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

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Abstract

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

Objectives: To evlauate 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 in 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 prostaste 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 multigenic, multifactorial inheritance [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 enviromental carcinogens. Sequence alterations, in particular missense mutations, within some of the relevent 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 Val/Val polymorphism in the *CYP1A1*, and the Leu432Val polymorphism in *CYP1B1*, both members of the cytochrome p450 gene

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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 I105V *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 (His113) within the microsomal epoxid hydroxylase (*mEPHX*) gene was expressed somatically in more than 90% of prostate cancer tissue analyzed [14]. The more active form of the enzyme (Tyr113) 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 venopuncture 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.J. 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 vizualized 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 Thr541 missense mutations were detected as previously described [6]. The c1088 C $\geqslant$ 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 Jewish Israeli patients with prostate cancer. The age range of diagnosis was 45–81 years (64.6 $\pm$ 7.4 years) (mean $\pm$ 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-Askenazi (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

PCR = polymerase chain reaction

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), moderately 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) Iraqi-Iranian born, and the rest were either mixed Ashkenazi–non-Ashkenazi (n=31, 12.4%) or Israeli-born (n=15, 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.

PSA = prostate-specific antigen

Genetic analyses

Homozygosity 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 encounterd less commonly among grade I tumors (2/36, 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 (25/75, 33.3%) than smokers (35/145, 6.9%) ($P < 0.001$). 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

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

		EPHX			P	CYP1A1			P	ER		P	GSTM1		P	GSTP1			P	
Age at Dx	>60 years	HH	HY	YY		HH	Hh	hh		M	N		N	P		II	IV	VV		
		58\154	63\154	33\154		4\154	42\154	108\154		18\154	136/154		63\154	91\154		72\154	66\154	16\154		
	<60 years	4\70	62\70	4\70		1\66	15\66	50\66		7\66	59\66		30\66	36\66		32\66	27\66	7\66		
					<0.001				0.765			0.957			0.74				0.987	
Grade + Age	>60 + Grade1	8\36	26\36	2\36		2\202	52\202	148\202		3\28	25\28		10\24	14\24		5\9	3\9	1\9		
	<60 + Grade >2	74\188	80\188	34\188		0\18	8\18	10\18		23\192	169\192		82\196	114\196		101\211	86\211	24\211		
					0.004				0.24			0.904			0.838				0.894	
Smoking	YES	51\172	92\172	29\172		3\154	46\154	105\154		8\98	90\98		62\146	84\146		55\118	51\118	12\118		
	NO	29\52	15\52	8\52		1\66	20\66	25\66		10\54	44\54		31\74	43\74		51\102	40\102	11\102		
					<0.001				0.219			0.103			0.949				0.834	

		GSTT1		P	P53 R72P			P	VITDBsm			P	VITDApa			P	VITDTaq			P
		N	P		RR	RP	PP		BB	Bb	bb		AA	Aa	aa		TT	Tt	tt	
Age at Dx	> 60 years	43\154	111\154		58\154	79\154	17\154		38\154	64\154	52\154		52\154	83\154	19\154		53\154	74\154	27\154	
	< 60 years	20\66	46\66		19\70	36\70	15\70		22\70	30\70	18\70		37\70	28\70	5\70		15\70	38\70	17\70	
				0.948				0.073				0.349				0.038				0.132
Grade+Age	>60+Grade 1	56\195	139\195		70\192	97\192	25\192		51\191	81\191	59\191		70\193	100\193	23\193		64\194	92\194	38\194	
	<60+Grade >2	5\25	20\25		7\32	20\32	5\32		10\33	13\33	10\33		14\31	15\31	2\31		7\30	17\30	6\30	
				0.497				0.274				0.406				0.512				0.523
Smoking	YES	35\145	110\145		52\147	69\147	26\147		55\154	60\154	39\154		74\148	63\148	11\148		32\150	80\150	38\150	
	NO	25\75	50\75		25\77	42\77	10\77		5\70	29\70	36\70		16\76	49\76	11\76		38\74	32\74	4\74	
				<0.001				0.494				<0.001				<0.001				<0.001

For each tested polymorphism, the common and rare homozygote and the heterozygote geneotype are shown, according to various clinical and pathologic characteristics. The *P* values for whole-group comparisons are shown. For the ER polymorphism, M denotes a heterozygote as there were no homozygotes for the rare allele. For the *GSTM1* and *GSTT1* polymorphisms, N denotes normal wild type, and P denotes the polymorphic allele. For the *GSTP1*, *EPHX*, and the *p53* missense mutations, the single letter symbols of the encoded amino acids are shown. For the vitamin D receptor polymorphisms the capital letters denote an uncut allele, and lower case letters denote an allele that was cleaved by the specified restriction enzyme.

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% (29/250) 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 predispisition 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 genes, 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 or 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

1. Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics. *CA Cancer J Clin* 1999;49:8–31.
2. Lesko SM, Rosenberg L, Shapiro S. Family history and prostate cancer risk. *Am J Epidemiol* 1996;144:1041–7.
3. Carpten J, Nupponen N, Isaacs S, et al. Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. *Nat Genet* 2002;30(2):181–4.
4. Suarez BK, Lin J, Burmester JK, et al. Chromosome screen of multiplex sibships with prostate cancer. *Am J Hum Genet* 2000;66:933–44.
5. Gibbs M, Chakrabarti L, Stanford JL, et al. Analysis of chromosome 1q42.2-43 in 152 families with high risk of prostate cancer. *Am J Hum Genet* 1999;64:1087–95.
6. Rebbeck TR, Walker AH, Zeigler-Johnson C, et al. Association of HPC2/ELAC2 genotypes and prostate cancer. *Am J Hum Genet* 2000;67:1014–19.
7. Raunio H, Husgafvel Pursiainen K, Anttlia S, Hietanen E, Hirvonen A, Pelkonen O. Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility. *Gene* 1995;159:113–21.
8. Hengstler JG, Arand M, Herrero ME, Oesch F. Polymorphisms of N-Acetyltransferases, Glutathione S-Transferase, Microsomal Epoxide Hydrolase and Sulfotransferases: Influence on Cancer Susceptibility. Recent Results in Cancer Research. Vol. 154. Berlin: Springer, 1998.
9. Stanford JL, Noonan EA, Iwasaki L, et al. A polymorphism in the CYP17 gene and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2002;11:243–7.
10. Tang YM, Green BL, Chen GF, et al. Human CYP1B1 Leu432Val gene polymorphism: ethnic distribution in African-Americans, Caucasians and Chinese; oestradiol hydroxylase activity; and distribution in prostate cancer cases and controls. *Pharmacogenetics* 2000;10:761–6.
11. Kote-Jarai Z, Easton D, Edwards SM, et al., for the CRC/BPG UK Familial Prostate Cancer Study Collaborators. Relationship between glutathione S-transferase M1, P1 and T1 polymorphisms and early onset prostate cancer. *Pharmacogenetics* 2001;11:325–30.
12. Rakozy C, Gringon DJ, Li Y, et al. P53 gene alterations in prostate cancer after irradiation failure and their association with clinical outcome: a molecular and immunohistochemical analysis. *Pathol Res Pract* 1999; 195:129–35.
13. Henner WD, Evans AJ, Hough KM, Harris EL, Lowe BA, Beer TM. Association of codon 72 polymorphism of p53 with lower prostate cancer risk. *Prostate* 2001;49:263–6.
14. Murray GI, Taylor VE, McKay JA, et al. The immunohistochemical localization of drug metabolizing enzymes in prostate cancer. *J Pathol* 2000;177:147–52.
15. Lancaster JM, Brownlee HA, Bell DA, et al. Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. *Mol Carcinog* 1996;17:160–2.
16. Taylor JA, Hirvonen A, Watson M, Pittman G, Mohler JL, Bell DA. Association of prostate cancer with vitamin D receptor gene polymorphism. *Cancer Res* 1996;56:4108–10.
17. Gsur A, Madersbacher S, Haidinger G, et al. Vitamin D receptor gene polymorphism and prostate cancer risk. *Prostate* 2002;51:30–4.
18. Wang YC, Chen CY, Chen SK, Chang YY, Lin P. P53 codon 72 polymorphism in Taiwanese lung cancer patients: association with lung cancer susceptibility and prognosis. *Clin Cancer Res* 1999;5:129–34.
19. Riggs BL. Vitamin D receptor genotypes and bone density. *N Engl J Med* 1997;337:125–6.
20. Bailey LR, Roodi N, Varrier CS, Yee CJ, Dupont WD, Parl FF. Breast cancer and CYP1A1, GSTM1, and GSTT1 polymorphisms: evidence of a lack of association in Caucasians and African Americans. *Cancer Res* 1998;58:65–70.

21. Harries LW, Stubbins MJ, Forman D, Howard GC, Wolf CR. Identification of genetic polymorphism at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis* 1997;18:641–4.
22. Hassett C, Aicher L, Sidhu JS, Omiecinski CJ. Human microsomal epoxide hydroxilase: genetic polymorphism and and functional expression in vitro of amino acid variants. *Hum Mol Genet* 1994;3:412–28.
23. Vesprini D, Nam RK, Trachtenberg J, et al. HPC2 variants and screen detected prostate cancer. *Am J Hum Genet* 2001;68:912–17.
24. Xu J, Zheng SL, Carpten JD, et al. Evaluation of linkage and association of HPC2/ELAC2 in patients with familial or sporadic prostate cancer. *Am J Hum Genet* 2001;68:901–11
25. Wang L, McDonnell SK, Elkins DA, et al. Role of HPC2/ELAC2 in hereditary prostate cancer. *Cancer Res* 2001;61:6494–9

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