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Peutz-Jeghers Syndrome: Confirmation of Linkage to Chromosome 19p13.3 and Identification of a Potential Second Locus, on 19q13.4

Hamid Mehenni,[1] Jean-Louis Blouin,[1] Uppala Radhakrishna,[1] Shiv Shanker Bhardwaj,[2] Kamla Bhardwaj,[2] V. B. Dixit,[2] Kent F. Richards,[3] Ambrosio Bermejo-Fenoll,[4] Antonio Silva Leal,[5] Ranjan C. Raval,[6] and Stylianos E. Antonarakis[1]

[1]Laboratory of Human Molecular Genetics, Department of Genetics and Microbiology, Geneva University Medical School, and Division of Medical Genetics, Cantonal Hospital, Geneva; [2]Government General Hospital, Bhiwani, India; [3]University Hospital of Utah Medical Center, Salt lake City; [4]Catedratico de Medicina Bucal, Universidad de Murcia, Murcia, Spain; [5]Department of Surgery, University of Porto, Porto, Portugal; and [6]Department of Dermatology, Civil Hospital and Medical College, Ahmedabad, India

Summary

Peutz-Jeghers syndrome (PJS) is an autosomal dominant disease with variable expression and incomplete penetrance, characterized by mucocutaneous pigmentation and hamartomatous polyposis. Patients with PJS have increased frequency of gastrointestinal and extraintestinal malignancies (ovaries, testes, and breast). In order to map the locus (or loci) associated with PJS, we performed a genomewide linkage analysis, using DNA polymorphisms in six families (two from Spain, two from India, one from the United States, and one from Portugal) comprising a total of 93 individuals, including 39 affected and 48 unaffected individuals and 6 individuals with unknown status. During this study, localization of a PJS gene to 19p13.3 (around marker D19S886) had been reported elsewhere. For our families, marker D19S886 yielded a maximum LOD score of 4.74 at a recombination fraction (θ) of .045; multipoint linkage analysis resulted in a LOD score of 7.51 for the interval between D19S886 and 19pter. However, markers on 19q13.4 also showed significant evidence for linkage. For example, D19S880 resulted in a maximum LOD score of 3.8 at $\theta = .13$. Most of this positive linkage was contributed by a single family, PJS07. These results confirm the mapping of a common PJS locus on 19p13.3 but also suggest the existence, in a minority of families, of a potential second PJS locus, on 19q13.4. Positional cloning and characterization of the PJS mutations will clarify the genetics of the syndrome and the implication of the gene(s) in the predisposition to neoplasias.

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Address for correspondence and reprints: Prof. Stylianos E. Antonarakis, Division of Medical Genetics, Centre Médical Universitaire, 1 rue Michel Servet, 1211 Genève, Switzerland. E-mail: Stylianos.Antonarakis@medecine.unige.ch

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Introduction

The identification of genes involved in the predisposition to human neoplasias is of importance, since it provides a better understanding of the pathogenesis of cancer and contributes to the possibility of diagnosis, prognosis, and therapeutic interventions. Monogenic hereditary syndromes that predispose to cancers are of particular interest, because they could provide the genomic mapping position of the gene involved and, subsequently, the cloning, characterization, and functional analysis of the affected gene. Peutz-Jeghers syndrome (PJS) is a rare, autosomal dominant disease with variable and incomplete penetrance, characterized by gastrointestinal hamartomatous polyposis and mucocutaneous hypermelanotic macules (melanin spots) on the lips, buccal mucosa, and digits (Jeghers et al. 1949; Foley et al. 1988). Patients with PJS demonstrate a high risk for gastrointestinal cancers (small bowel and colon, pancreatic, and stomach) (Giardiello et al. 1987) and cancers of other organs, such as the ovaries, testes, uterine cervix, and breast (Young et al. 1982; Lehur et al. 1984; Gilks et al. 1989; Buck et al. 1992). In addition, benign ovarian tumors may occur in female PJS patients (Young et al. 1995). The hamartomatous polyps usually are considered to be of low malignant potential, but many reports have documented adenomatous and carcinomatous changes arising from these hamartomas (Perzin and Bridge 1982; Foley et al. 1988).

In order to map the locus (or loci) associated with PJS, we collected DNA from members of six PJS families (two from Spain, one from Portugal, one from Utah, and two from India), including 39 affected and 48 unaffected individuals and 6 individuals with an unknown diagnostic status. While performing a genomewide genotyping, using short-sequence-repeat (SSR) polymorphic markers, Hemminki et al. (1997) reported localization of a PJS gene to chromosome 19p13.3. Their highest two-point LOD score was 5.40 at a recombination fraction (θ) of .00 for polymorphic marker D19S886.

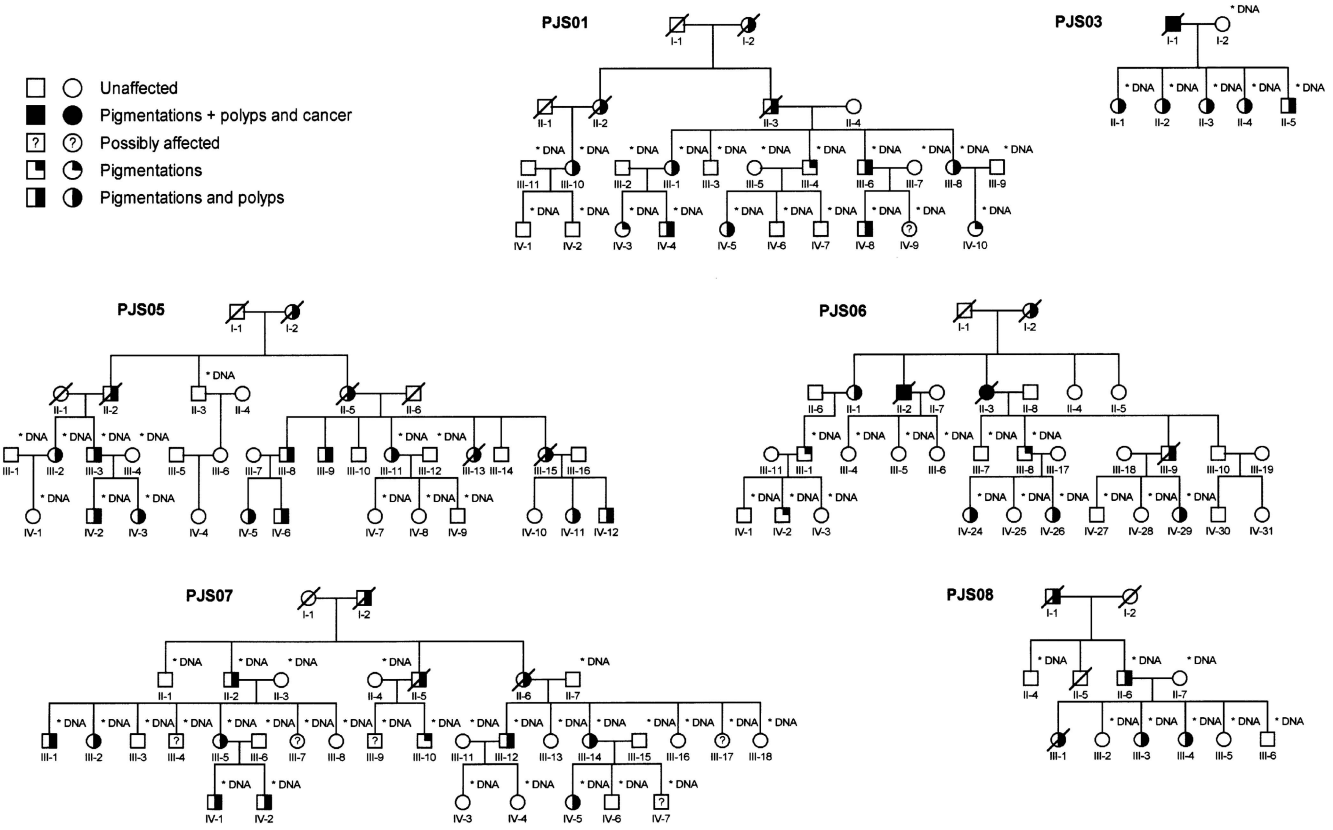

Figure 1 Pedigrees of the PJS families. "*DNA" indicates individuals for whom genotypes have been determined.

Furthermore, comparative genomic hybridization, combined with studies of loss of heterozygosity (LOH), provided additional evidence for the involvement of the 19p13.3 genomic region, in PJS (Hemminki et al. 1997). In this article, we confirm linkage of a PJS locus, in the families we studied, to chromosome 19p13.3, since marker D19S886 yielded a maximum two-point LOD score of 4.74 at $\theta = .04$. The multipoint-analysis LOD score was 7.51 for the interval between D19S886 and 19pter. Furthermore, during the genomewide genotyping, we observed another genomic region with evidence suggestive of linkage, around marker D19S880 on chromosome 19q13.4. For this marker, the maximum LOD score, determined by use of all the families, was 3.80 at $\theta = .13$. Most of this positive linkage was contributed by a single family, PJS07. This family alone gave a LOD score of 3.52 at $\theta = .00$ for markers D19S877 and D19S891. Multipoint analysis using genotypes from family PJS07 yielded a LOD score of 3.9 for the interval between D19S180 and D19S254 on 19q13.4. These results confirm the existence of a PJS locus on 19p13.3 but also raise the possibility of a second locus, on chromosome 19q13.4.

Subjects and Methods

Families with PJS

We identified six families with affected individuals who fulfilled the criteria for the diagnosis of PJS (OMIM 175200 [http://www3.ncbi.nlm.nih.gov/omim/]), and we collected blood samples from their affected and unaffected members. Each of these families contained more than one individual with PJS. The ascertainment of the families was from a literature search or through personal contacts with gastroenterologists. Two of the families were from Spain (PJS01 and PJS03), one was from Portugal (PJS06), one was from the United States (PJS05), and two were from India (PJS07 and PJS08) (fig. 1). We classified as affecteds those individuals with mucocutaneous hypermelanotic macules and/or hamartomatous polyps. There were 39 affecteds in our sample. The diagnostic status of six individuals was considered to be unknown, either because invasive diagnostic procedures were not performed or because they were too young to demonstrate the diagnostic signs. A total of 48 individuals were classified as unaffected; however, in view of

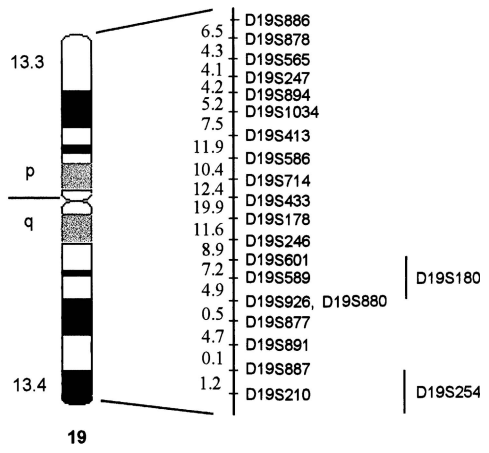

Figure 2 Ideogram of chromosome 19, showing locations of the markers analyzed in this study. The order of these markers was obtained from the Généthon linkage map (http://www.genethon.fr/), the CHLC linkage map (http://www.chlc.org/), and the Third International Workshop on Chromosome 19 Mapping (Mohrenweiser et al. 1996).

the reduced penetrance of the disease, it is not unlikely that some of these individuals are carriers of a mutant PJS gene. All participants were enrolled in the study in accordance with approved clinical protocols from the University of Geneva and the local institutions, and they were asked to provide blood after signing informed consents.

DNA Polymorphism and Linkage Analysis

Genomic DNA was purified from peripheral blood lymphocytes, in accordance with the standard SDS–proteinase K and phenol/chloroform extraction method. DNA polymorphisms were analyzed by PCR amplification of SSRs. These markers were selected from the Généthon and Cooperative Human Linkage Center (CHLC) collections (NIH/CEPH Collaborative Mapping Group 1992; Buetow et al. 1994; Gyapay et al. 1994; Murray et al. 1994; Dib et al. 1996). One oligonucleotide primer of each marker was labeled with 5 μCi of γ-[^{32}P]ATP with T4 polynucleotide kinase. PCR was performed with MJ Research PTC-100 and Biometra UNO I thermocyclers, to amplify 90 ng of genomic DNA in a total volume of 15 μl per reaction, containing 0.4 pmol of labeled forward primer, 2.6 pmol of unlabeled reverse primer, 1.3 μM of each dNTP, and 0.25 U of *Taq* polymerase. PCR products were separated by electrophoresis on a 6% denaturing urea/polyacrylamide gel (Blouin et al. 1995). Genotypes were scored independently by two different investigators, after autoradiography. Family information and marker genotypes were stored in the pedigree computer program Cyrillic. Linkage analysis was performed by use of the ILINK, MLINK, and LINKMAP programs of LINKAGE version 5.2 (Lathrop et al. 1984) and the FASTLINK version 3.0 (Cottingham et al. 1993) and VITESSE (O'Connell and Weeks 1995) software packages. Multipoint linkage analysis was performed with the help of the computer facility of the U.K. Human Genome Mapping Project Resource Centre (http://www.hgmp.mrc.ac.uk/). Maximum LOD and location scores were calculated for each marker locus by assuming an autosomal dominant mode of inheritance with 90% penetrance. The gene frequency of PJS was estimated as 1/10,000. For all polymorphic markers, the allele frequencies were considered to be equal. For the multipoint linkage analysis, the intermarker genetic distances were based on the published Généthon/CHLC maps and were as reported from the Third International Workshop on Chromosome 19 Mapping (Mohrenweiser et al. 1996).

Results

The alleles of a total of 168 polymorphic markers from throughout the human genome were genotyped for the members of the six PJS families collected (a list of these markers can be supplied via E-mail; contact H.M. at Hamid.Mehenni@medecine.unige.ch). Approximately 60% of the genome was analyzed for linkage, by use of these markers. A region on chromosome 19q13.4 that showed pairwise positive LOD scores >3 (see below) was identified. During the course of this analysis, Hemminki et al. (1997) reported linkage of a PJS locus to markers on chromosome 19p13.3; no heterogeneity was found in the 12 families (containing 34 affected individuals) that they reported. We therefore focused on markers on the entire chromosome 19 (fig. 2). More than 22 markers have been analyzed on this chromosome, and the results of two-point linkage analysis are shown in table 1. A maximum two-point LOD score of 4.74 was obtained for D19S886 on 19p13.3 at θ = .045, by use of 90% penetrance. Analysis with the HOMOG program showed no evidence of heterogeneity (α = 1.0). In the study by Hemminki et al. (1997), a maximum LOD score also was detected with locus D19S886. A likely recombinant between D19S886 and the PJS phenotype was detected in family PJS06 (male individual IV-27). Additional markers in the 19p13.3 region also yielded positive LOD scores >3. Multipoint linkage analysis, shown in figure 3, resulted in a maximum LOD score of 7.51 for the interval between D19S886 and 19pter. The locus order and genetic distances between the loci used in this linkage analysis are D19S886–0.053 cM–D19S565–0.083 cM–D19S894 (Généthon map; Dib et al. 1996). The use of different estimates of penetrance and disease-allele frequency did not significantly affect the LOD score. Inspection of hap-

Table 1

Results of Pairwise Two-Point Linkage Analysis between the PJS Phenotype and Selected Markers Covering the Entire Chromosome 19, at Various θ's, for the PJS Families

Marker and Family	LOD Score at θ =							Z_{max}	θ_{max}
	.00	.01	.05	.10	.20	.30	.40		
D19S886:	1.51	4.39	4.74	4.50	3.48	2.18	.89	4.74	.045
PJS01	2.12	2.08	1.92	1.70	1.25	.77	.29		
PJS08	.03	.05	.10	.13	.12	.07	.02		
PJS07	.65	.66	.67	.65	.53	.36	.17		
PJS03	.57	.55	.48	.40	.25	.12	.03		
PJS06	−2.73	.19	.76	.88	.78	.51	.21		
PJS05	.87	.86	.81	.73	.55	.35	.16		
D19S878:	−11.75	−.84	1.72	2.48	2.45	1.68	.68	2.61	.142
PJS01	−5.67	−.21	.95	1.25	1.21	.86	.39		
PJS08	−1.15	−1.07	−.82	−.58	−.27	−.10	−.02		
PJS07	−2.61	.32	.86	.95	.81	.52	.18		
PJS03	.78	.76	.68	.58	.38	.20	.06		
PJS06	−3.34	−.88	−.20	.03	.14	.10	.05		
PJS05	.33	.32	.31	.28	.20	.11	.03		
D19S565:	−5.75	2.08	3.96	4.36	3.79	2.5	1.04	4.36	.1
PJS01	−1.60	1.05	1.55	1.58	1.33	.9	.39		
PJS08	−1.50	−1.40	−1.01	−.67	−.3	−.12	−.03		
PJS07	2.54	2.49	2.29	2.02	1.45	.86	.32		
PJS03	.78	.76	.68	.58	.38	.20	.06		
PJS06	−2.44	.08	.70	.86	.78	.51	.20		
PJS05	−3.54	−.90	−.24	−.01	.14	.15	.09		
D19S247:	−7.17	.82	2.61	3.09	2.83	1.91	.79	3.12	.122
PJS01	−1.60	1.35	1.82	1.84	1.53	1.05	.47		
PJS08	.03	.03	.03	.02	.01	.01	.00		
PJS07	2.47	2.42	2.21	1.94	1.38	.78	.24		
PJS03	.57	.55	.48	.4	.25	.12	.03		
PJS06	−5.12	−2.65	−1.69	−1.09	−.42	−.10	.02		
PJS05	−3.52	−.88	−.24	−.02	.08	.06	.02		
D19S894:	−5.92	.98	3.38	3.95	3.56	2.41	1.01	3.97	.118
PJS01	−2.16	−.35	.83	1.14	1.13	.82	.38		
PJS08	.03	.03	.03	.02	.01	.01	.00		
PJS07	2.26	2.25	2.18	2.03	1.59	1.03	.41		
PJS03	.78	.76	.68	.58	.38	.20	.06		
PJS06	−3.29	−.81	−.08	.21	.37	.30	.15		
PJS05	−3.55	−.91	−.26	−.04	.07	.06	.02		
D19S1034:	−13.52	−3.96	−.16	1.14	1.67	1.27	.61	1.67	.188
PJS01	−3.64	−2.10	−.71	−.17	.21	.25	.16		
PJS08	−4.89	−.68	−.08	.10	.15	.09	.02		
PJS07	−2.63	.31	.92	1.07	.96	.66	.32		
PJS03	.78	.76	.68	.58	.38	.20	.06		
PJS06	−3.41	−2.52	−1.26	−.73	−.29	−.13	−.06		
PJS05	.27	.28	.29	.29	.26	.20	.11		
D19S413	−20.02	−6.22	−1.88	−.11	1.06	1.06	.55	1.15	.246
D19S586	−16.68	−1.84	1.27	2.19	2.35	1.74	.84	2.42	.158
D19S714	−26.62	−10.81	−4.72	−2.16	−.15	.41	.32	.43	.328
D19S433	−12.83	−4.97	−1.8	−.36	.64	.73	.45	.75	.265
D19S178	−27.92	−11.54	−5.46	−2.78	−.57	.16	.26	.27	.372
D19S246	−27.59	−13.2	−6.92	−4.00	−1.32	−.22	.15	.15	.42
D19S601	−9.57	−3.44	−.44	.76	1.43	1.19	.59	1.43	.21
D19S589	−4.03	−1.19	.03	.68	1.08	.93	.52	1.08	.22
D19S180	−7.09	−3.78	−.88	.40	1.21	1.15	.69	1.25	.235

(*continued*)

Table 1 (continued)

Marker and Family	LOD Score at θ = .00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
D19S926:	−15.96	−5.54	−1.25	.44	1.48	1.34	.69	1.52	.229
PJS01	−3.31	−.76	.10	.48	.70	.60	.32		
PJS08	.08	.10	.14	.15	.12	.06	.01		
PJS07	2.96	2.91	2.68	2.39	1.77	1.11	.47		
PJS03	−4.77	−2.80	−1.44	−.89	−.39	−.15	−.04		
PJS06	−2.91	−1.76	−.98	−.60	−.25	−.09	−.03		
PJS05	−8.01	−3.22	−1.75	−1.09	−.47	−.18	−.04		
D19S880:	−10.31	.96	3.07	3.72	3.56	2.55	1.17	3.80	.13
PJS01	−2.98	−.38	.38	.67	.79	.62	.32		
PJS08	−.36	−.31	−.16	−.04	.06	.06	.02		
PJS07	−4.41	1.86	2.31	2.27	1.85	1.22	.50		
PJS03	.78	.76	.68	.58	.38	.20	.06		
PJS06	−3.13	−.77	.01	.34	.54	.47	.27		
PJS05	−.21	−.20	−.15	−.11	−.05	−.02	.00		
D19S877:	−15.76	−4.71	−.48	1.09	1.88	1.52	.73	1.88	.20
PJS01	−8.13	−3.06	−1.46	−.73	−.10	.10	.10		
PJS08	.08	.10	.14	.15	.13	.07	.02		
PJS07	3.52	3.46	3.20	2.87	2.15	1.38	.61		
PJS03	−4.77	−2.80	−1.44	−.89	−.39	−.15	−.04		
PJS06	−2.76	−1.42	−.62	−.27	−.01	.04	.02		
PJS05	−3.71	−1.00	−.30	−.04	.10	.08	.02		
D19S891:	−6.42	−.77	1.37	2.12	2.23	1.62	.75	2.31	.156
PJS01	−3.79	−1.24	−.36	.05	.34	.33	.18		
PJS08	.03	.03	.03	.02	.01	.01	.00		
PJS07	3.52	3.46	3.20	2.87	2.15	1.38	.61		
PJS03	.78	.76	.68	.58	.38	.20	.06		
PJS06	−3.2	−2.29	−1.43	−.97	−.51	−.25	−.10		
PJS05	−3.77	−1.5	−.75	−.43	−.15	−.05	−.01		
D19S887:	−25.01	−9.74	−4.39	−2.03	−.12	.37	.29	.38	.325
PJS01	−4.54	−3.59	−1.86	−.98	−.15	.16	.19		
PJS08	−9.30	−2.18	−1.32	−.88	−.41	−.17	−.04		
PJS07	1.11	1.09	1.00	.88	.65	.41	.19		
PJS03	−4.83	−2.8	−1.44	−.89	−.39	−.15	−.04		
PJS06	.64	.67	.74	.73	.55	.26	.02		
PJS05	−8.09	−2.93	−1.51	−.91	−.37	−.14	−.03		
D19S210:	−20.55	−8.22	−3.13	−.94	.75	1.05	.62	1.06	.283
PJS01	−3.97	−3.17	−1.55	−.75	−.04	.21	.20		
PJS08	.08	.10	.16	.19	.17	.10	.03		
PJS07	−3.76	2.46	2.86	2.78	2.26	1.52	.66		
PJS03	−4.83	−2.8	−1.44	−.89	−.39	−.15	−.04		
PJS06	−3.6	−2.67	−1.81	−1.33	−.77	−.42	−.18		
PJS05	−4.47	−2.14	−1.35	−.95	−.48	−.20	−.05		
D19S254:	−11.24	−.34	1.8	2.52	2.53	1.81	.87	2.66	.146
PJS01	.93	.94	.97	.94	.77	.52	.26		
PJS08	−1.24	−1.16	−.88	−.62	−.28	−.11	−.02		
PJS07	−4.30	1.56	2.02	2.00	1.63	1.08	.51		
PJS03	−.22	−.20	−.14	−.08	−.03	−.01	.00		
PJS06	−1.84	−.19	.41	.56	.53	.33	.13		
PJS05	−4.57	−1.30	−.58	−.29	−.07	−.01	.01		

Note.—LOD scores determined by use of the MLINK and ILINK programs. The order of markers is from 19pter to 19qter, as determined in the CHLC linkage map, version 4 (http://www.chlc.org/), the Généthon linkage map (http://www.genethon.fr/), and the report from the Third International Workshop on Chromosome 19 Mapping (Mohrenweiser et al. 1996). For each marker, the first line of data indicates the results determined by use of genotypes from all six families; the LOD scores for each family also are shown. For some markers only the LOD scores for all six families are shown.

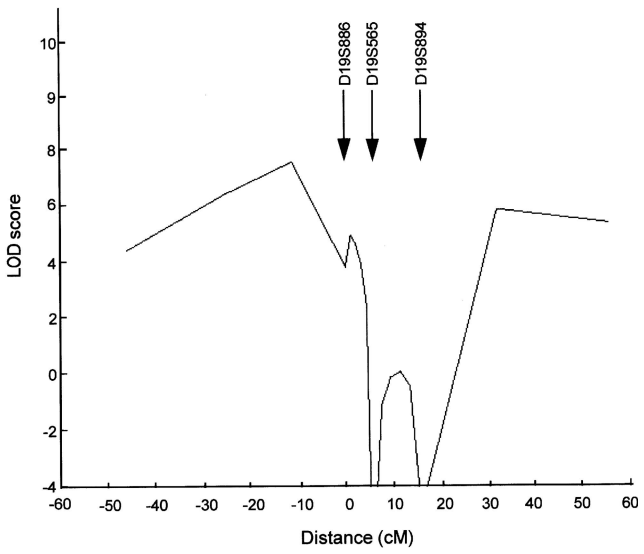

Figure 3 Multipoint linkage analysis between the PJS phenotype and three selected chromosome 19p13.3 markers (D19S886–D19S565–D19S894), determined by use of genotypes from all the families. Marker D19S886 was set at map position zero. The map distances were calculated by use of the Haldane mapping function.

lotypes for polymorphic markers in 19p13.3 did not reveal any association between a particular haplotype and the PJS locus. This is compatible with the independent occurrence of mutations at the PJS locus in different populations and families.

As stated earlier, a genomic region on chromosome 19q13.4 that is unlinked to 19p13.3 also yielded significant LOD scores. Marker D19S880 resulted in a maximum two-point LOD score of 3.80 at $\theta = .13$. Most of the evidence for linkage with this marker was obtained from Indian family PJS07, which alone yielded a maximum LOD score of 3.52 at $\theta = .00$, for neighboring markers D19S877 and D19S891. Analysis with the HOMOG program showed evidence for heterogeneity ($\alpha = .21$ and .36 for markers D19S877 and D19S891, respectively). Multipoint linkage analysis using genotypes of family PJS07 resulted in a maximum LOD score of 3.9 for the interval between D19S180 and D19S254 (fig. 4).

Discussion

We have used linkage analysis to map the locus (loci) for autosomal dominant PJS. Using DNA from members of six families, with 39 affected individuals, we were able to confirm linkage to marker D19S886 on 19p13.3, recently reported by Hemminki et al. (1997). Unlike this latter study, in which no crossover was observed with marker D19S886, the linkage analysis of the families that we studied revealed that at least one of these families (PJS06) showed a recombination with this marker. However, it is unknown whether family PJS06 is linked to the 19p13.3 chromosomal region. In addition, the apparent recombination occurred in an unaffected individual who may represent an example of incomplete penetrance. Multipoint linkage analysis that included the genotyping data from all the families studied indicated that the PJS locus maps distal to D19S886. However, it is possible that the locus for family PJS06 maps elsewhere in the genome, and, therefore, the results from the multipoint linkage analysis may be misleading. A project to develop the physical map of chromosome 19, comprising cosmids and other cloning vectors, has been nearly completed by the Lawrence Livermore National Laboratory (LLNL), and a considerable number of genes already have been mapped to 19p13.3 (see the LLNL gene ideogram for human chromosome 19 [http://www-bio.llnl.gov/genome/html/gene_ideogram.html]). Some of these genes are candidates for the PJS phenotype. Clearly, more families and more polymorphic markers distal to D19S886 need to be studied, since this is the most distal 19pter marker available to narrow the critical region. It is unlikely that linkage disequilibrium will help in further localization of the PJS region on 19p13.3, because the disorder is dominant and because new mu-

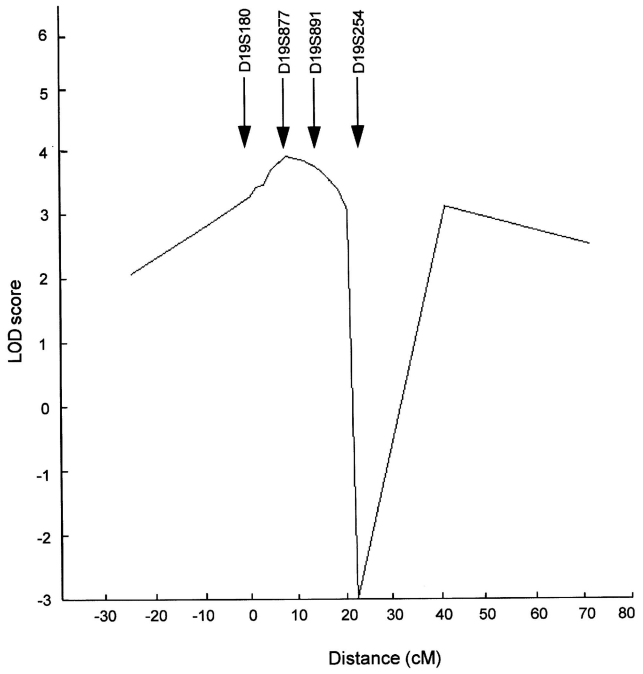

Figure 4 Multipoint-linkage-analysis curve for markers on chromosome 19q13.4 (D19S180–D19S877–D19S891–D19S254), determined by use of genotypes from family PJS07. Marker D19S180 was set at map position zero. The map distances were calculated by use of the Haldane mapping function.

tations are more likely to occur in the most common haplotypes. On the other hand, as suggested by Hemminki et al. (1997), studies of LOH in PJS polyps or tumors may better demarcate the borders of the critical region that harbors the mutated gene.

Interestingly, one family from India (PJS07) showed high LOD scores with polymorphic markers on 19q13.4. For example, for PJS07, the maximum LOD score for markers D19S877 and D19S891 was 3.52 at $\theta = .00$ (for D19S880, the maximum LOD score determined by use of genotypes from all the families was 3.80 at $\theta = .11$). The same family, PJS07, was not very informative for the most 19p telomeric markers but yielded maximum LOD scores of 2.54 and 2.26 for markers D19S565 and D19S894, respectively, at $\theta = .00$. Multipoint linkage analysis with markers D19S886–D19S565–D19S894 on 19p13.3, using genotypes from only family PJS07, resulted in a maximum LOD score of 3.21 for the intervals on either side of marker D19S565. Since the 19p13.3 markers are unlinked to those at 19q13.4, it is possible that the considerably high LOD score for 19q13.4 in family PJS07 is due to chance only. Alternatively, it is possible that there is a second locus for PJS, on 19q13.4. Mutations in this second locus may result in PJS in a subset of PJS families. Furthermore, the gene products of these two loci may interact, and the development of a PJS phenotype may be the result of the interaction of mutant proteins encoded by both loci. An example of so-called digenic inheritance already has been well documented in one form of autosomal dominant retinitis pigmentosa (Kajiwara et al. 1994). It would be of interest to determine the genotypes of markers on 19q13.4 in the families described by Hemminki et al. (1997), in order to study the contribution of a potential locus on 19q13.4 in these families. Evidence of LOH on chromosome 19q13.4 was not observed in the data reported by Hemminki et al. (1997). Positional cloning experiments and mutation analyses of candidate genes (e.g., see the National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov/SCIENCE96/]) are now in progress for the cloning and characterization of the PJS gene on 19p13.3.

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References

Blouin J-L, Christie DH, Gos A, Lynn A, Morris MA, Ledbetter DH, Chakravarti A, et al (1995) A new dinucleotide repeat polymorphism at the telomere of chromosome 21q reveals a significant difference between male and female rates of recombination. Am J Hum Genet 57:388–394

Buck JL, Harned RK, Lichtenstein JE (1992) Peutz-Jeghers syndrome. Radiographics 12:366–378

Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Duyk GM, Sheffield VC, Wang Z, et al (1994) Integrated human genome-wide maps constructed using the CEPH reference panel. Nat Genet 6:391–393

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–263

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Foley TR, McGarrity TJ, Abt AR (1988) Peutz-Jeghers syndrome: a clinicopathologic survey of the "Harrisburg family" with a 49-year follow-up. Gastroenterology 95: 1535–1540

Giardiello FM, Welsh SB, Hamilton SR, Offerhaus GJA, Gittelsohn AM, Booker SV, Krush AJ, et al (1987) Increased risk of cancer in the Peutz-Jeghers syndrome. N Engl J Med 316:1511–1513

Gilks CB, Young RH, Aguirre P, DeLellis RA, Scully RE (1989) Adenoma malignum (minimal deviation adenocarcinoma) of the uterine cervix. Am J Surg Pathol 13:717–729

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–94 Généthon human genetic linkage map. Nat Genet 7:246–249

Hemminki A, Tomlinson I, Markie D, Jarvinen H, Sistonen P, Bjorkqvist A-M, Knuutila S, et al (1997) Localization of susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. Nat Genet 15:1587–1590

Jeghers H, McKusick VA, Katz KH (1949) Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits. N Engl J Med 241:992–1036

Kajiwara K, Berson EL, Dryja TP (1994) Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 264:1604–1607

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lehur P-A, Madarnas P, Devroede G, Perey BJ, Menard DB, Hamade N (1984) Peutz-Jeghers syndrome: association of duodenal and bilateral breast cancers in the same patient. Dig Dis Sci 29:178–182

Mohrenweiser H, Olsen A, Archibald A, Beattie C, Burmeister

M, Lamerdin J, Lennon G, et al (1996) Report and abstracts of the Third International Workshop on Human Chromosome 19 Mapping 1996. Cytogenet Cell Genet 74:161–186

Murray JC, Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Manion F, Quillen J, et al (1994) A comprehensive human linkage map with centimorgan density: Cooperative Human Linkage Center (CHLC). Science 265: 2049–2054

NIH/CEPH Collaborative Mapping Group (1992) A comprehensive genetic linkage map of the human genome. Science 258:67–86

O'Connell JR, Weeks DE (1995) The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. Nat Genet 11:402–408

Perzin KH, Bridge MF (1982) Adenomatous and carcinomatous changes in hamartomatous polyps of the small intestine: Peutz-Jeghers syndrome. Cancer 49:971–983

Young RH, Welch WR, Dickerson GE, Scully RE (1982) Ovarian sex cord tumor with annular tubules: review of 74 cases, including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. Cancer 50:1384–1402

Young S, Gooneratne S, Straus FH, Zeller WP, Belun SE, Rosentha IM (1995) Feminizing Sertoli cell tumors in boys with Peutz-Jeghers syndrome. Am J Surg Pathol 19:50–58