Absence of *PTEN* Germ-Line Mutations in Men with a Potential Inherited Predisposition to Prostate Cancer[1]

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ABSTRACT

Epidemiological studies have demonstrated that men with a family history of prostate cancer are at an increased risk for this disease. This important observation has led a number of research teams, including our own, to collect DNA samples and clinical data from prostate cancer families, with the goal of localizing and characterizing prostate cancer susceptibility genes. The candidate tumor suppressor gene *PTEN* (also called *MMAC1*) has recently been shown to be somatically altered in several common malignancies, including cancers of the brain, kidney, skin, thyroid, endometrium, breast, and prostate. Germ-line mutations in this gene, which maps to chromosome 10q23, have been associated with Cowden disease, an autosomal dominant cancer predisposition syndrome that is characterized by multiple hamartomas. Although prostate cancer is not typically associated with Cowden disease, previous studies of sporadic prostate cancers demonstrate loss of heterozygosity at 10q23 loci in ~25% of cases. We, therefore, hypothesized that germ-line mutations in the *PTEN* gene may predispose to prostate cancer in a subset of families, particularly those in which cancers of the breast, kidney, and/or thyroid also segregate. To test this hypothesis, DNA was isolated from whole blood of 11 prostate cancer patients from 10 unrelated families. Four of the 10 families met the previously established clinical criteria for hereditary prostate cancer. Eight of the 11 men had at least one second primary malignancy, including cases of neuroendocrine cancer, glioblastoma multiforme, melanoma, kidney, and thyroid cancer. Although we identified some common as well as some unique polymorphisms, no nonsense or missense mutations were identified in any of the 11 samples. To further examine the possibility that *PTEN* mutations contribute to prostate cancer predisposition, we also studied the probands from each of 10 families with early-onset and/or multiple individuals with prostate cancer. Sequence analysis of the *PTEN* gene in these 10 men also revealed no mutations or novel polymorphisms. We conclude that germ-line mutations in the *PTEN* are unlikely to contribute in a significant way to the inherited predisposition to prostate cancer.

INTRODUCTION

The recognition that prostate cancer frequently clusters within families has led to the search for prostate cancer predisposition genes. Although many laboratories, including our own, have performed linkage studies using high-density prostate cancer families to identify associated genetic loci, these methods are complicated by the relatively late onset as well as the high phenocopy rate of this disease. Despite these inherent obstacles, three genetic regions have recently been reported to harbor prostate cancer susceptibility genes, namely 1q24–25 (*HPC1*; Refs. 1–3), 1q42–43 (4), and Xq26–28 (5). A second strategy that may be used to identify prostate cancer susceptibility loci is a candidate gene approach. One advantage of this approach is that high-density families are not required, and even single individuals can be analyzed. The *PTEN* gene (also called *MMAC1*) was identified independently by two research teams (6, 7) using positional cloning techniques. The *PTEN* gene encodes a 403-amino acid protein product that is a dual-specificity protein phosphatase. The genetic region that contains *PTEN*, namely, 10q23.3, is deleted in a large number of common cancers, including prostate cancer. Mutations in the *PTEN* gene are associated with CD[3] (OMIM 158350), an autosomal dominant genetic syndrome that is characterized by multiple hamartomas as well as an increased risk of breast, renal, thyroid, endometrial, and neuroendocrine malignancies (8). Recent studies of prostate cancer specimens have also shown that biallelic inactivation of the *PTEN* gene may occur in a small subset of sporadic prostate cancers (9–14). Because germ-line mutations in the *PTEN* gene predispose to multiple histologically distinct forms of cancer and because somatic inactivation of this gene occurs in sporadic prostate cancer, we speculated that inherited mutations in this gene may contribute to early-onset autosomal dominant prostate cancer predisposition. We elected to directly

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[3] The abbreviations used are: CD, Cowden disease; HPC, hereditary prostate cancer.

Table 1 Clinical description of prostate cancer patients and their families[a]

Clinical features of the 11 prostate cancer patients selected for examination of the *PTEN* gene are illustrated in the left side of the table. Individuals 9 and 10 are brothers. The clinical data include age at diagnosis in years, Gleason score of the primary tumor, prediagnosis serum prostate-specific antigen values, and other primary sites of cancer. All primary malignancies in the 11 men were confirmed by review of pathology records, unless otherwise indicated. Family history, including number of prostate cancer cases in the family, whether or not the family met the proposed clinical definition for HPC (15), and other primary sites of cancer reported to occur in first- and/or second-degree family members, is displayed on the right.

	Prostate cancer characteristics			Personal history of cancer	Family history of cancer		
Patient no.	Age at diagnosis (yr)	Gleason score	Prediagnosis PSA (ng/ml)	Other primary cancer(s)	No. of PC cases	HPC	Other cancer(s) in family
1	49	7	2467	Neuroendocrine tumor, age 51	2	No	Lung, gynecologic (NOS)
2	58	6	3.1	No	4	Yes	Testes, bladder
3	72	6	9.4	No	4	Yes	Lung, medullary thyroid cancer
4	57	7	8.1	No	4	Yes	Brain
5	47	7	5.9	Melanoma, age 40 Glioblastoma multiforme, age 48	1	No	Gynecologic (NOS)
6	66	5	7.2	Melanoma,[b] age 55	2	No	Stomach, bladder
7	64	7	4.4	Kidney, age 65	2	No	Liver
8	69	7	22.1	Basal cell carcinomas,[b] ages 55 and 66	2	No	Melanoma
9	62	6	7.3	Melanoma	3	Yes	Kidney, melanoma, pancreas
10	69	7	19	Squamous cell carcinoma (skin), age 67 Basal cell carcinoma, age 67 High-grade sarcoma, age 69			
11	55	6	5.7	Papillary thyroid cancer,[b] age 44 Kidney, age 57	4	No	Bladder, kidney, colon, breast

[a] PSA, prostate-specific antigen; PC, prostate cancer; NOS, not otherwise specified.
[b] Pathology records not available to confirm diagnosis.

analyze the gene for mutations in a subset of prostate cancer patients with early-onset disease and/or a personal/family history of other cancers, especially those cancers previously associated with *PTEN* mutations, because we estimated that the contribution of this gene to HPC may be small and difficult to detect through traditional linkage studies.

MATERIALS AND METHODS

Study Population. All subjects described here are participants in the University of Michigan Prostate Cancer Genetics Project. The overall goal of this project is to define the molecular basis of HPC. The selection criteria for enrollment in this study included: (*a*) families that fulfilled one or more of the proposed clinical criteria for the diagnosis of HPC [three or more affected individuals within one nuclear family; affected individuals occurring in three successive generations (maternal or paternal lineage); or a cluster of two or more relatives each affected before the age of 55 years (15)]; (*b*) families with two or more living men affected with prostate cancer; and (*c*) men affected with prostate cancer prior to the age of 55 years. Research protocols and consent forms were approved by the Institutional Review Board at the University of Michigan. All cases of prostate cancer and, when possible, other primary cancers were confirmed by review of pathology records from the 11 men. Family history was provided by study participants for pedigree analysis. DNA was extracted from whole blood using commercial reagents (Puregene kit; Gentra Systems, Plymouth, MN).

Mutation Screening. Nested PCR amplifications were performed using genomic DNA, and the resulting amplicons were screened for sequence variants, as described previously (7), with the following modifications. (*a*) Exon 6 was screened as a single secondary amplicon amplified using the exon 6 FB-RR primer pair. (*b*) Exon 8 was screened as two secondary amplicons using the following FB-RQ and FC-RR primers: CA6.HB (5′-GTTTTCCCAGTCACGACGAGGTGACAGATTTTCTTTTTTA-3′), CA6.HQ (5′-AGGAAACAGCTATGACCATTCGGTTGGCTTTGTCTTTA-3′), CA6.HC (5′-GTTTTCCCAGTCACGACGCATTTGCAGTATAGAGCGT-3′), and CA6.HR (5′-AGGAAACAGCTATGACCATAGCTGTACTCCTAGAATTA-3′). (*c*) Because mononucleotide runs in certain introns caused poor dye-primer sequencing, we obtained dye-terminator sequence data on secondary amplicons exon 8 FB-RQ and exon 9 FB-RR using the nested primers 5′-TTTTTTTTTAGGACAAAATGTTTC-3′ and 5′-AATTCAGACTTTTGTAATTTGTG-3′. Any sequence variants were confirmed by analysis of an independently amplified PCR product. Approximately 95% double-stranded coverage of the *PTEN* coding sequence was achieved using this strategy.

RESULTS AND DISCUSSION

Clinical information describing the 11 patients and their families is provided in Table 1. Patients 9 and 10 are brothers. The average age of prostate cancer diagnosis in the 11 men reported here was 60.7 years (range, 47–72 years). Six of the cases of prostate cancer were assigned a Gleason score of 7; the remaining five cases were Gleason score 5 or 6 tumors. Ten of the 11 men had a serum prostate-specific antigen value of <20 ng/μl at the time of diagnosis of prostate cancer. Patient 1 had an extraordinarily high serum prostate-specific antigen value (2457 ng/μl) and was found to have widespread metastatic disease at presentation. Eight of the 11 men had at least one

Table 2 Summary of sequencing of the *PTEN* gene in 11 patients with prostate cancer

The results of sequence analysis of the *PTEN* gene in the 11 patients are presented. All changes are intronic except for the silent mutation, 132C→T, observed in patient 9.

Patient no.	Summary of *PTEN* sequencing
1	IVS1-68A→G
2	IVS1-68A→G
3	IVS1-68A→G
4	
5	IVS1-68A→G
6	IVS1-68A→G
7	IVS1-68A→G
8	IVS4-29, insT
9	132C→T exon 2 (silent)
10	IVS1-68A→G
11	IVS1-68A→G

Table 3 Description of clinical features of families with early-onset and/or multiple cases of prostate cancer

The probands (patients 12–21) from 10 prostate cancer families were studied for potential germ-line mutations in the *PTEN* gene. The diagnosis of prostate cancer was confirmed by review of medical records when possible. Deceased men were also coded as affected if the diagnosis of prostate cancer could be verified independently by two family members. The average age of prostate cancer diagnosis within a family was determined only from those men who provided a blood sample for DNA analysis.

Patient no.	Age at prostate cancer diagnosis (yr)	Total no. of affected men in family	No. of affected men available for genotyping	Average age of prostate cancer diagnosis in family (yr)
12	60	7	4	60.3
13	52	5	5	60.4
14	43	3	3	53.0
15	68	3	3	61.3
16	50	4	4	58.8
17	65	8	4	62.5
18	60	3	3	58.7
19	60	4	3	66.7
20	66	4	3	70.0
21	71	6	4	69.3

second primary malignancy, including: kidney, thyroid, and skin cancer (both basal cell and squamous cell histology); melanoma; sarcoma; glioblastoma multiforme; and neuroendocrine tumor of the lung.

All individuals except patient 5 reported that at least one family member had been previously diagnosed with prostate cancer. The average number of cases of prostate cancer in first- and/or second-degree relatives was 2.8 (range, 1–4). Eleven of 18 cases of prostate cancer in family members were confirmed by review of pathology records. Four of the 10 families were classified as HPC families using the proposed clinical definitions for HPC described above (15). Other primary malignancies reported to occur in first- and/or second-degree family members include cancer of the lung, testes, bladder, thyroid, brain, stomach, liver, kidney, pancreas, colon, and breast as well as melanoma.

All nine exons of the *PTEN* gene were screened for mutation in the 11 men with prostate cancer from 10 unrelated families. Patient 9 was noted to have a silent mutation in codon 44 of the *PTEN* gene; a C→T transition was observed at position 132, which does not result in an amino acid substitution (Table 2). A common intron 1 polymorphism was observed in 8 of 11 patients. Patient 8 was discovered to have a novel intronic polymorphism (IVS4–29, insT). Using allele-specific oligonucleotide hybridization, we did not find this allele among 60 control alleles (data not shown). However, because this polymorphism is not in a splice site, it is unlikely to have functional consequences. No missense or nonsense germ-line mutations were discovered in any of the 11 prostate cancer patients.

To further investigate whether mutations in the *PTEN* gene predispose to prostate cancer, we studied the probands from an additional 10 families with early-onset and/or multiple cases of prostate cancer (Table 3). All families except that of individual 18 met at least one of the criteria for HPC (15). Individuals 12 and 17 are African-American; all other probands are Caucasian. DNA from each proband was screened for mutations in the *PTEN* gene, as described above. No germ-line mutations or novel polymorphisms were identified in any of the probands from these 10 prostate cancer families.

The molecular basis of HPC remains elusive. There are a variety of factors that make identification of prostate cancer susceptibility genes a particularly formidable task. First, prostate cancer is a late-onset disease. Therefore, it is often difficult to obtain accurate medical information and/or DNA specimens from individuals in vertical generations. Furthermore, at this time, there are no definitive clinical or pathological criteria that allow one to differentiate between the inherited and sporadic forms of the disease (16). Prostate cancer is also extremely common in the general population, with a one in five lifetime probability that a man will develop clinically detectable prostate cancer (17). This high phenocopy rate significantly complicates prostate cancer linkage studies.

The candidate gene approach is an effective strategy to complement linkage studies. The *PTEN* gene is a logical candidate gene for HPC for several reasons. First, germ-line mutations at this locus are associated with at least two genetic syndromes, namely CD (OMIM 158350) and Bannayan-Ruvalcaba-Riley syndrome (OMIM 153480). Hamartomas are a common feature of both syndromes, whereas cancer of the breast and/or thyroid gland and neuroendocrine cancers of the skin are typically seen in CD. Although prostate cancer is not generally considered to be a feature of CD, there is tantalizing biological data implicating the *PTEN* gene in prostate carcinogenesis. Homozygous inactivation of the mouse *Pten* gene by homologous recombination results in early embryonic lethality (18). Mice that are heterozygous for *Pten* inactivation (*Pten* +/−) have been shown to develop prostatic hyperplasia and dysplasia, although progression to prostate cancer has not been observed. This is perhaps due to premature death of these mice from other malignancies.

Chromosome 10q23–25 is frequently deleted in sporadic prostate cancer (19, 20). It has been hypothesized that the *MXI* gene at 10q24–25 is the tumor suppressor gene that is targeted by these observed deletions (21). This gene is a member of the helix-loop-helix-leucine zipper family that alters the transcrip-

tional activity of Myc. Additional studies in an expanded set of prostate cancer specimens, however, failed to confirm a significant frequency of *MXI1* mutations in sporadic prostate cancer (22, 23). When the *PTEN* gene was identified, a number of research groups studied both prostate cancer cell lines and human tissue samples to determine the frequency of inactivation of this gene via mutation and/or deletion. The LNCaP cell line has been observed to have a frameshift mutation, and the DU145 cell line has a missense mutation at amino acid 132 resulting in a substitution of Leu for Met (6, 7). Studies of an additional 4 prostate cancer cell lines and 11 xenografts reported by Vlietstra *et al.* (12) also demonstrated a significant frequency of *PTEN* mutation and/or deletions (60%). The frequency of biallelic inactivation of *PTEN* in primary prostate cancer samples has been reported to be in the range of 10–20% (10–14). For example, Cairns *et al.* (9) reported allelic loss at 10q23 in 11 of 60 prostate specimens obtained from radical prostatectomy cases and 12 of 20 pelvic lymph node metastases. A second inactivation event was observed in 10 of 23 cancers. Because 7 of these 10 cases were observed to be from individuals with regional lymph node metastases at the time of diagnosis, *PTEN* inactivation may be more common in locally advanced and/or metastatic prostate cancers.

The *PTEN* gene has also been reported to be altered in several other types of cancer including cancers that are a part of CD as well as those tumors with LOH at 10q23–24. Somatic hemizygous deletions at 10q22–23 have been observed in a subset of benign and malignant thyroid tumors without inactivation of the second allele (24). Rhei *et al.* (25) examined the frequency of germ-line and somatic *PTEN* mutations in 54 cases of breast cancer. Although a number of polymorphisms and missense mutations of unknown function were found, only one germ-line and one somatic mutation were observed (25). Several studies of brain tumors have described that, although 10q23 LOH occurs in meningiomas and gliomas of all grades, somatic mutation of the nondeleted *PTEN* allele is uniquely observed in a significant percentage of high-grade gliomas (25–40%; Refs. 18, 26, and 27). Similarly, somatic *PTEN* mutations have been noted in ~30–50% of endometrial carcinomas but not in other types of gynecological cancer (28, 29). Finally, disruption of the *PTEN* has been found in 43% of melanoma cell lines, although studies of clinical specimens have not been reported (30). Taken together, these studies suggest that, although germ-line *PTEN* mutations result in CD, somatic mutation/deletion of this tumor suppressor gene contributes to the development of specific histological types of cancer, particularly high-grade brain tumors and endometrial cancer. The fact that these cancers are not typically observed in individuals with germ-line mutations is puzzling.

Given the constellation of tumors that have been associated with *PTEN* alterations, we anticipated that one or more of the individuals that we analyzed would have a germ-line mutation in this gene. For example, patient 5 reported a personal history of melanoma, early-onset prostate cancer and glioblastoma multiforme, all tumors shown to have a significant frequency of somatic *PTEN* alterations. Of note, no *p16* gene mutations were detected in the constitutional DNA isolated from patient 5 (data not shown). Mutations in *p16* have been associated with some cases of familial melanoma (31). There is a significant possibility that individuals with multiple primary tumors occurring at a relatively young age, regardless of family history, may carry a germ-line mutation in a cancer predisposition gene. Candidate gene analysis is the optimal way to test the hypothesis in this type of individual because the lack of affected family members precludes formal linkage analysis.

In conclusion, our study of prostate cancer patients with personal and/or family history of prostate and other cancers failed to reveal germ-line *PTEN* mutations. Our data, therefore, suggest that the *PTEN* gene is unlikely to play a significant role in the inherited predisposition to prostate cancer. Because hamartomas are a hallmark of syndromes associated with germ-line *PTEN* mutations, future studies may attempt to identify individuals with hamartomas and prostate cancer to identify a subset of prostate cancer families that may be associated with *PTEN* mutations.

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