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Advances in Brief

Fine Mapping of a Genetic Locus for Peutz-Jeghers Syndrome on Chromosome 19p[1]

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Abstract

Peutz-Jeghers syndrome (PJS) was recently mapped in a single report to the telomeric region of chromosome 19p (A. Hemminki *et al.*, Nat. Genet., *15:* 87–90, 1997). Our studies confirm this location and provide further localization of the PJS locus. In the five families examined, there were no recombinants with the marker *D19S886*. The multipoint log odds score at *D19S886* is 7.52, and we found no evidence for genetic heterogeneity. We also found that all carriers expressed the PJS phenotype and no noncarriers displayed PJS sequellae, indicating complete penetrance with no sporadic cases.

Introduction

PJS[3] is a rare autosomal dominant syndrome characterized by cutaneous hypermelanocytic macules and associated hamartomatous polyp development in the stomach and the small and large bowel (1, 2). More than 95% of carriers show buccal hypermelanocytic lesions (3). The hypermelanocytic lesions are rarely present at birth, become prominent within the first five years of life, and fade in adulthood. The development of hamartomatous polyps constitutes the most significant clinical component of PJS; 90% of patients report this symptom (4). Hamartomas from PJS carriers have low malignant potential, but many reports have documented adenomatous and carcinomatous changes arising from hamartomas (2, 5). In addition to small bowel and colonic neoplasias, pancreatic and gastric carcinomas are relatively common among PJS carriers (6). A single report recently provided localization of the PJS gene to the telomeric region of chromosome 19p (7) with no evidence for heterogeneity. Here, we confirm linkage of all of the families that we have studied to this region and provide information to refine the critical PJS region.

Materials and Methods

Family Accrual. Families were accrued by collaborators either at the University of Texas M. D. Anderson Cancer Center or Pennsylvania State University, Hershey Medical Center (Hershey, PA). All participants were enrolled on Institutional Review Board-approved protocols and were asked to provide blood or buccal smears after signing informed consent forms. Medical history information was requested from all individuals. Participants also completed a questionnaire designed to elicit information about pigmentary changes typical of PJS, as well as history of polyp resections and abdominal symptoms. We classified participants as affected if they had a first-degree relative with PJS and pigmentary changes and/or surgeries for polyps. One individual classified as unaffected had been hospitalized once because of vague abdominal complaints but had no history of pigmentary changes and had never had any subsequent surgeries or abdominal complaints. All PJS carriers reported a history of pigmentary changes.

DNA Marker Analyses. DNA templates for PCR analyses were prepared either from peripheral blood mononuclear cells using modifications of standard techniques (8) or prepared from buccal cell brushings. Briefly, for buccal cell DNA, after cheek surfaces were brushed, brushes were placed in 600 ml of 50 mM NaOH. After being heated at 95°C for 10 min and vortexed, the sample was neutralized with 50 ml of 1.0 M Tris/HCl, pH 8.0. For working solutions, stock DNA was sheared and resuspended in T10E1 at a 1:4 dilution, and 1 ml was used in 5-μl PCR reactions.

Polymorphic microsatellite markers were analyzed using a 373A DNA sequencer (Applied Biosystems) and a multiplex protocol similar to that described previously. (9). Briefly, PCRs were performed in 96-well microtiter plates in a 5-μl reaction volume using 10 ng of sheared human DNA, 0.5 μl of 10 × reaction buffer (Perkin-Elmer Corp.), 3.5 μM final concentration of each dNTP, 80 nM final concentration of forward and reverse primers, and 0.25 units of Taq DNA polymerase (Life Technologies, Inc.). PCRs were performed in either a BIOOVEN III (Biotherm Corporation) or GeneAmp PCR System 9600 (Perkin-Elmer Corp.) using mostly Weissenbach conditions (92°C for 5 min followed by 35 cycles of 40 s at 92°C, 30 s at 55°C, and final extension of 2 min at 72°C), with some variations made in the annealing temperatures, $MgCl_2$ concentration, and number of cycles performed to optimize the individual marker conditions. For semiautomated data collection and analysis, 672 GeneScan Collection software (Version 1.1) was used (Applied Biosystems).

Allele Assignment and Analysis. DNA fragment analysis software Genotyper Version 1.1 (Applied Biosystems) was used for semiautomated allele size assignment in bp following the manufacturer's manual. The allele size of the PCR product was determined in reference to an internal lane size standard.

Linkage Analysis. Prior to performing genetic linkage analyses, we estimated the mean allele frequencies from the data. We used the estimated allele frequencies for the markers and assumed the PJS-causing mutation occurred in 1 of 10,000 individuals. We assumed that the penetrance for PJS among carriers was 95% and that 2% of noncarriers might show symptoms that could be confused with PJS (*e.g.*, have pigmentary changes). We also fitted a model with 100% penetrance and no sporadic cases. We used the optimized version of the LINKAGE program (10) for all two-point analyses, assuming equal male and female recombination fractions (θ). Results of two-point analyses are presented in terms of the LOD score, which summarizes the $\log_{10}$ of a ratio of the likelihood of the data assuming linkage with $\theta < 0.5$ to the likelihood of the data with $\theta = 0.5$. Generally, a LOD score of 3.3 (11) was taken as evidence in favor of linkage, whereas LOD scores less than −2.0 were taken

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[3] The abbreviations used are: PJS, Peutz-Jeghers syndrome; LOD, log odds in favor of linkage.

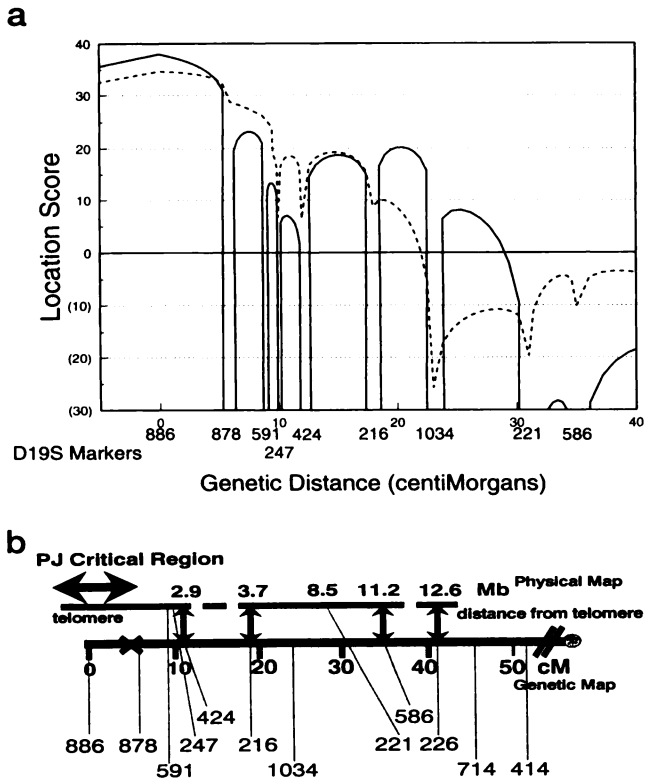

Fig. 1. Multipoint mapping results for PJS (*a*) *versus* the nine most telomeric markers (*b*). *a*, the location of the markers is indicated along the X axis. The composite map was constructed by combining five-point sliding maps (four markers and the disease) at each point. ——, simple autosomal dominant model with complete penetrance; - - - - -, autosomal dominant model with 95% penetrance and 2% sporadic risk. *b*, genetic and physical relationships of the PJS critical region. Shown at the *top* are the distances in Mb from the telomere, derived from the Lawrence Livermore Laboratory contig map (*D19S591*, 2.6 Mb; *D19S247*, 2.7 Mb; *D19S424*, 2.9 Mb; *D19S216*, 3.7 Mb; *D19S586*, 8.5 Mb; *D19S221*, 11.2 Mb; and *D19S226*, 12.6 Mb). *Bottom*, positions on the genetic map in cM from the most distal marker (*D19S886*), from the sex-averaged Genethon map (*D19S878*, 6.5 cM; *D19S424*, 10.8 cM; *D19S216*, 19.1 cM; *D19S221*, 35.5 cM; *D19S226*, 41.7 cM; and *D19S414*, 53.2 cM). Genetic map positions for *D19S1034*, *D19S586*, and *D19S714* were estimated by comparison of the position of these markers in the sex-averaged Marshfield genetic map (which also includes *D19S886*, *D19S878*, *D19S591*, and *D19S247*) with the Livermore physical map and Genethon genetic map. The most distal crossover (depicted to the *left* of *D19S878*) defines the PJS critical region.

as evidence against linkage. For multipoint mapping, we used VITESSE software (12). We set the male recombination fraction at 9 times that for females as suggested by the genetic map for the extreme telomeric region of chromosome 19 (13). We fixed the genetic distances among the markers according to map distances shown in Fig. 1*b*.

Results

Prior to the report of Hemminki *et al.* (7), we had systematically excluded chromosomes 1–3, 6–8, and 10–12 and regions of chromosomes 4, 5, 9, 13, 14, 15, and 22 (results not shown). Complete typings of all available family members were performed for 11 microsatellite markers on 19p13.3 (Fig. 1*b*). Two-point mapping studies for these markers are presented in Table 1. The strongest evidence of linkage was found at the most telomeric marker (*D19S886*), with a LOD score (under the model with 95% penetrance) of 4.45 at $\theta = 0$. Under a model with 100% penetrance and no sporadic risk, the two-point LOD score was 4.75 for *D19S886* (results not shown). As shown in Fig. 1*a*, under the model with 95% penetrance, the multipoint location score was 34.63, corresponding to a multipoint LOD score of 7.52 (*i.e.*, 3.5×10^8 times more likely to be linked with no recombination than unlinked) maximizing at *D19S886*. Under a model with complete penetrance (Fig. 1*a*), the location score at *D19S886* was 37.98, which yields a multipoint LOD score of 8.25. Haplotype analysis (family 4, individual III-4; Fig. 2) provides strong evidence for a single recombination between PJS susceptibility and *D19S878*. These data localize PJS to the region telomeric to *D19S878* (shown schematically in Fig. 1*b*). The five disease-associated haplotypes in these families are unique, although further haplotype studies are needed once further genetic markers become available in this region.

Discussion

This study provides the first confirmation that susceptibility to PJS localizes to telomeric chromosome 19p. As shown by Fig. 1, recombination events in this region are relatively more common than for other regions of the genome. Therefore, positional cloning of the PJS locus may be more straightforward than would be expected for other genomic regions. Haplotype analysis in our families demonstrates that the PJS locus must be telomeric to *D19S878*. This localizes the region to a region comprising approximately 3 Mb of the extreme telomeric region of chromosome 19p. It is notable that most of the crossovers occur from male meiotic events, consistent with the increased meiotic frequency in males for this part of the genome. We did not observe any obvious commonality among the five disease-associated haplotypes that were studied. This observation needs to be confirmed in studies of larger numbers of families but suggests that positional cloning of PJS through linkage disequilibrium studies may not be feasible. Further family studies are needed to document a lack of genetic heterogeneity for the syndrome and further characterize the risks for neoplasias. Our results, along with those of Hemminki *et al.* (7), show that all PJS families studied to date are linked to chromosome 19p, and genetic linkage studies in PJS can be used for genetic counseling.

Table 1 *Two-point linkage analysis between PJS and various markers on chromosome 19p*

	LOD score at recombination fraction						
Marker locus	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D19S886	4.45	4.37	4.01	3.55	2.62	1.67	0.77
D19S878	4.05	4.11	4.06	3.77	2.94	1.97	0.96
D19S591	2.33	2.46	2.63	2.54	2.00	1.29	0.57
D19S247	3.35	3.43	3.45	3.24	2.56	1.72	0.83
D19S424	0.93	3.80	4.31	4.15	3.28	2.17	1.01
D19S216	0.21	2.75	3.37	3.33	2.72	1.84	0.88
D19S1034	1.43	1.75	2.14	2.14	1.77	1.22	0.62
D19S221	−6.00	−2.71	−0.29	0.70	1.27	1.15	0.70
D19S586	−2.98	−1.40	−0.12	0.42	0.68	0.56	0.31
D19S714	0.01	1.33	1.98	2.12	1.90	1.39	0.73
D19S414	−3.99	−3.37	−2.09	−1.31	−0.57	−0.25	−0.10

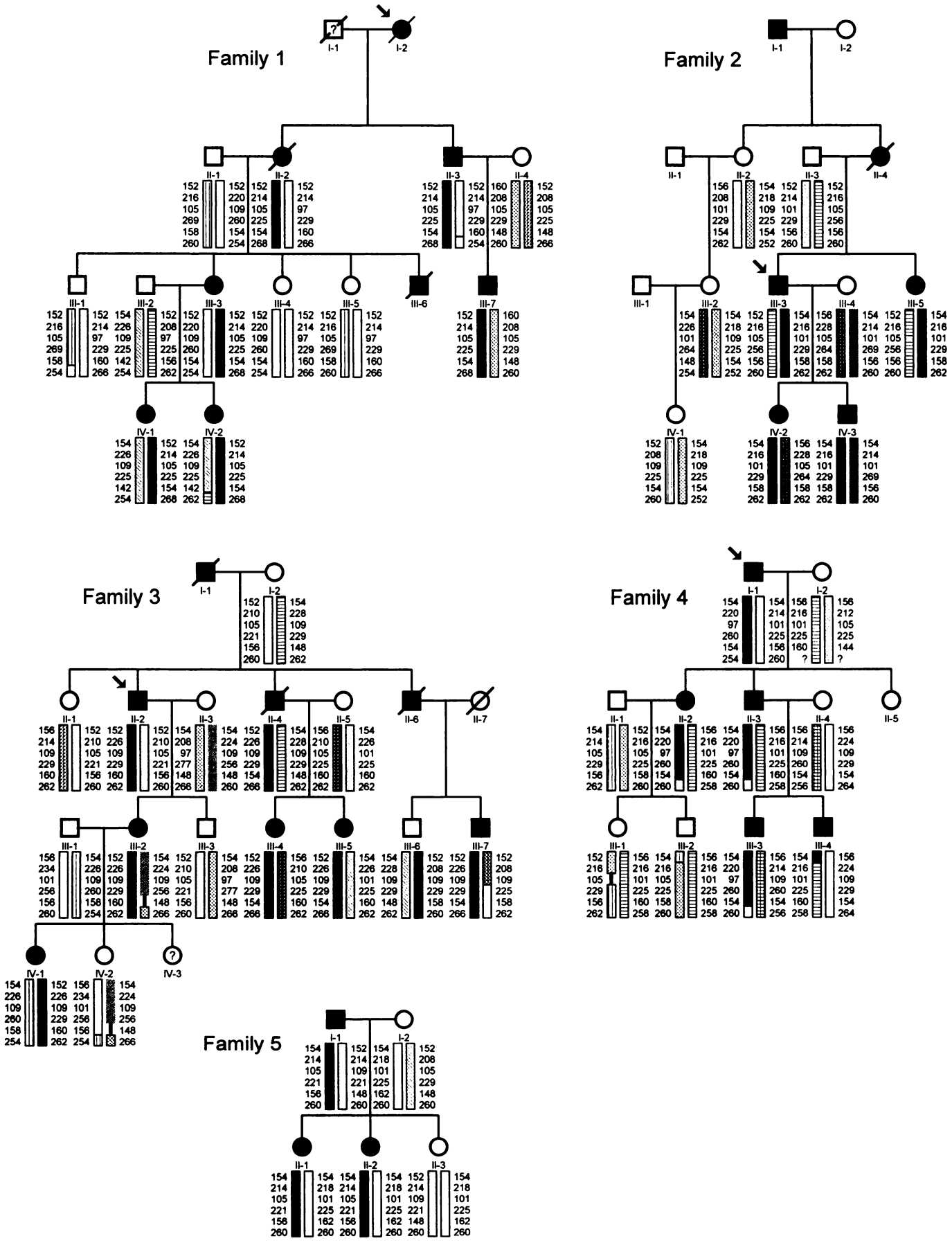

Fig. 2. Haplotypes of five families with PJS. Markers included are *D19S886, D19S878, D19S591, D19S247,* and *D19S424.*

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