Analysis of Polymorphic Patterns in Candidate Genes in Israeli Patients with Prostate Cancer

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Abstract

Background: The specific genes involved in conferring prostate cancer risk in sporadic and familial cases are not fully known.

Objectives: To evaluate the genetic profile within several candidate genes of unselected prostate cancer cases and to correlate this profile with disease parameters.

Methods: Jewish Israeli prostate cancer patients (n = 224) were genotyped for polymorphisms within candidate genes: p53, ER, VDR, GSTT1, CYP1A1, GSTP1, GSTM1, EPHX and HPC2/ELAC2, followed by analysis of the genotype with relevant clinical and pathologic parameters.

Results: The EPHX gene His113 allele was detected in 21.4% (33/154) of patients whom disease was diagnosed above 61 years, compared with 5.7% (4/70) in earlier onset disease (P < 0.001). Within the group of late-onset disease, the same allele was noted in 5.5% (2/36) with grade I tumors compared with 18% (34/188) with grade II and up (P = 0.004). All other tested polymorphisms were not associated with a distinct clinical or pathologic feature in a statistically significant manner.

Conclusions: In Israeli prostate cancer patients, the EPHX His113 allele is seemingly associated with a more advanced, late-onset disease. These preliminary data need to be confirmed by a larger and more ethnically diverse study.

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Prostate cancer is the most common solid tumor diagnosed and the second leading cause of cancer-related death among American men, with 175,000 new cases diagnosed annually in the USA [1]. The worldwide estimate is 239,000 prostate cancer-related deaths per year. The majority of prostate cancer cases occur sporadically, most commonly in the seventh decade of life. In about 10% of prostate cancer cases familial clustering occurs, clinically heralded by an earlier age at onset (i.e., below age 60 years). These familial cases usually exhibit an autosomal dominant mode of transmission and are putatively attributable to germline mutation(s) in major cancer susceptibility gene(s) [2]. Yet, the precise genetic factors associated with inherited predisposition to prostate cancer have not yet been fully elucidated. A small subset of inherited prostate cancer cases segregate with a locus on chromosome 1 (1q24-q25)-HPC1 locus; and recently, germline mutations and polymorphisms within a candidate gene in that region (RNASEL) were detected in two families with two or more prostate cancer cases [3]. Several potentially important chromosomal regions have been associated with early and late-onset familial prostate cancer: a region proximal to the site of HPC1 locus on chromosome 1 [4], CAPB [5], as well as two missense mutations (Leu127 and Thr541) in the HPC2/ELAC2 gene [6].

Genetic factors may also be involved in sporadic disease. These genes presumably confer a mild or moderate prostate cancer susceptibility, and the inheritance pattern is compatible with a multifactorial, multifactorial inheritance pattern [7]. The precise genes involved in conferring prostate cancer risk in non-familial cases are currently unknown, but several have been suggested and tested as candidate genes. These include genes that are somatically involved in disease pathogenesis (e.g., p53), genes involved in prostate tumorigenesis based on theoretical considerations (e.g., estrogen receptor), and genes whose protein products affect the metabolism and detoxification of environmental carcinogens. Sequence alterations, in particular missense mutations, within some of the relevant genes have been tested for an association with prostate cancer risk [7] and for a less favorable prognosis in affected individuals [8].

A polymorphism of the CYP17 gene, a member of the cytochrome p450 gene family responsible for biosynthesis of testosterone, was reportedly associated with prostate cancer risk in Caucasians with a family history of the disease [9]. Similarly, the ValVal polymorphism in the CYP1A1, and the Leu432Val polymorphism in CYP1B1, both members of the cytochrome p450 gene...
family, have also been associated with prostate cancer risk in ethnically diverse populations [10].

Functional polymorphisms within genes whose products promote detoxification of potentially carcinogenic substances, in particular the GST superfamily, have been tested for association with prostate cancer risk. The 1105V GSTP1 gene polymorphism, but not polymorphisms within other GST supergene family members, was associated with early-onset prostate cancer [11].

The role that $p53$ mutations play in the pathogenesis of the disease are well established, and the finding that somatic overexpression, taken as an indication for the presence of a mutant allele, was associated with clinical failure [12], may serve to further support its pivotal role in predisposition to prostate cancer. Indeed, a missense mutation at codon 72 (R72P) of the $p53$ gene was reported to be associated with a reduction of risk to prostate cancer in carriers of the codon 72 pro/pro alleles [13].

A homozygous pattern of a missense mutation (His13) within the microsomal epoxide hydrolase (mEPHX) gene was expressed somatically in more than 90% of prostate cancer tissue analyzed [14]. The more active form of the enzyme (Tyr13) is associated with increased risk of ovarian cancer [15], but this polymorphism was never tested in prostate cancer risk. Of three neutral polymorphisms in the vitamin D receptor (VDR) gene, one was reportedly associated with an increased risk of developing prostate cancer [16], whereas no association with prostate cancer risk was reported in other studies [17].

To gain insight into the possible contribution of some of these polymorphisms and the two HPC2 missense mutations to prostate cancer predisposition and pathogenesis in Israeli patients, we genotyped unselected Jewish Israeli prostate cancer patients for polymorphisms within some of these genes, and correlated the resulting genotype with clinical and histopathologic parameters.

**Materials and Methods**

**Study population**

The study population comprised unselected Jewish Israeli men with pathologically confirmed prostate cancer, who were treated at one of the three participating medical centers between January 1998 and June 2000. Demographic and relevant clinical data were obtained from medical files and a detailed questionnaire that was completed during a personal or telephone interview. The Institutional Review Boards approved the study, and a written informed consent was obtained from each patient. Cases with at least one additional first-degree relative with prostate cancer or other seemingly associated cancer types (breast, ovary) were designated familial. All others were considered sporadic.

**Control population**

The control population used to assess the rate of the HPC/ELAC2 missense mutations in an unaffected population included ethnically and age-matched individuals who were recruited from among consultees at the Genetics Institute of Sheba Medical Center and from patients with non-cancer related problems who attended the hospital's Urology outpatient clinic. Their medical status (i.e., healthy with normal prostate-specific antigen levels and no suspicious mass on digital rectal examination) was ascertained by a personal interview, physical examination and, in cases of doubt, by contact with their treating physician.

**DNA extraction**

DNA was extracted from peripheral blood leukocytes obtained by venepuncture using standard procedures, and using the Puregene Gentra kit (Gentra Systems Inc., Minneapolis, USA) according to the manufacturer's recommended protocol.

**Polymerase chain reaction amplifications**

PCR amplifications were carried out in a thermocycler (PTC-100-60, M.I. Research Inc., Watertown, MA, USA), in a final reaction volume of 50 μl, containing 15 pmol of each primer, 50–100 nanograms of genomic DNA, 200 μM dNTPs, 0.5 units of thermostable Taq DNA polymerase (BioTaq, Appligene, France) and standard 10x PCR buffer. Following PCR, 10% of the PCR product (5 μl) was analyzed on 2% agarose gels to ensure success and specificity of the PCR and visualized by ultraviolet transillumination of the ethidium bromide stained gels.

The primer sequences, PCR amplification conditions, and the detection techniques for the $p53$ (P72R) polymorphism [18] and the three polymorphisms in the VDR gene [19] were performed as previously described. The polymorphisms of the GSTT1, GSTM1, and CYP1A1 genes [20], the polymorphic valine to isoleucine change at codon 105 in the GSTP1 gene [21], and the histidine to tyrosine change at codon 113 in the EPHX gene [22] were all carried out as previously described. The Leu217 and Thr941 missense mutations were detected as previously described [6]. The c1088 C → T (R243R) polymorphism in the estrogen receptor was detected by employing the DGGE technique.

**Statistical analysis**

Analysis of relationship between gene exposure and discrete (nominal or ordinal) variables was performed using Pearson's chi-square test for appropriate cross-tabulation. The difference between mean values of continuous variables and gene exposure was analyzed using one-way ANOVA. All calculations used SAS6.12 for Windows software.

**Results**

**Patients' characteristics**

The study included 224 Israeli patients with prostate cancer. The age range of diagnosis was 45–81 years (64.6 ± 7.4 years) (mean ± SD). Regarding the patients' origin, 100 (44.6%) were Ashkenazi (East European), 78 (34.8%) were non-Ashkenazi – mostly (n=48) Asian (i.e., Iraqi, Iranian), and the remaining patients (n=46) were mixed Ashkenazi-non-Ashkenazi (n=29, 12.9%) or Israeli-born for more than four generations (n=17, 7.5%). Prostate cancer was diagnosed in 70 patients prior to or at 60 years of age, and in 154 patients older than 61 years. A family history of cancer could be elicited in 122 patients only, primarily because of truncated family trees as a result of the Holocaust or immigration to Israel at an
early age with loss of contact with other family members. Of these, 12 of 122 (9.8%) had prostate, breast and/or ovarian cancer in at least one first-degree relative, 25 (20.5%) had a more remote family history of cancer (in second-degree relatives and cancer types other than prostate, breast, or ovary), and in the remaining 85 patients (69.7%) prostate cancer was designated sporadic. There were 35 tumors at stage T1 disease, 135 at stage T2, 46 at stage T3, and 8 at stage T4 (staging was assigned by the revised TNM system from 1997) and was based on digital rectal exam, transrectal and pelvic ultrasonography, abdominal computerized tomography and bone scan. Tumor grades were as follows: well-differentiated (Gleason scores 2–4) collectively referred to as grade I (n=36), moderately differentiated (Gleason scores 5, 6) or grade II (n=122), poorly to poorly differentiated (Gleason score 7) or grade III (n=60), poorly differentiated (Gleason scores 8–10) or grade IV tumors (n=6).

**Control population characteristics**

Overall, 250 men were genotyped for the two missense mutations in the HPC2/ELAC2 gene. Their ages ranged from 35 to 83 years (61.7 ± 9.7 years), 113 (45.2%) were Ashkenazi, 91 (36.4%) were of non-Ashkenazi origin, mostly (n=63) Iranian-Iranian born, and the rest were either mixed Ashkenazi-non-Ashkenazi (n=31, 12.4%) or Israeli-born (n=13, 6%) for more than four generations. All were asymptomatic, with no personal history of cancer, no abnormal masses on digital rectal exam, and PSA levels within the normal range during the preceding 12 months.

**Table 1.** Selected clinical, histologic and genotype data of study participants

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**Genetic analyses**

Homzygosity for the low activity genotype of the EPHX gene (His113 allele) was significantly less common in patients with early-onset prostate cancer (< 60 years) (4/70, 5.7%) as compared with late-onset disease (≥ 61 years) (33/154, 21.4%) (P < 0.001). In addition, within the late-onset group of patients, the same low activity genotype was encountered less commonly among grade I tumors (23/61, 5.5%) compared with higher grade tumors (grades 2 and up) (34/188, 18%) (P = 0.004). Patients with a family history of cancer other than prostate, breast or ovary, displayed the BB genotype of the VDR gene less frequently than those with no family history of cancer (1/25, 4% vs. 31/97, 31.9%) (P = 0.023) (data not shown in the table). Patients with prostate cancer who were non-smokers more often displayed the TT (38/74, 51.3%) Aa (49/76, 64.4%) and bb (36/70, 51.4%) pattern in the VDR gene polymorphisms than did smokers (TT 32/150, 21.3%, Aa 63/148, 42.5%, bb 39/154, 25.3%) – P < 0.001 for all three comparisons. In addition, non-smokers more often carried the wild-type GSTT1 allele (26/75, 33.3%) than smokers (35/146, 23.9%) (P < 0.001). For all other tested polymorphisms, no significant associations were noted between the specific polymorphism and age at diagnosis, ethnic origin, family history of cancer, smoking history, disease stage and grade. The distribution of polymorphisms in all candidate genes within the study population analyzed by age at diagnosis, tumor grade and smoking history is shown in Table 1.

In addition to the data presented in Table 1, the rate of the Leu217 missense mutation among prostate cancer patients was 36.6% (82/224) and 37.2% (93/250) among healthy, ethnically and
age-matched asymptomatic controls. Similarly, the rate of the Thr541 missense mutation in the same gene was 4.9% (11/224) in the prostate cancer group and 5.8% (29/520) in the controls. Both were statistically insignificant differences.

Discussion
In this study, a missense mutation in the EPHX gene His113Tyr (H113Y) was associated with diagnosis at age older than 61 years and a more advanced grade prostate cancer in Israeli patients. The biologically more active Tyr113 allele (YY genotype) was associated with an increased risk for ovarian cancer [15]. This association may reflect enhanced activation of endogenous or exogenous carcinogens to more mutagenic derivatives by the high activity genotypes. Alternatively, this polymorphic variation in EPHX activity could modify the penetrance of other prostate cancer susceptibility gene(s).

The initial enthusiasm sparked by the findings of the role that the Thr541 and Leu217 missense mutations play in prostate cancer predisposition and pathogenesis [6] has somewhat abated. Subsequent studies failed to show a more frequent occurrence of these mutations in prostate cancer patients than in controls [23,24], and even in the selected group of familial prostate cancer cases the role of HPC2/ELAC2 mutations may be limited [25]. Our data support the limited role, if any, of these polymorphisms in prostate cancer pathogenesis in Israeli patients.

Polymorphic patterns in the vitamin D receptor and the functional polymorphism in the GSTT1 significantly differed between smokers and non-smokers in the present study. This finding, if confirmed in other populations, may help to identify individuals who smoke and are genetically at higher risk for developing prostate cancer. While the involvement of the GSTT1 in the detoxification pathway is well established, no such role has been proposed for the VDR, and its presumed involvement in prostate cancer pathogenesis has been attributed to its role in cellular proliferation. Our finding may indicate that the VDR may be a modulator of some of the carcinogenic substances in cigarette smoke. The other polymorphisms tested in this study appear not to be involved in prostate cancer tumorigenesis in Israeli patients. However, analysis of other polymorphisms within the same gene, preferably single nucleotide polymorphisms, in a larger group of patients may help provide a more accurate answer regarding the putative role of these genes in prostate cancer risk and progression.

The clinical implications of this study, if confirmed, may affect several aspects of prostate cancer detection and prevention. Analysis of the EPHX gene polymorphism may help to identify asymptomatic individuals at high risk for developing late-onset prostate cancer in the general, moderate risk, population. It may also help to target prostate cancer patients who are likely to have a more advanced disease and hence should be placed under a more strict surveillance scheme. Lastly, it may provide a genetic tool for identifying individuals who smoke and are at a higher than average risk for developing prostate cancer. Nonetheless, caution is called for in interpreting and extrapolating these results. Certainly, confirmation of these preliminary data based on a larger number of patients with diversified ethnic origin is needed.

References
Research Projects

Mechanism of expression of the HBV polymerase gene

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Hepatitis B virus (HBV) is an enveloped DNA virus that infects human liver and replicates via reverse-transcription of the pregenomic RNA by a viral encoded polymerase. The infection often develops to chronic hepatitis with high risk of liver cirrhosis and cancer. HBV has a unique mode of replication and gene expression. We investigated the HBV transcription program in order to understand how the viral polymerase gene is expressed. Using genetics and biochemical tools we identified two functional RNA species and resolved the mechanisms of their expression. We found that an exact genome-size RNA species is produced as a result of the HBV composite poly(A) signal with embedded TATA-box. This unprecedented DNA regulatory box acts both to initiate and to terminate the synthesis of a given RNA. Our data also suggest that this composite box is essential for virus replication, possibly because it is required for the synthesis of the polymerase. The second RNA to be identified is a large transcript about 1.3 times the size of the genome. We found that this RNA encodes the smallest protein, named pX. Unexpectedly, this large transcript accumulates in the nucleus but its nuclear function was not resolved. These data clarify some very basic aspects in HBV gene expression and life cycle.

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Capsule

Antidepressants and hippocampal neurogenesis

Antidepressants can cause rapid increases in serotonin and noradrenaline levels in patients, but usually the clinical benefits are not seen until 3 or 4 weeks have passed. One possible explanation for this delay is that antidepressant drugs change the mood of patients by stimulating neurogenesis. Santarelli et al. studied genetically engineered mice lacking the serotonin 1A receptor and mice whose ability to undergo hippocampal neurogenesis had been blocked by focal irradiation. In both cases, antidepressant-induced neurogenesis was inhibited and the behavioral actions of the antidepressants were abolished.

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