lus onset to the stimulus offset. This response image was divided by the image obtained during a blank screen period ("blank reference") to compensate for the difference in the background level of reflected light. From the image, then, was subtracted the image obtained by division between the image obtained in the 1-s period just before the stimulus onset and the corresponding image of the blank reference, in order to compensate for slow changes in signals over several minutes. No spatial filtering except smoothing (high-cut filtering) was performed.
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14. The t values calculated on a trial-by-trial basis for the deviation from 1 of the mean for the pixels below the sulcus gave $P < 0.01$ for all of the 19 stimuli examined, whereas those calculated for pixels above the sulcus gave $P > 0.1$ for 17 stimuli and $P > 0.05$ for the remaining 2 stimuli.
15. The coefficient of cross-correlation calculated on a pixel-by-pixel basis for the anterior IT region was 0.65 ± 0.04 ($n = 19$). These values had no overlap with the correlation coefficients calculated between images obtained with different critical features (0.30 ± 0.08, $n = 91$).
16. The sites of electrode penetration were pinpointed with ink directly on the cortical surface and were successfully identified at the time of optical imaging for 13 penetrations. In 10 out of the 13 cases, the penetration site was enclosed by the contour of the dark spot defined by a drop from the peak by $1/e$ of the difference between the peak and the average of the surrounding regions. In another case, the penetration site was at a $2/e$ drop from the peak, and in the other two cases the penetration site was distant from the dark spots.
17. Calculation of t values for individual pixels, in reference to the cocktail references, showed that the darkening evoked by the critical feature determined at the site was statistically significant ($P < 0.01$) but that the other stimuli evoked no significant darkening around the region ($P > 0.10$).
18. Only optical imaging was conducted in these two hemispheres. The main purpose of these experiments was to repeat the systematic movement of the activation spot with the rotation of the face. Ten or 14 nonface stimuli were also presented in order to examine the selectivity of activation.
19. G. Wang, K. Tanaka, M. Tanifuji, data not shown.
20. None of the nonface stimuli [10 stimuli in the cases shown in Fig. 3, A and C, and 14 stimuli in the case shown in Fig. 3B] evoked a statistically significant darkening around the regions ($P > 0.10$).
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Human Homolog of *patched*, a Candidate Gene for the Basal Cell Nevus Syndrome

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The basal cell nevus syndrome (BCNS) is characterized by developmental abnormalities and by the postnatal occurrence of cancers, especially basal cell carcinomas (BCCs), the most common human cancer. Heritable mutations in BCNS patients and a somatic mutation in a sporadic BCC were identified in a human homolog of the *Drosophila patched (ptc)* gene. The *ptc* gene encodes a transmembrane protein that in *Drosophila* acts in opposition to the Hedgehog signaling protein, controlling cell fates, patterning, and growth in numerous tissues. The human *PTC* gene appears to be crucial for proper embryonic development and for tumor suppression.

Patients with the BCNS (also called Gorlin syndrome; Mendelian Inheritance in Man, No. 109400) have diverse developmental abnormalities, often including rib and craniofacial alterations and, less often, polydactyly, syndactyly, and spina bifida (*1*). In addition, these patients suffer from a multitude of tumor types. These include fibromas of the ovaries and heart; cysts of the skin, jaws, and mesentery; and the more devastating cancers of the central nervous system—medulloblastomas and meningiomas. The most frequent of the BCNS tumors are BCCs. BCCs are the most common human cancer, with an estimated 750,000 cases occurring each year in the United States alone (*2*). The great majority of BCCs arise sporadically and in small numbers on sun-exposed skin of middle-aged or older people of northern European ancestry. By contrast, BCCs in the BCNS family members appear earlier, during the second decade of life, and occur in large numbers. The BCNS is inherited as an autosomal dominant disease, and patients are expected to be heterozygotes for the causative mutation.

The *ptc* gene, identified initially as a *Drosophila* segment polarity gene (*3*), encodes a transmembrane protein that is produced in precise spatial patterns in developing flies (*4*). The *ptc* gene controls development by repressing transcription, in specific cells, of genes encoding members of the transforming growth factor-β (TGF-β) and Wnt families of signaling proteins. These genes are induced by a signal from the Hedgehog (Hh) protein, which counters repression by Patched (Ptc). Ectopic expression of Hh in *Drosophila* imaginal discs, which are precursors of adult body structures, stimulates growth and causes appendage duplication (*5*, *6*), whereas ectopic expression of Ptc inhibits growth, causing development of smaller than normal wings (*7*).

Vertebrate homologs of *hh* and *ptc* have been identified in mice, chickens, and zebrafish (*8*–*11*). Human *HH* genes have also been described (*12*). The patterns of *ptc* and *hh* expression in vertebrates suggest that these genes have important roles in organizing many tissues—including neural tube, skeleton, limbs, craniofacial structures, and skin. As in insect wings, ectopic expression of Hh in chick limb buds causes dramatic outgrowths (*9*). Hh induces transcription of a variety of genes in vertebrates, including the genes for bone morphogenetic proteins, which are TGF-β family members, and *ptc* itself (*13*). The induction of *ptc* transcription by Hh in *Drosophila* and vertebrates suggests that the Hh–Ptc pathway is evolutionarily conserved (*10*, *11*, *14*). Thus, vertebrate Ptc may oppose the stimulation of growth by Hh, as in *Drosophila*.

To investigate possible links of *ptc* to human disease, we have used a positional candidate strategy (*15*). We cloned the human *PTC* homolog by screening a human lung complementary DNA (cDNA) library with several mouse *ptc* cDNA probes (*16*). Several partial human *PTC* cDNAs were identified and sequenced to obtain the entire coding sequence. Thus far we have found only one human gene, although we have not performed an exhaustive search for related genes. The assembled 5.1 kilobases (kb) of contiguous sequence contains a 4.5-kb open reading frame (ORF) encod-

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ing a 1447–amino acid protein (Fig. 1). At a position 225 nucleotides upstream of the first AUG of the ORF is an in-frame stop codon, which indicates that this AUG encodes the initiating methionine. In addition, the NH_2- and COOH-terminal sequences of human *PTC* agree precisely with those of the mouse homolog. The predicted amino acid sequence has 96% and 40% identity to mouse and *Drosophila* Ptc proteins, respectively. Like the previously cloned homologs, human PTC is predicted to contain 12 hydrophobic membrane-spanning domains and two large hydrophilic extracellular loops (*10*).

We developed a polymerase chain reaction (PCR)–based assay for the human *PTC* gene, and by radiation hybrid mapping we determined that the gene resides on chromosome 9, very near the meiotic marker D9S287, which lies between D9S196 and D9S176 on the Genethon genetic map (*17*). These markers are located in band 9q22.3, a region implicated in the BCNS. We refined the position of the *PTC* gene to the centromeric portion of our ~5.5-megabase (Mb) integrated yeast artificial chromosome (YAC) contig (*18*) that spans the closest genetically defined flanking loci, D9S196 and D9S287 (Fig. 2) (*19*, *20*).

We looked for altered *PTC* sequences in germline DNA from BCNS individuals and from BCCs. An initial Southern (DNA) blot screen of Eco RI–digested DNA from probands of 60 BCNS kindreds did not reveal major rearrangements of the *PTC* gene, so we screened for more subtle sequence abnormalities. Using a PCR approach and a BAC (bacterial artificial chromosome) that contains genomic DNA for the entire coding region of *PTC*, we determined the intronic sequences flanking 20 of at least 23 exons (*21*). Single-strand conformation polymorphism (SSCP) analysis of PCR-amplified DNA from normal individuals, BCNS patients, and sporadic BCCs was performed for 20 exons of *PTC* coding sequence (*22*). Samples that produced abnormal banding patterns were then sequenced (*23*).

We identified two independent sequence changes in exon 15 in blood cell DNA from BCNS individuals. One 49-year-old affected man has an insertion of 9 base pairs (bp) (CCGAATATC) at nucleotide 2445 of the coding sequence, resulting in the insertion of three amino acids (Pro-Asn-Ile) after amino acid 815 (Fig. 3, A through C). Because the normal sequence preceding the insertion is also Pro-Asn-Ile, this change creates a tandem duplication of three amino acids. The patient's affected sister and daughter have the same alteration, but three unaffected relatives do not.

The second case of an exon 15 change was an 18-year-old woman who developed BCCs at age 6 and jaw cysts at age 8. In addition, she has other BCNS manifestations, including palmar and plantar pits, pectus carinatum (chest displacement), and rib abnormalities. The developmental defects and tumors are diagnostic of the BCNS. We detected an SSCP alteration in her DNA that is not present in DNA from her unaffected parents, who have the same SSCP pattern as 84 other control chromosomes. The patient's blood cell DNA has a

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MASAGNAAEPQDRGGGGSGCIGAPGRPAGGGRRRRTGGLRRAAAPDRDYLHRPSYCDAAFALEQISKGKATGRKAPLWLR   80
                    ____________1__________
AKFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFAVGLKAANLETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQ  160

TPKEEGANVLTTEALLQHLDSALQASRVHVYMYNRQWKLEHLCYKSGELITETGYMDQIIEYLYPCLIITPLDCFWEGAK  240

LQSGTAYLLGKPPLRWTNFDPLEFLEELKKINYQVDSWEEMLNKAEVGHGYMDRPCLNPADPDCPATAPNKNSTKPLDMA  320

LVLNGGCHGLSRKYMHWQEELIVGGTVKNSTGKLVSAHALQTMFQLMTPKQMYEHFKGYEYVSHINWNEDKAAAILEAWQ  400
                                          _________2__________           _________
RTYVEVVHQSVAQNSTQKVLSFTTTTLDDILKSFSDVSVIRVASGYLLMLAYACLTMLRWDCSKSQGAVGLAGVLLVALS  480
_____3______             __________4_________                          _____5______
VAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAFSETGQNKRIPFEDRTGECLKRTGASVALTSISNVTAFF  560
_________        ___________6____________
MAALIPIPALRAFSLQAAVVVVFNFAMVLLIFPAILSMDLYRREDRRLDIFCCFTSPCVSRVIQVEPQAYTDTHDNTRYS  640

PPPPYSSHSFAHETQITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDTLSCQSPESTSSTRDLLSQFSDSSL  720
                                    __________7__________
HCLEPPCTKWTLSSFAEKHYAPFLLKPKAKVVVIFLFLGLLGVSLYGTTRVRDGLDLTDIVPRETREYDFIAAQFKYFSF  800

YNMYIVTQKADYPNIQHLLYDLHRSFSNVKYVMLEENKQLPKMWLHYFRDWLQGLQDAFDSDWETGKIMPNNYKNGSDDG  880

VLAYKLLVQTGSRDKPIDISQLTKQRLVDADGIINPSAFYIYLTAWVSNDPVAYAASQANIRPHRPEWVHDKADYMPETR  960
                                                                       _____8______
LRIPAAEPIEYAQFPPYLNGLRDTSDFVEAIEKVRTICSNYTSLGLSSYPNGYPFLFWEQYIGLRHWLLLFISVVLACTF 1040
________    ___________9___________    __________10_________
LVCAVFLLNPWTAGIIVMVLALMTVELFGMMGLIGIKLSAVPVVILIASVGIGVEFTVHVALAFLTAIGDKNRRAVLALE 1120
 __________11_________            ___________12____________
HMFAPVLDGAVSTLLGVLMLAGSEFDFIVRYFFAVLAILTILGVLNGLVLLPVLLSFFGPYPEVSPANGLNRLPTPSPEP 1200

PPSVVRFAMPPGHTHSGSDSSDSEYSSQTTVSGLSEELRHYEAQQGAGGPAHQVIVEATENPVFAHSTVVHPESRHHPPS 1280

NPRQQPHLDSGSLPPGRQGQQPRRDPPREGLWPPLYRPRRDAFEISTEGHSGPSNRARWGPRGARSHNPRNPASTAMGSS 1360

VPGYCQPITTVTASASVTVAVHPPPVPGPGRNPRGGLCPGYPETDHGLFEDPHVPFHVRCERRDSKVEVIELQDVECEER 1440

PRGSSSN                                                                          1447
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Fig. 1. Predicted amino acid sequence of the human PATCHED protein. Putative transmembrane domains are overlined. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The nucleotide sequence has been deposited in GenBank (accession number U59464).

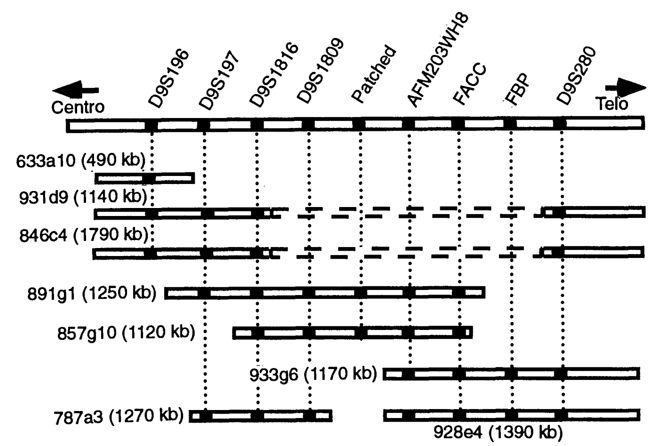

Fig. 2. Mapping of the *PTC* gene on the centromeric portion of a YAC contig spanning the genetically defined BCNS region, D9S197–D9S287. Each locus is shown arbitrarily as equidistant from adjacent loci. YACs are represented as horizontal boxes, and the dashed horizontal lines indicate deletions in Y931d9 and Y846c4, which previously were reported to span D9S197 and D9S280 (*35*). Vertical dotted lines with dark bands indicate the STS content of each YAC. The directions toward the centromere and telomere are indicated. D9S287 is located to the right of the region shown. FACC, Fanconi Anemia Complementation Group C; FBP, Fructose-1,6, Bisphosphatase.

heterozygous deletion of 11 bp that removes nucleotides 2442 to 2452 (TATCCAG-CACT) from the coding sequence. The resulting frameshift truncates the ORF by creating a stop codon nine amino acids after amino acid 813, just after the putative seventh transmembrane domain (Fig. 3, D through F, and Fig. 4). In *Drosophila*, a Ptc protein that is truncated after the sixth transmembrane domain is inactive when ectopically expressed (*24*), in contrast to full-length protein (*7*), suggesting that the human protein is inactivated by the exon 15 alteration. The patient with this mutation is the first affected member of this family, because her parents, age 48 and 50, have had neither BCCs nor other signs of the BCNS. We confirmed the presence of the deletion in the patient and its absence in her parents by restriction enzyme digestion of PCR-amplified DNA. The relevant Mae III band seen in the parents' DNA is diminished by 50% in the patient DNA, and a new band, 11 bp smaller, was seen only in the patient's DNA (Fig. 3D). These results indicate that the alteration in exon 15 arose in the same generation as did the BCNS phenotype. Hence, her disease is the result of a new mutation.

To investigate whether *PTC* is involved in the far more common, sporadic BCCs that are not associated with the BCNS or germline changes, we examined the DNA of 12 BCCs. In one of these tumors, a C to T transition in exon 3 at nucleotide 523 of the coding sequence changes Leu-175 to Phe in the putative first extracellular loop (Figs. 3G and 4). Blood cell DNA from the same individual does not have the alteration, suggesting that it arose somatically in the tumor. This leucine residue is present in all reported Ptc sequences—*Drosophila*, mouse, chicken, and human (*10, 11*). We used SSCP analysis to examine exon 3 DNA from 60 individuals (120 alleles) who do not have the BCNS and found no changes from the normal sequence.

We conclude that human *PTC* is a strong candidate gene for the BCNS. The expression of vertebrate *ptc* genes in developing sclerotome, branchial arches, limbs, and spinal cord (*10, 11*) is consistent with the rib and craniofacial anomalies and the polydactyly, syndactyly, and spina bifida development seen in BCNS. Expression of *ptc* and *hh* in vertebrate skin is consistent with a role in BCC suppression; we have found by reverse transcription and PCR that *PTC* is expressed in adult human skin (*25*). We do not know whether the developmental defects in the BCNS are due to haploinsufficiency or loss of heterozygosity, or both. In addition, we cannot rule out the possibility that some kindreds have a chromosomal deletion that affects not only *PTC* but also adjacent genes that might be responsible for some of the less commonly observed abnormalities.

The existence of sporadic and hereditary forms of BCCs is reminiscent of the characteristics of the two forms of retinoblastoma. The genetic alteration in the sporadic BCC is consistent with previous reports that a substantial fraction of both sporadic and hereditary BCCs have single-copy deletions of chromosome 9q22.3. The limited sensitivity of SSCP, together with the possibility of mutations outside the exons we have examined, may account for the low number of mutations detected to date. In tumors of BCNS patients, the allele deleted is that predicted by linkage to be the normal one, leaving the tumors hemizygous for the mutant allele (*19, 26*). Taken together, these data place the *PTC* gene well in the tradition of other tumor suppressor genes. In *Drosophila*, *ptc* represses a variety of genes and it may act similarly in human skin, a hypothesis consistent with the genetic evidence for its tumor suppressor function. The large body size of BCNS patients also could be due to reduced *PTC* function, perhaps because growth factor gene transcription is derepressed. C to T transitions like the one identified in *PTC* in the sporadic BCC are common genetic changes in the *P53* gene in BCCs and are consistent with the suspected role of sunlight in causing these tumors (*27*). By contrast, the inherited deletion and insertion mutations identified in BCNS patients, as expected, are not those characteristic of ultraviolet light-induced mutagenesis.

The *ptc* gene is the third *Drosophila* segment polarity gene whose vertebrate homologs are implicated in cancer. The mouse mammary oncogene *Wnt1* (*28*) and the human glioblastoma oncogene *GLI* (*29*) are homologs of the *Drosophila* segment polarity genes *wingless* and *Cubitus interruptus* (*Ci*). The *Ci* gene is a component of the Hh-Ptc signal transduction pathway (*30, 31*), and its homolog *GLI1* is expressed in a pattern

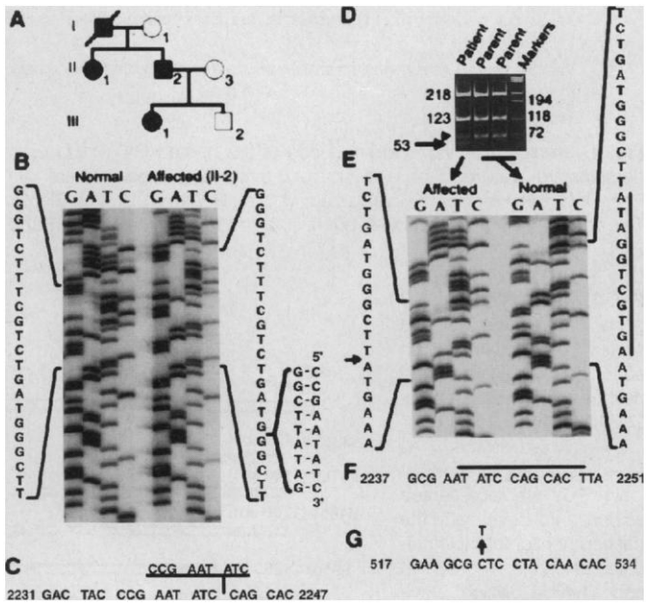

Fig. 3. Mutations in *PTC* in BCNS individuals and a sporadic BCC tumor. (**A** through **C**) Analysis of the BCNS family carrying a 9-bp duplication. (A) Pedigree. (B) Sequence of cloned DNA from an unaffected and an affected individual. The reverse complement of the coding strand is shown and the 9-bp duplication is indicated at right as a double-stranded sequence. (C) The duplication (underlined) shown in context of the coding sequence. (**D** through **F**) Analysis of a newly arising 11-bp deletion in a BCNS patient. (D) Ethidium bromide–stained gel showing a Mae III digestion of PCR-amplified DNA from exon 15. Blood cell DNA from the patient and her two parents was used as templates. The intensity of the smallest band (64 bp) is diminished by 50% (arrowhead) in the patient as compared to that of her parents, and a new band at 53 bp (arrow) is observed. (E) Sequences of cloned exon 15 DNA from the patient and one parent. The vertical line at right indicates the 11-bp sequence present in the parent but not in the patient. (F) The deletion (overlined) shown in context of the coding sequence. (**G**) Analysis of DNA from a sporadic BCC revealed a C to T transition in *PTC* exon 3 that is not observed in blood cell DNA from the same person.

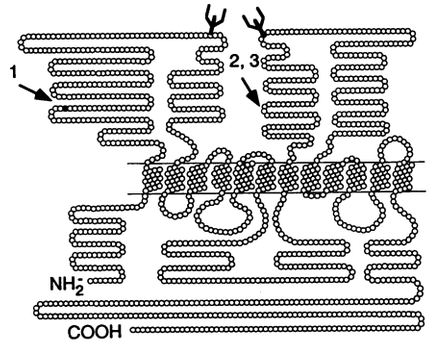

Fig. 4. A schematic model of the PATCHED protein (*10*) indicating the positions affected by mutations in the human gene. In a sporadic BCC, a Leu to Phe change (1) was found in the first putative extracellular loop. In separate BCNS individuals, a three-amino–acid duplication (2) and a frameshift mutation (3) were identified in the second putative extracellular loop.

similar to that of *ptc* in mice (*32*) and may therefore be part of the vertebrate Hh-Ptc pathway. Other classes of developmental regulators also contribute to tumorigenesis. For example, the *extradenticle* and *trithorax* regulators of fly homeotic genes are related to the *PBX1* and *HRX* dominant human leukemia oncogenes, and a homolog of the Mad protein involved in the TGF-β signal transduction pathway in *Drosophila* has been implicated in human pancreatic carcinoma (*33*).

The identification of *PTC* mutations as a cause of BCNS links a large body of developmental genetic information to this important human disease. The *Drosophila ptc* gene plays a key role in controlling where signaling proteins are produced, and one consequence of lost *ptc* function is signal transmission in the wrong cells. This results in changed patterns of growth and also changed cell fates and polarities, such as mirror-image duplications of body segments and appendages. The patterning changes in *Drosophila ptc* mutants are due in part to derepression of another segment polarity gene, *wingless* (*wg*) (*5*), which encodes a *Wnt*-class secreted signaling protein (*34*). In normal embryonic development, *ptc* repression of *wg* is relieved by the Hh signaling protein that emanates from adjacent cells in the posterior part of each segment (*5*, *6*). The resulting localized *wg* expression in each segment primordium organizes the pattern of bristles on the surface of the animal. The consequences of diminished human *ptc* function for development and for tumorigenesis are likely to reflect similarly deregulated signaling proteins.

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16. Plaques (2×10^5) from a human lung cDNA library (HL3022a, Clontech) were hybridized at 60°C with a 1.1-kb Eco RI fragment of mouse *ptc* cDNA, M2 and an additional 6×10^5 plaques were screened in duplicate using 0.4-kb Eco RI and 0.8-kb Xho I fragments of M2. Of the 12 isolated clones, 6 were subcloned into pBSII (Stratagene). To obtain the full coding sequence, we completely sequenced H2 and partially sequenced H14, H20, and H21. MacVector and GCG programs were used for sequence analysis.
17. To perform radiation hybrid mapping of the human *PTC* gene, we first developed oligonucleotide primers and conditions for specifically amplifying a portion of the gene from genomic DNA by PCR. These primers were designated sequence-tagged site (STS) SHGC-8725, and PCR generated a 196-bp product that was observed on agarose gel electrophoresis when human DNA was used as template but not when rodent DNA was used as template. We then scored in duplicate for the presence or absence of the 196-bp product in 83 radiation hybrid DNA samples from the Stanford G3 Radiation Hybrid Panel purchased from Research Genetics, Inc. By comparing the pattern of G3 panel scores with those from a series of Genethon meiotic linkage markers, we determined that no radiation hybrid breaks were observed between *PTC* and D9S287 in the 83 hybrid cell lines. These results indicate that the *PTC* gene lies within 50 to 100 kb of the marker D9S287. Subsequent physical mapping in YAC and BAC clones confirmed that this close linkage estimate was accurate. Detailed map information can be obtained from http://www-shgc.stanford.edu
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