Results of the linkage analysis of the markers on chromosome 19q13 are shown in tables 3 and 4. When all the Scandinavian families were analyzed together, significant evidence for linkage to several markers on 19q13 was detected, with a maximum LOD score of 5.59 for marker MK4, at $\theta = .02$ (table 3). Two markers,

Table 3

Results from the Linkage Analysis of the Scandinavian PLO-SL Families

Marker	LOD_{Hom}[a]	LOD_{Het}[b]
D19S208	.05	.32
D19S191	.87	1.34
D19S609	.51	.61
MK4	5.59	5.79
MK5	2.29	2.97
D19S610	4.70	5.40
D19S608	3.69	4.46
D19S224	2.86	3.92
D19S896	3.09	3.19
D19S228	2.04	2.04
D19S421	.24	.47
D19S422	2.67	3.68
D19S223	.54	.86
D19S420	.71	.75
D19S217	1.25	1.26

[a] Maximum LOD score under homogeneity.
[b] Maximum LOD score under heterogeneity.

Table 4

Results from the Linkage and Association Analyses of the Finnish PLO-SL Families

| | LOD Score at θ = | | | | | | P | |
Marker	.00	.01	.05	.1	.2	χ^2 LRT [a]	value	λ[b]
D19S208	$-\infty$	.11	.65	.72	.54	.00000	.500000	.000000
D19S191	$-\infty$	1.39	1.81	1.72	1.20	1.32827	.124547	.749818
D19S609	.98	.98	.96	.89	.69	.00000	.500000	.000000
MK4	6.28	6.10	5.36	4.45	2.75	10.55326	.000583	.750131
MK5	4.71	4.58	4.04	3.38	2.12	1.11148	.145872	.599820
D19S610	6.37	6.19	5.44	4.53	2.83	20.96025	.000002	.943770
D19S608	5.08	4.91	4.26	3.48	2.07	13.36223	.000129	.883339
D19S224	5.12	4.97	4.36	3.62	2.25	15.44040	.000043	1.000000
D19S896	4.67	4.55	4.03	3.38	2.12	6.80221	.004561	.774917
D19S228	1.50	1.46	1.28	1.06	.65	.00000	.500000	.000000
D19S421	1.76	1.72	1.56	1.33	.87	13.50329	.000120	1.000000
D19S422	3.42	3.35	3.01	2.55	1.60	.00000	.500000	.000000
D19S223	2.01	1.98	1.82	1.59	1.05	.00000	.500000	.000000
D19S420	$-\infty$	.31	1.09	1.28	1.07	.00000	.500000	.000000
D19S217	1.85	1.85	1.80	1.64	1.17	.00000	.500000	.000000

[a] The χ^2 statistic of the likelihood-ratio test (Terwilliger 1995, 1996).

[b] The proportion of excess of a certain allele in the chromosomes carrying the PLO-SL gene.

D19S224 and D19S422, gave significant evidence for linkage only when heterogeneity was allowed. All markers gave higher LOD scores under heterogeneity (table 3), but none of them revealed statistically significant evidence for heterogeneity.

When the Finnish families were analyzed separately, no evidence for locus heterogeneity was obtained. All markers in a 17-cM region showed evidence for linkage, with a maximum LOD score of 6.37, to marker D19S610 (table 4). The critical region was bordered by obligatory recombinations, observed with markers D19S191 and D19S420, which are separated by 9 cM (Ashworth et al. 1995; Dib et al. 1996).

When chromosome 19 markers were analyzed in the Swedish and Norwegian families, no evidence for linkage or for heterogeneity was obtained. All the markers, except for D19S228, which was uninformative in the families, showed exclusion of linkage to 19q13 (LOD score <-2 at $\theta = .00$). Haplotype analysis of 19q13 markers in these families revealed clear nonsegregation in Norwegian family T (fig. 2) and in Swedish family X, whereas linkage to chromosome 19 markers could not be excluded in two other Swedish families (U and V in fig. 1).

Linkage-Disequilibrium Analysis of the Finnish PLO-SL Families

The results of the linkage-disequilibrium analysis of the Finnish PLO-SL families are shown in table 4. Several markers in a 3-cM region between markers MK4 and D19S421 showed evidence for allelic association in the PLO-SL chromosomes (Ashworth et al. 1995; Männikkö et al. 1995; Dib et al. 1996; Kestilä et al., in press).

Data from all markers were combined in the multipoint association analysis, by use of the program HRRMULT (Terwilliger 1995, 1996). The peak of the likelihood curve was reached between markers D19S610 and D19S224, within the vicinity of marker D19S608, with $\chi^2 = 67.3$, which had a P value of $<10^{-15}$ (fig. 3). The estimated α value was 1.00.

SSCP Analysis of the APLP1 Gene

The gene for APLP1 (Wasco et al. 1992, 1993) is located within the vicinity of marker D19S610 (Männikkö et al. 1995), the alleles of which show the highest linkage disequilibrium with the PLO-SL chromosomes. Even though the physiological function of the gene product is not known, this gene was considered to be an interesting candidate for PLO-SL, because of its homology to the amyloid-precursor protein (APP) that is defective in a subgroup of Alzheimer patients. The APLP1 gene is ~10.8 kb and codes for 17 exons (Lenkkeri et al., in press). Intronic primers for all exons were designed, and the exons were amplified, by PCR, from two Finnish PLO-SL patients and two healthy individuals. Differences in the mobility of the SSCP bands, between patients and controls, were searched under two running conditions, but none were detected.

Discussion

Similarity Search

In this study, we assigned the locus for PLO-SL to chromosome 19q13, using a modification of the haplotype-sharing method. In the haplotype-sharing
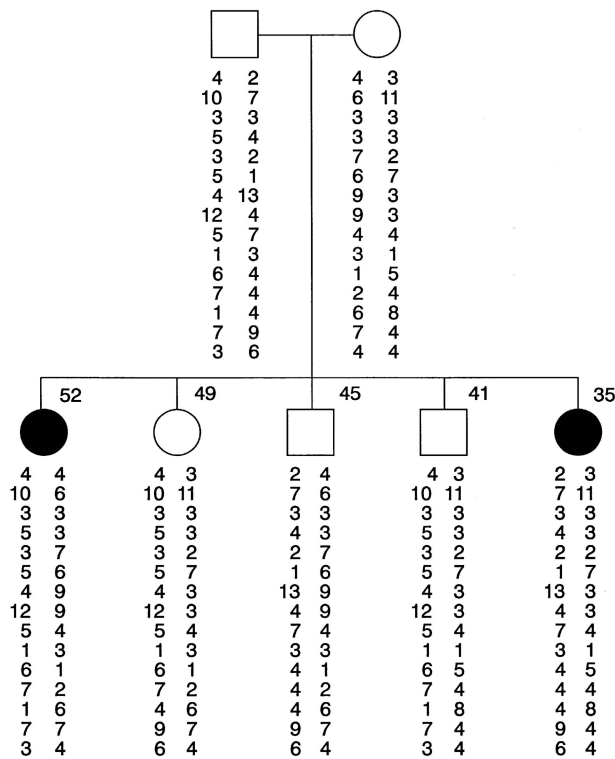
Figure 2 Chromosome 19 haplotypes for Norwegian PLO-SL family T. Marker order (top to bottom) is as follows: D19S208, D19S191, D19S609, MK4, MK5, D19S610, D19S608, D19S224, D19S896, D19S228, D19S421, D19S422, D19S223, D19S420, and D19S217. Blackened symbols indicate PLO-SL patients, and a number above a symbol indicates age at time of study.

method, chromosome segments shared identical-by-descent (IBD) by affected individuals, owing to linkage disequilibrium, are used to locate a disease gene (Houwen et al. 1994). The usefulness of this method depends on the degree of heterogeneity and on the distance of the relationship between the affected individuals. When these factors increase, more samples and a denser marker map are required (Houwen et al. 1994; Meerman et al. 1995). As a result, the haplotype-sharing method has been adopted mainly for the analysis of affected individuals from homogeneous populations who are known to be related (Houwen et al. 1994; Nikali et al. 1995). In Finland, most of the rare recessive diseases are caused by one major mutation descending from a single founder chromosome (de la Chapelle 1993; Peltonen et al. 1995). The size of the region where linkage disequilibrium is detected in disease chromosomes varies by 2–13 cM (Peltonen et al. 1995). In our study, the two sibships analyzed in the first stage of the genome screening were not known to be related, and, consequently, a map as dense as 2 cM would have been required to detect linkage disequilibrium. Therefore, we did not try to identify a

haplotype shared by all five affected individuals, but, rather, we tried to pinpoint for further analysis those genomic regions in which both sibships would show extensive haplotype sharing. Terwilliger et al. (1997) have shown that patients tend to share segments IBD that overlap the disease gene and that are larger than would be expected to occur by chance. Six times during the genome screening, the five affected siblings in the two Finnish sibships shared marker genotypes at two or more adjacent markers simultaneously. Only two chromosomal regions showed extensive haplotype sharing at a >10-cM region, of which one was found to be linked to PLO-SL. As was expected, we did not find a conserved haplotype shared by all affected individuals during the first stage of the genome screening (table 2), since this conserved haplotype was too short (3 cM) to be detected with our map density.

Heterogeneity of PLO-SL

Our extensive genome screening, heterogeneity analysis, and haplotype analysis of the Norwegian and Swedish families suggest that PLO-SL is a heterogeneous disease. Our primary assumption was that all the Scandinavian families would be linked to the same locus. This seemed especially valid with respect to the Swedish families, since all the Swedish PLO-SL families originated in northern Sweden, where the Finnish influence has been strong. Northern Sweden has received several waves of immigration from Finland, and it has been estimated that 60%–80% of the gene pool is Finnish in origin (Nylander and Beckman 1991). The ancestors of two families (U and V in fig. 1) could actually be traced back to Finland (Nylander et al. 1996). According to our haplotype analysis, the Norwegian family and the Swedish family not originating directly from Finland (X
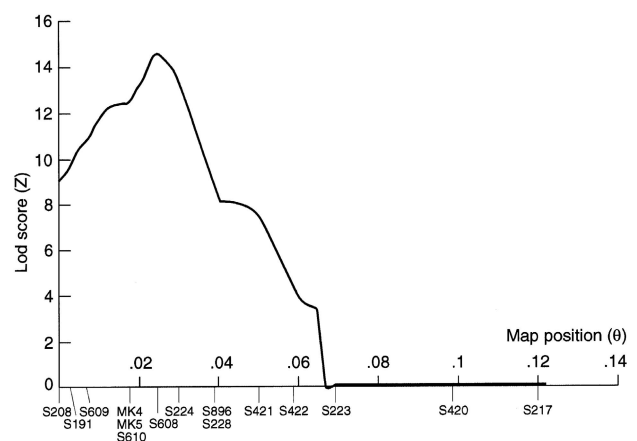

Figure 3 Multipoint association analysis of chromosome 19 markers, for the Finnish PLO-SL families.

in fig. 1) are not linked to the chromosome 19 locus. On the other hand, we cannot exclude linkage to chromosome 19 in Swedish families U and V, which are known to have Finnish ancestors. Unfortunately, the analysis of the two unlinked families, T and X, did not have enough power to detect linkage alone. When the mutation in the Finnish PLO-SL families is identified, it might be possible to search for members with the same metabolic pathway or gene family, to identify the gene defect in the unlinked families as well.

Locus Restriction by Linkage Disequilibrium

The critical region for PLO-SL is bordered by recombinations in markers D19S191 and D19S420, which are separated by 9 cM (fig. 3) (Ashworth et al. 1995; Dib et al. 1996). This region on chromosome 19q13.1, harboring tens of genes, is one of the most gene-rich regions in the whole human genome (Schuler et al. 1996). Cloning of the PLO-SL gene from this region would be an exhausting task. However, linkage disequilibrium in the Finnish PLO-SL families was detected only with markers in the region extending from MK4 to D19S421, restricting the area to a maximum of 3 cM (fig. 3). Physically, this distance corresponds to ~1.8 Mb (Ashworth et al. 1995; Männikkö et al. 1995; Dib et al. 1996; Kestilä et al., in press), which is already feasible for transcript mapping and even for direct sequencing.

Candidate Genes in the Critical Region

Within the 3-cM region between markers MK4 and D19S421, at least seven genes are known to exist (Ashworth et al. 1995): the genes for the α-polypeptide of the H^+/K^+-transporting ATPase (ATP4A; Maeda et al. 1990), cytochrome c oxidase subunit VIB (COX6B; Taanman et al. 1989), muscle-specific polypeptide 1 of cytochrome c oxidase subunit VII (COX7A1; Fabrizi et al. 1989), amyloid precursor–like protein 1 (APLP1; Wasco et al. 1992, 1993), and the small polypeptide of calpain (CANPS; Ohno et al. 1986). ATP4A is found in gastric parietal cells and is responsible for acid secretion in the stomach. Cytochrome c oxidase forms complex IV of the mitochondrial respiratory chain, the defects of which are associated with chronic lactic acidemia and myopathy or multiorgan syndrome (De Vivo 1993). Lactic acidosis has not been reported in the PLO-SL patients (Hakola 1972, 1990). APLP1 and CANPS appear to be the more interesting candidates for PLO-SL.

APLP1 was cloned in 1992 (Wasco et al. 1992, 1993), on the basis of its homology to APP, which is the source of the βA4-peptide forming the major component of the core of amyloid plaques in Alzheimer disease. APLP1 belongs to a conserved APP gene family, members of which also have been cloned from mouse, rat, and drosophila. There are few studies on the biological function

of APLP1, but mouse APLP1 exhibits brain-specific expression and is localized to postsynaptic density of the synapse (Kim et al. 1995). Therefore, it is hypothesized to play a role in brain synaptic function (Kim et al. 1995). The expression of APLP1 in bone tissue has not been investigated. We studied the exonic sequences of the APLP1 gene, by SSCP analysis, and did not detect mobility differences between the samples from the Finnish PLO-SL patients and those from controls. The sensitivity of SSCP analysis varies by 50%–98%, depending on the sequence and the SSCP conditions (Glavac and Dean 1993). In our laboratory, the sensitivity has been as high as 98% (Ikonen et al. 1991; Kauppinen 1992). However, we cannot exclude the possibility of a conformationally silent mutation in the coding sequences or of a mutation in the noncoding sequences, such as the introns and the 5' regulatory regions.

Another interesting gene in the PLO-SL region is CANPS, the gene for the small regulatory subunit of calcium-dependent cysteine proteinase (Ohno et al. 1986). Calpain has been shown to mediate the parathyroid-hormone induced osteoblastic retraction (Tram et al. 1993), and calpain activation has been proposed to affect long-term potentiation of memory as well as neuropathological processes such as transient ischemia-induced neurodegeneration and Alzheimer disease (Suzuki et al. 1995). Therefore, as a candidate for PLO-SL, CANPS also warrants further study.

Significance of the PLO-SL Gene

PLO-SL belongs to a clinically and genetically heterogeneous group of degenerative dementias (Miller 1997), but it is unique because of its combination of dementia and bone cysts. The PLO-SL locus does not overlap with any other locus reported for degenerative dementias. Genetic loci for other frontotemporal dementias, which usually are dominantly inherited and have a typical age at onset of senilium, have been reported on chromosomes 3 (Brown et al. 1995) and 17 (Wilhelmsen et al. 1994; Wijker et al. 1996; Yamaoka et al. 1996). Another dementia gene, Notch3—which is defective in CADASIL (<u>c</u>erebral <u>a</u>utosomal <u>d</u>ominant <u>a</u>rteriopathy with <u>s</u>ubcortical <u>i</u>nfarcts and <u>l</u>eukoencephalopathy), a hereditary adult-onset condition causing stroke and dementia—also localizes to chromosome 19 but is on the p arm (Joutel et al. 1996).

Even though PLO-SL is a rare disease, it deserves special attention because some of its symptoms and pathological features resemble those of common diseases, such as Alzheimer disease, vascular and other dementias, and major psychoses. It also has been suggested that PLO-SL is incompletely recessive, since single bone cysts also have been found in otherwise clinically healthy siblings of the PLO-SL patients (Hakola 1972). Investiga-

tion of whether one copy of the PLO-SL gene renders carriers more susceptible to common diseases, such as common dementias and major psychoses, would be worthwhile.

There is a long latent period between the early skeletal and the incapacitating neuropsychiatric symptoms of PLO-SL, which can be as long as 20 years. At present, there is no curative treatment available for patients who are diagnosed in the relatively asymptotic skeletal phase. Cloning of the defective gene could make it possible to interfere in the neurodegenerative process and prevent the deterioration.

Acknowledgments

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