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Advances in Brief

Mutations in the Human Homologue of the Drosophila *patched* Gene in Caucasian and African-American Nevoid Basal Cell Carcinoma Syndrome Patients[1]

Abirami Chidambaram, Alisa M. Goldstein, Mae R. Gailani, Bernard Gerrard, Sherri J. Bale, John J. DiGiovanna, Allen E. Bale, and Michael Dean[2]

Intramural Research Support Program, Scientific Applications International Corp., Frederick [A. C., B. G.] and Human Genetics Section, Laboratory of Genomic Diversity [M. D.], Frederick Cancer Research and Development Center, National Cancer Institute, NIH, Frederick, Maryland 21702; Genetic Epidemiology Branch, National Cancer Institute, NIH, Bethesda, Maryland 20852 [A. M. G.]; Departments of Pediatrics, Dermatology, and Genetics, Yale University School of Medicine, New Haven, Connecticut 06520 [M. R. G., A. E. B.]; and Intramural Research Program, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland 20892-2757 [S. J. B., J. J. D.]

Abstract

The nevoid basal cell carcinoma syndrome (NBCCS), or Gorlin syndrome, is a multisystem autosomal dominant disorder. The salient features of this syndrome include multiple basal cell carcinomas, palmar and/or plantar pits, odontogenic keratocysts, skeletal and developmental anomalies, and ectopic calcification. Other features include such tumors as ovarian fibromas and medulloblastomas. There is extensive interfamilial as well as intrafamilial variability with respect to the manifestation and severity of the phenotype. Alterations in the human homologue (PTCH) of the *Drosophila* segment polarity gene *patched* have been identified in NBCCS patients as well as tumors associated with this syndrome. We report several mutations in this gene in NBCCS patients and present the clinical phenotypes of the individuals in whom these mutations were identified.

Introduction

NBCCS[3] (Online Mendelian Inheritance in Man number 109400), or Gorlin syndrome, is an autosomal dominant disorder characterized primarily by multiple BCCs, developmental and skeletal anomalies, plantar and/or palmar pits, odontogenic keratocysts, and ectopic calcification (1, 2). The NBCCS locus was mapped to human chromosome 9q22.3 by linkage analysis (3–5). Familial as well as sporadic forms of BCCs showed loss of heterozygosity of marker alleles in this genomic region, suggesting that the gene functions as a tumor suppressor (4, 5). Similar loss of heterozygosity is also exhibited by tumors such as ovarian fibromas and medulloblastomas that are less common features in NBCCS (6, 7). The human homologue (*PTCH*) of the *Drosophila* segment polarity gene *patched* (ptc) has now been identified as the gene for NBCCS (8–10). The human *patched* gene consists of at least 23 exons and spans approximately 34 kb (9). We screened 59 unrelated NBCCS patients for mutations in PTCH (23 exons) and have thus far identified deletions, insertions, splice site alterations, and nonsense and missense mutations distributed throughout the gene. Mutations have been detected in both Caucasian and African-American NBCCS families and appear to be unique to the families screened.

Materials and Methods

Patient DNA samples used in this study were obtained from the National Cancer Institute (Bethesda, MD) and New Haven, CT, NBCCS collections. Cell lines from NBCCS patients deposited in the NIGMS human genetic mutant cell repository were also utilized in this analysis. The patients were examined by medical geneticists and other specialists. Criteria for diagnosis of NBCCS (Gorlin syndrome) included the presence of at least two major features of the syndrome such as multiple BCCs or onset of one BCC before the age of 20, pits of palms and/or soles, jaw cysts, calcification of the falx cerebri, and having a first-degree relative with NBCCS. Tumor DNA was obtained from patients when available.

Primers were designed (Table 1) to amplify entire exons to screen the coding regions of the gene. Patient DNA samples (100 ng) were screened for mutations occurring within exons by radiolabeled ([α-^{32}P]dCTP) PCR amplification followed by simultaneous SSCP and heteroduplex analysis (11–13). DNA samples were amplified in PCR buffer (0.6 mM dNTPs; 1.5 mM $MgCl_2$ final concentration) and [α-^{32}P]dCTP for 35 cycles (94°C for 30 s, 57°C for 30 s, and 72°C for 30 s). Amplified products were diluted 1:3 in stop solution, denatured for 8 min at 94°C, and chilled briefly on ice before loading onto 8% acrylamide:Bis gel (2.6% cross-linking) in 1× Tris-borate EDTA buffer. Gels were run at 70 W for 2–3 h at 4°C and then dried and exposed to X-ray film for 4–12 h. Heteroduplexes were identified from the double-stranded DNA at the bottom of the gels, and SSCPs were identified from the upper single-stranded region. PCR products with SSCP or heteroduplex variants were analyzed further by treatment with shrimp alkaline phosphatase and exonuclease (United States Biochemical Corp.) and cycle sequenced with AmpliTaq FS (Perkin-Elmer Corp.). The sequence was analyzed on an Applied Biosystems model 373A DNA sequencer.

Results and Discussion

A PCR-based screening strategy was used for rapid, large-scale identification of variants that were then characterized by sequence analysis and restriction endonuclease digests where appropriate as described in the previous section. Besides the three mutations described previously (9), we found eight additional mutations in eight exons. Table 2 shows these results (four insertions, four substitutions, one deletion, one deletion + substitution, and one splice site alteration, each unique to the DNA sample in which it was identified) and the associated phenotypes in patients for whom confirmed clinical data were available. Although several of these mutations occurred in highly conserved regions of the gene (Fig. 1), the mutations identified thus far appear to be distributed randomly throughout the gene.

The predominant type of mutation that we have characterized disrupts the coding region with small insertions or substitutions. One putative splice site variant replaces the G at the +1 position of the splice donor site of exon 16. Alterations in the gene that would not predict truncation of the protein product included a missense mutation

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[2] To whom requests for reprints should be addressed, at Frederick Cancer Research and Development Center, Building 560, Room 21-18, National Cancer Institute, NIH, Frederick, MD 21702-1201. E-mail: dean@ncifcrf.gov.

[3] The abbreviations used are: NBCCS, nevoid basal cell carcinoma syndrome; BCC, basal cell carcinoma; *PTCH*, human homologue of *Drosophila patched* gene; SSCP, single-strand conformation polymorphism; NIGMS, National Institute of General Medical Science.

Table 1 *PTCH gene primers*

Exon[a]	Size product	Primers				
9	230	PTCF23	GTGCT	GTCGA	GGCTT	GTG
		PTC1204R	AGAAG	CAGGA	GCAGT	CATGG
10	250	PTC1336F	TTCGG	CTTTT	GTTCT	GTGC
		PTC1336R	CCGGT	GGCAT	TTGTC	AAC
21	240	PTCX21F	CAGGT	AAATG	GACAA	GAAC
		PTCX21R	CTGTG	TGATG	TGCTG	CTC
22	227	PTCF10	TCTAA	CCCAC	CCTCA	CCCTT
		PTC31R	ATTGT	TAGGG	CCAGA	ATGCC
	265	PTCF35	TTCTG	CCTCC	GTGAC	TGTC
		PTCR35-2	CTCTA	GGTCC	CTTGG	CTGC

[a] Sequence is from GenBank U43148. Primers used for the amplification of regions of the *PTCH* gene not covered previously (9). Exons 9 and 10 are amplified by separate primers; PTCX21F and -R amplify all of exon 21, and two primer pairs are used to amplify exon 22.

Table 2 *PTCH mutations identified in NBCCS patients*

Pedigree	Source[a]	Exon[b]	Mutation[c]	Ethnicity[d]	No. affected	Phenotype[e]
NBCCS1[f]	F	2	269insT	C	(Proband)[g]	(−)
NBCCS2[h]	F	2	277AA→C	C	(Proband)[g]	B
NBCCS3	S	6	853insC	C	1	(−)
NBCCS4[i]	F	8	C1081T (Q365X)	A	7	B3, C7, R2, P7
NBCCS5	F	11	G1513 (G509R)	A	2	B1, M1, C2, P1
NBCCS6	F	11	G1514T (G509V)	C	3	(−)
NBCCS7[i]	S	13	2000insC	C	1	B, M, R, P
NBCCS8[i]	F	13	2047insCT	C	(Proband)[g]	(−)
NBCCS9	F	14	2434del3 (ΔQ815)	C	8	B7, C7, P8
NBCCS10	S	16	2875+1G→C	C	1	B, C, P
NBCCS11	F	19	C3383A (S1132Y)	C	8	B7, C3, P4

[a] S, nonfamilial/new mutation; F, familial.
[b] Exons are numbered as in Hahn *et al.* (9).
[c] Mutations denote nucleotide alterations; corresponding amino acid substitutions (where relevant) are shown in parentheses.
[d] Ethnicity: C, Caucasian; A, African-American.
[e] Phenotype: (−), NBCCS patients diagnosed according to criteria outlined in "Materials and Methods," for whom detailed clinical information was unavailable. B, BCC; C, odontogenic keratocytes; R, rib anomalies; P, palmar and/or plantar pits; M, medulloblastoma. Numbers denote the number of NBCCS patients in that family with these clinical features.
[f] NIGMS cell line GM02139B.
[g] Information available only on proband.
[h] NIGMS cell line GM01656.
[i] Mutations reported previously (9).

in codon 509 of kindreds NBCCS 5 and 6 and a 3-bp deletion, ΔQ815 (deletion of glutamine residue), in kindred NBCCS 9. None of these alterations were found in 72 unrelated normal Caucasian chromosomes (data not shown). In kindred NBCCS 11, a S1132Y (serine-to-tyrosine substitution) mutation was observed in all of the eight affected family members but in none of the unaffected individuals. Additional genetic and functional data should provide insights into the mechanisms by which these alterations disrupt the function of the *PTCH* gene.

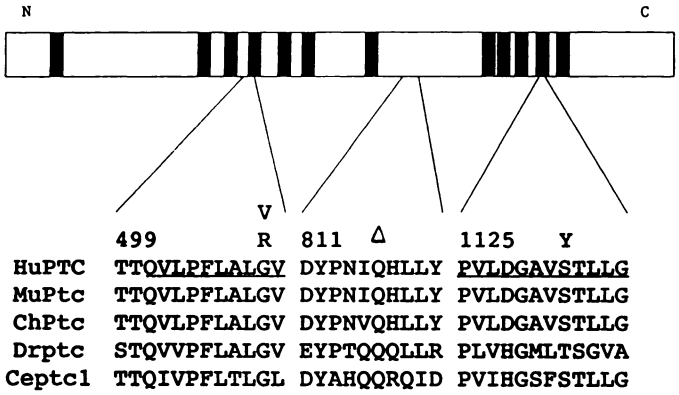

Fig. 1. Schematic representation of the *patched* gene protein showing PTCH mutations in conserved regions of the gene. *N*, amino terminal; *C*, carboxyl terminal. *HuPTC*, human PTCH; *MuPtc*, mouse PTCH; *ChPtc*, chicken PTCH. *patched* sequences to show identity: *Drptc, Drosophila; Ceptc1, Caenorhabditis elegans. Numbers* indicate codon/amino acid positions. *Underlined sequences*, regions of the human gene predicted to encode a transmembrane domain.

The previously reported 2000insC mutation (9) was identified in an 8-year-old female patient with severe clinical manifestations of the disorder, including multiple BCCs, hypertelorism, and bifid ribs. She was ascertained at the age of 5 years because of a medulloblastoma and is presumed to be a new mutation, because her parents are not symptomatic for the disease. This patient also has mild mental retardation. Although mental retardation is not observed commonly in NBCCS, previous studies suggested that contiguous genes might be responsible for this feature (14). The observations that PTCH is expressed in both fetal and adult brain tissue and that dysgenesis of the corpus callosum is observed in many NBCCS patients (15) suggest that this gene may play a critical role in the development of the nervous system. Although malformations of the corpus callosum themselves are not usually associated with retardation, other related variations in brain morphology or biochemistry might be correlated with abnormal cerebral function. The functional role of the 2000insC mutation and this region of the gene in normal developmental processes and its possible contribution to clinical features marginally associated with NBCCS remains to be assessed.

Two different missense alterations within the same codon, G509V

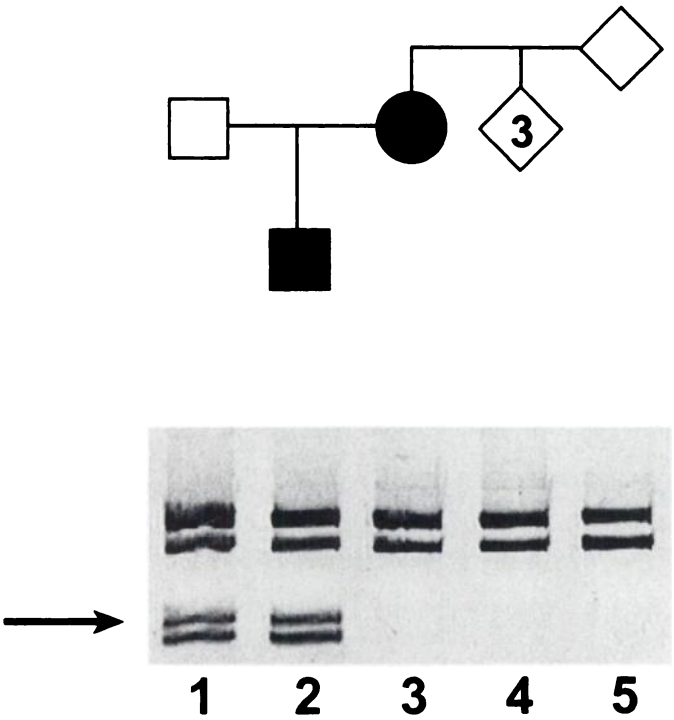

Fig. 2. *EarI* restriction endonuclease digestion of PCR-amplified products using PTCH primers F5 and R5 (9) in an African-American pedigree demonstrating transmission of the *G509R* allele from the unaffected parent (*1*) to the affected child (*2*). Affected parent (*3*) and child (*2*) are represented as *filled symbols*. Restriction enzyme digestion data on four other family members, three unaffected siblings (*4*) and an unaffected parent (*5*) of the affected parent (*3*), are also shown.

and G509R were identified in a Caucasian and an African-American family, respectively. In the Caucasian family, segregation of G509V was concordant with disease status. The proband, an affected parent, and an affected sibling had this mutation, whereas the unaffected individuals in this family did not. In the African-American family, however, G509R was shared by the proband and his unaffected parent (father; Fig. 2). This alteration was not present in any of 84 unrelated, normal African-American chromosomes (data not shown). DNA extracted from a medulloblastoma sample from the proband demonstrated loss of the "normal" (wild-type) paternal allele but retention of the maternal (affected) allele. This is consistent with the two-hit model of neoplastic transformation wherein both copies of the normal allele are lost by one of several mechanisms that include loss of the wild-type allele and retention of the mutant allele as observed in this case (16). It is interesting to note that the proband displays a more severe phenotype than his mother, whose mutation remains to be identified (17). It is possible that the paternal G509R allele contributes to severity of disease in the proband. It is also likely that this amino acid substitution is neutral with respect to the functional aspect of the protein and therefore exerts no effect on the clinical phenotype seen in this patient. Given the considerable intrafamilial variability seen in NBCCS kindreds (15, 18) with regard to the spectrum of clinical manifestations and their severity, other genetic and/or environmental factors may contribute to the disease phenotype in this patient.

In this study, we have identified and characterized eight new PTCH mutations in NBCCS, each unique to the patient/kindred analyzed. We have also presented all available clinical data on the patients in whose DNA these mutations were found. Considering the complexity and variability of the NBCCS phenotype, molecular analysis of mutations will undoubtedly provide valuable clues regarding basic mechanisms contributing to the clinical phenotype. These clinical and molecular features will, however, have to be assessed in conjunction with other modifying factors such as ethnicity, genetic background of the patients, and interaction with environmental factors such as exposure to UV and X radiation. Combined data from many sources to evaluate genotype-phenotype correlations and gene-environment interactions will help elucidate the factors that confer predisposition to NBCCS and the role of the PTCH mutations underlying this disorder.

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