New Paraoxonase 1 Polymorphism I102V and the Risk of Prostate Cancer in Finnish Men

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Background: Human serum paraoxonase eliminates carcinogenic lipid-soluble radicals. Because expression of the main human paraoxonase gene PON1 varies widely in humans, certain PON1 polymorphisms might be associated with increased risks of cancer. We sought new functional mutations in PON1 and determined whether known or new PON1 mutations were associated with the risk for prostate cancer in a prospective, random, population-based sample of Finnish men and in a case–control study.

Methods: Serum paraoxonase activity was measured in 835 healthy men in the Kuopio Ischaemic Heart Disease Risk Factor Study. PON1 mutations were identified by hierarchical phenotype-targeted sequencing in DNAs from the 100 men with the lowest paraoxonase activity in this cohort, and 1595 men in the cohort were genotyped for PON1 mutations by restriction fragment length polymorphism. Multivariable analysis was used to investigate the association of known and new PON1 mutations with incident prostate cancer in 1569 cancer-free men in the cohort followed for 9–14 years. In a case–control study of Finnish men, the association of prostate cancer with the PON1 mutation identified in the cohort study was investigated in 69 case patients with familial prostate cancer and 69 unmatched healthy control subjects.

Results: We identified a new single-nucleotide PON1 polymorphism associated with decreased serum paraoxonase activity that caused an isoleucine→valine change at codon 102 in exon 4 (I102V). Of the 1569 men cancer-free at baseline, 56 (3.6%) were carriers of the I102V mutation. After adjusting for age and cholesterol-lowering medications, the relative risk for developing prostate cancer during follow-up was 6.3 (95% confidence interval [CI] = 2.1 to 19.2) among 102V allele carriers compared with noncarriers. Other PON1 alleles were not statistically significantly associated with prostate cancer. In the case–control study, patients with familial prostate cancer were more likely to be carriers of the PON1 I102V mutation than control subjects (odds ratio = 4.3, 95% CI = 0.9 to 21.5).

Conclusion: The PON1 102V allele appears to be associated with an increased risk for prostate cancer. [J Natl Cancer Inst 2003;95:812–8]

Prostate cancer is the most commonly diagnosed cancer among men in industrialized countries, such as the United States (1) and Finland (2). The familial aggregation of prostate cancer has been described (3). Men who have multiple relatives with prostate cancer and relatives diagnosed with prostate cancer at an early age appear to be at increased risk of this disease (4,5). An analysis of 44 788 twin pairs indicated that as many as 42% of prostate cancers may be explained genetically (6).

Oxidative stress and free radicals have been associated with the increased risks of various cancers (7,8). The human body has a number of endogenous free-radical scavenging systems, including paraoxonase. Serum paraoxonase binds to high-density lipoprotein and contributes to the detoxification of organophosphorus compounds, such as paraoxon, and carcinogenic lipid-soluble radicals from lipid peroxidation (9–11). The main paraoxonase-encoding gene PON1 (Online Mendelian Inheritance in Man [OMIM] No. 168820), located at 7q21.3, is polymorphic in human populations (9), and the expression of paraoxonase varies...
widely in human populations (9). It has been hypothesized that PON1 polymorphisms might contribute to the increased risks of cancer associated with pollutants and other environmental chemicals (12–14).

Two common functional polymorphisms, Q192R and L55M, have been described in the coding region of human PON1, and both affect serum paraoxonase activity (9,14). Other mutations in paraoxonase genes are the (−107)T/C, (−824)G/A, and (−907)G/C promoter polymorphisms of the PON1 gene (15) and the C311S and A148G polymorphisms of the PON2 gene (16,17).

The purpose of this study was to identify additional functional mutations in the nine exons of the PON1 gene by hierarchical phenotype-targeted sequencing and to determine whether the new and previously known mutations are associated with the risk of incident prostate cancer in Finnish men.

SUBJECTS AND METHODS

Study Population

The first study population was a prospective cohort among participants of the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD), a population-based study that is investigating genetic and other risk factors for cardiovascular diseases, diabetes, and cancer (18). The KIHD study protocol was approved by the Research Ethics Committee of the University of Kuopio. A total of 2682 men from Eastern Finland aged 42, 48, 54, or 60 years were examined between March 1984 and December 1989. All participants provided written informed consent. Fifty-one men with a history of cancer, either self-reported or registered in the national cancer registry before entry into KIHD, were excluded from the study. Of the remaining 2631 men, a DNA sample was available for 1609, but for technical reasons, complete PON1 genotypic information was available for only 1543 men (data on M55L was missing for 34 subjects, on I102V for 14 subjects, and on Q192R for 18 subjects). No statistically significant difference in the prevalence or incidence of prostate cancer was observed between the men with and without a DNA sample (data not shown). The average follow-up time was 11.2 years, resulting in 17,262 person-years of follow-up.

The second study, which started on June 20, 1995, is planned to continue through at least December 31, 2010. This confirmatory set consisted of 69 patients derived from nonrelated families, each family with three or more cases of prostate cancer or two cases if the age of the proband at diagnosis was less than 60 years (54 and 15 families, respectively). The youngest affected subject from each family was selected for genotyping. Detailed family identification and sample collection of the Finnish hereditary prostate cancer families have been described elsewhere (19).

The families were ascertained by a number of methods, as described (19), with appropriate approval from the Ministry of Health and Social Affairs, the Ethical Committee of the Tampere University Hospital, and the local ethics committees of regional hospitals. The study was also approved by the National Human Genome Research Institute’s Institutional Review Board. Written informed consent was obtained from all patients. For control subjects, 69 samples from healthy, anonymized voluntary male blood donors from the Tampere region were analyzed. These subjects were not matched.

Ascertaining of Cancers

Since 1953, every cancer in the health care system has been reported in a countrywide and population-based manner in Finland. Coverage of the national cancer registry is virtually complete (20). Our study cohort was record-linked with the cancer registry data by using the unique personal identification code (social security number) that all Finns have. The first occurrence of prostate cancer after the KIHD baseline examination was registered and validated for 20 men in the Finnish Cancer Registry from 1984 through 1998. None of these incident prostate cancers were registered during the first 5 years of follow-up. No systematic prostate-specific antigen screening programs in the Kuopio area occurred during the study period. Prostate cancer diagnoses in the case-control study were also confirmed by the Finnish Cancer Registry or individual patient records from regional hospitals.

Measurement of Paraoxonase Activity

Serum paraoxonase activity was measured in 835 healthy men at the 11-year follow-up of KIHD, from 1998 through 2000. Briefly, 100 mL of diluted serum (1:25 dilution in 0.1 M Tris–HCl, pH 8.0) was mixed with 100 mL of paraoxon (Dr. Ehrenstorfer GmbH, Augsburg, Germany; 0.1 g in 66.1 mL of 0.1 M Tris–HCl, pH 8.0). Formation of p-nitrophenol was monitored photometrically at 405 nm at 30 °C; 1 U of paraoxonase activity is defined as the conversion of 1 nmol of paraoxon to p-nitrophenol per 1 mL/minute.

PON1 Gene Amplification

Polymerase chain reaction (PCR) amplifications were conducted in 25 μL containing 150 ng of genomic DNA, 1× PCR buffer, all four deoxyribonucleoside triphosphates (each at 10 mM), 20 pmol of each primer, and 1 U of DNA polymerase (DyNAmo DNA polymerase kit; Finnzymes, Espoo, Finland). Samples were amplified with a Biometra UNO programmable thermoblock (Biometra, Göttingen, Germany). The primer pairs and amplification conditions are presented in Table 1 (see Table 4 for clinical details and the PON1 single-nucleotide polymorphism genotype of the 20 patients with histologically verified prostate carcinoma from the KIHD cohort).

Hierarchical Phenotype-Targeted Sequencing

Sequencing was carried out in a 16-capillary ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA). The sequencing reactions were made with an FS ABI PRISM sequencing kit (PE Biosystems, Foster City, CA). The sequencing primers were the same as the PCR primers. Cycle sequencing was made in the GeneAmp PCR system 9600 (PE Biosystems) according to the manufacturer’s instructions. Dye terminator removal and sequencing reaction cleanup were conducted in 96-well filtration plates (MultiScreen-HV clear plates; Millipore, Bedford, MA). We first sequenced PON1 DNA (exons 1–13) from the 10 men with the lowest paraoxonase activities (of the 835 men whose paraoxonase activities were measured) and then sequenced PON1 exon 4 from the 90 men with the next to the lowest levels of paraoxonase activity.

Genotyping

Large-scale genotyping was based on restriction fragment length polymorphism analysis as follows: 10 mL of the PON1
Table 1. Primer pairs and amplification conditions for the human PON1 gene

<table>
<thead>
<tr>
<th>Exon(s)</th>
<th>Primer pair</th>
<th>Annealing temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5'-GTGCACTCTAGACACCTGGCTTG-3' 5'-CAGGTGGAAAGGCAAATGGG-3'</td>
<td>64†</td>
</tr>
<tr>
<td>2–3</td>
<td>5'-GGAGAATTTGGACACTGG-3' 5'-CCCAAGATCTAAAGATTTACC-3'</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>5'-CTCTCCATGTTATAAAGGG-3'</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>5'-GACTGTCATGTGTTCTCTCT-3' 5'-CCTGACGCTAAGAAGGAAAT-3'</td>
<td>58</td>
</tr>
<tr>
<td>6</td>
<td>5'-GCTAAGGATTGTATCGGC-3' 5'-CACTAGGCAAATCTGTTAAA-3'</td>
<td>57</td>
</tr>
<tr>
<td>7</td>
<td>5'-GTTGTTATCTTCTAGTACT-3'</td>
<td>52</td>
</tr>
<tr>
<td>8</td>
<td>5'-CTCTCCATGTTATAAAGGG-3'</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td>5'-CATGGTGACATGCGCTTG-3' 5'-GTCTAGATACTCTCCACCTC-3'</td>
<td>62†</td>
</tr>
<tr>
<td></td>
<td>5'-CTGAAACAGACATGGAACGGC-3'</td>
<td></td>
</tr>
</tbody>
</table>

*All amplifications were otherwise identical with conditions for the polymerase chain reaction as follows: denaturation 95 °C for 3 minutes; 30 cycles of denaturation at 95 °C for 30 seconds, annealing for 45 seconds at the temperature indicated above, and extension at 72 °C for 45 seconds; and a final extension at 72 °C for 5 minutes.
†Hot Start after 1 minute at 95 °C for the initial denaturation.

New Mutation in the PON1 Gene

In DNA samples from the 10 men with the lowest serum paraoxonase activity (from 20.4 U/L to 36.9 U/L), we identified a new human PON1 variant in exon 4, an A → G substitution (ATC to GTC) at nucleotide position 304 resulting in an isoleucine → valine substitution at codon 102 of the PON1 DNA (I102V), in three of the 10 men (GenBank accession number AF402963). To determine whether this mutation had wider occurrence, we sequenced DNA samples from an additional 90 men with the next lowest paraoxonase activities (from 36.9 U/L to 52.5 U/L) and found that 9.0% of the 100 men carried the I102V mutation. Consequently, we attempted to genotype all 1609 DNA samples that were available in the KIHD cohort with a restriction fragment length polymorphism-based analysis of exon 4 of PON1 and found the mutation in 61 of the 1595 successfully genotyped samples (i.e., 3.8% of the random population sample of middle-aged men). All carriers of PON1 I102V were heterozygotic. Selected phenotype characteristics of the mutation carriers and noncarriers are presented in Table 2.

PON1 Mutations and Paraoxonase Activity

PON1 mutations in codons 55, 102, and 192 accounted for 62% of the serum paraoxonase activity, and a fully saturated linear interaction model (including most of the parameters presented in Table 2) accounted for 68% of serum paraoxonase activity in 835 healthy men at the 11-year KIHD follow-up visit. All three mutations were associated with statistically significantly reduced paraoxonase activity (P<.001). Paraoxonase activities in the serum of men carrying various PON1 haplotypes are shown in Table 3.

The 55M and 192Q alleles are regarded as the low-activity alleles. The serum paraoxonase activity in men with at least one low-activity allele (55M or 192Q) was decreased on average by 57% from 339 U/L (95% CI = 320 to 358 U/L) to 144 U/L (95% CI = 138 to 150 U/L) (P<.001). The paraoxonase activity in men with either the 55M or 192Q allele and the 102V allele was decreased by another 51% from 144 U/L (95% CI = 138 to 150 U/L) to 70 U/L (95% CI = 63 to 77 U/L) (P<.001). The alleles associated with lower paraoxonase activity—55M, 102V, and 192Q—were in strong linkage disequilibrium (i.e., they were inherited as a block from generation to generation) (D statistics: D' = 0.60 for 55M-102V, D' = 0.80 for 55M-192Q, and D' = 0.83 for 102V-192Q). Allelic distributions of all three loci followed Hardy–Weinberg proportions.

PON1 Ile102Val Polymorphism and the Incidence of Prostate Cancer

Among the 1569 men genotyped who had not been diagnosed with clinical cancer before the baseline examination, 20 were diagnosed with prostate cancer by the end of 1998.
details of these patients are presented in Table 4. Of the 56 carriers of the PON1 I102V mutation, four (7.1%) were diagnosed with prostate cancer during the follow-up, whereas of the 56 noncarriers, 16 (1.1%) were diagnosed with prostate cancer. Thus, the I102V mutation was statistically significantly associated with increased risk of prostate cancer (odds ratio [OR] = 7.2, 95% confidence interval [CI] = 2.3 to 22.3; P = .005, two-sided Fisher’s exact test). In contrast, the 55M allele (M/M versus M/L + L/L; OR = 2.2, 95% CI = 0.32 to 215; P = .72) and the 192R allele (Q/Q + Q/R versus R/R; OR = 2.3, 95% CI = 0.33 to 15.4; P = .72) were not statistically significantly associated with increased risk of prostate cancer.

In a univariate Cox proportional hazards model of 1569 men for whom all follow-up information was available, men who carried the 102V allele were at a higher RR for developing their first prostate cancer (OR = 6.6, 95% CI = 2.2 to 19.8; P < .001) than men who did not carry the allele.

In a step-up Cox model of 1543 men, 24 potential risk factors for cancer, examination years, and all three genotyped PON1 mutations were tested for entry. The following three variables were statistically significantly associated with prostate cancer: the PON1 I102V mutation (RR = 6.3, 95% CI = 2.1 to 19.2; P = .001) (Fig. 1), age (RR = 1.2 per year, 95% CI = 1.0 to 1.3 per year; P = .007), and the use of a cholesterol-lowering medication (RR = 10.2, 95% CI = 1.3 to 78.7; P = .026). Because 3.6% of healthy men (at baseline) in our cohort carried the 102V allele, 9.3 (95% CI = 2.8 to 22; P = .007) were not statistically significantly associated with increased risk of prostate cancer.

In a Cox model containing all eight haplotypes found in the study population, age, and cholesterol-lowering medication, the only haplotypes that were statistically significantly associated with incident prostate cancers were haplotypes that included the 102V allele: haplotype 55L-102V-192Q (RR = 11.6, 95% CI = 2.1 to 57.5; P = .027). The reference haplotype in this analysis was 55MM and Q192R polymorphisms were not statistically significantly associated with the risk of prostate cancer.

To confirm the finding in an independent study population, we also genotyped DNA samples from 69 patients with familial prostate cancer and from 69 control subjects. We found the
carriers of the PON1 102V mutation were heterozygotic. The 95% CI for the cumulative incidence and the number of patients at risk, in parentheses, are 95% CI 0.002 to 0.003 (55 patients), 95% CI 0.012 to 0.016 (54 patients), 95% CI 0.054 to 0.067 (53 patients), and 95% CI 0.099 to 0.128 (52 patients).

Table 5. PON1 haplotype distribution among the 1543 men with complete PON1 genotype information in the Kuopio Ischaemic Heart Disease Risk Factor Studya

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>No. cancer-free during follow-up</th>
<th>No. diagnosed with prostate cancer during follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>55L-102I-192Q</td>
<td>1054</td>
<td>13</td>
</tr>
<tr>
<td>55L-102I-192R</td>
<td>943</td>
<td>11</td>
</tr>
<tr>
<td>55L-102V-192Q</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>55L-102V-192R</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>55M-102I-192Q</td>
<td>932</td>
<td>12</td>
</tr>
<tr>
<td>55M-102I-192R</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>55M-102V-192Q</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>55M-102V-192R</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

aNumber of haplotypes equals to twice the number of subjects.

PON1 102V allele in eight (11.6%) of the 69 patients with prostate cancer and in two (2.9%) of the 69 healthy control subjects (OR = 4.3, 95% CI = 0.9 to 21.5; P = .09). All carriers of the PON1 102V mutation were heterozygotic.

DISCUSSION

We found a new common mutation in the coding region of the PON1 gene, termed I102V, that was strongly associated with an increased risk of incident prostate cancer in a random population-based cohort of Finnish men. Men who carried the PON1 102V allele had a greater than sixfold increased RR for the first clinical manifestation of prostate cancer. Theoretically, as many as 16% of prostate cancers in this cohort were associated with the presence of the PON1 102V allele. Consequently, as many as 16% of these prostate cancers might have been detected early if the men had been screened for the presence of the PON1 1102V polymorphism. In populations similar to our cohort, there might be 300–400 mutation carriers per 10000 men. Of these, 20–30 men will develop prostate cancer in 10 years, of which almost all might be associated with the PON1 102V mutation. Thus, in the future, genetic screening may help to identify men with an increased risk for prostate cancer.

The strength of this association (the chance of a false-positive association is less than one per 1 million men) suggests, but does not prove, that the I102V mutation leads to an increased risk of prostate cancer. However, because Finns have a relatively small founder population and a slow inflow of new genes, the magnitude of the increased risk of prostate cancer associated with the I102V mutation may be different in different populations. In an independent analysis of 69 men with familial prostate cancer and 69 healthy control subjects, our results were almost identical: patients with familial prostate cancer were more likely than control subjects to be carriers of the PON1 I1102V mutation (OR = 4.3, 95% CI = 0.9 to 21.5). The direction and magnitude of this finding support the association of the I102V mutation with an increased risk of prostate cancer.

Because the association of I102V with prostate cancer was not any stronger for familial prostate cancer than for prostate cancer in the general population, PON1 is more likely to act as a low-penetrance allele rather than a high-penetrance allele. In addition, the haplotype analysis of the KIHD genotype data suggests that the new I102V mutation has the strongest association with prostate cancer of the three mutations investigated.

From segregation analyses, it was initially speculated that familial aggregation of prostate cancer among those diagnosed at an early age was best explained by the presence of a rare autosomal dominant, highly penetrant allele (25–27). It has been suggested that the HPC1 gene located at 1q24–25 is a potential prostate cancer susceptibility locus (28). Carpten et al. (29) found a rare autosomal variant, Glu265X, in tumor suppressor gene RNASEL in an HPC1-linked family. Tavtigian et al. (30) reported that the potential hereditary prostate cancer susceptibility gene HPC2/ELAC2 was located at 17p11. Rebbeck et al. (31) found the association of a rare autosomal variant Leu217/Thr541 at 17p11 with a 2.4-fold increased RR of prostate cancer, but other studies have not confirmed this association (32,33). Four other prostate cancer susceptibility loci—PCAP (34), HPCX (35), CAPB (36), and HPC20 (37)—have been reported from linkage studies. To date, corresponding genes have not been isolated for these chromosomal regions (38–40). Single-nucleotide mutations in SRD5A2 and an androgen receptor gene CAG repeat polymorphism may be associated with the risk of prostate cancer (41,42). From the large number of candidate genes, the hereditary component of prostate cancer may be polygenic (33,34,43). Highly penetrant prostate cancer susceptibility loci appear to be rare (43). In future studies, additional I102V
predicting and multivariable analysis should be done in other populations with a larger number of patients with prostate cancer to confirm the association of the I102V polymorphism with the risk of prostate cancer.

Free-radical stress appears to play a role in the development of prostate cancer (44). Our findings provide additional support for the role of oxidative stress in the development of prostate cancer. Our findings also indicate that, in future clinical trials testing the anticarcinogenic effect of antioxidative supplements, the screening or stratification of subjects according to the PON1 Ile102Val polymorphism may be warranted.

REFERENCES


NOTES

Editor’s note: M. Marchesani, T.-P. Tuomainen, J. Kaikkonen, and J. T. Salonen share in a patent application connected to the findings in this paper. Oy Jurilab Ltd. has applied for a patent concerning the Ile102Val mutation as well as the Hierarchical Phenotype-targeted sequencing (HPTS) method.

The KIHD study was funded by Public Health Service (PHS) grant HL44199 (to Professor George A. Kaplan) from the National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), Department of Health and Human Services (DHHS), and by grants 41471, 1041086, and 2041022 (to J. T. Salonen) from the Academy of Finland. Ascertainment of families with hereditary prostate cancer in Finland was supported by PHS grant N01-55389 from the National Human Genome Research Institute, NIH, DHHS. J. Kaikkonen is a fellow (supported by grant 80624) of the Academy of Finland.

We thank Rikka Rontu, Ph.D., for PON1 codon 55 genotyping; Kristiina Nyysönen, Ph.D., for supervising most of the biochemistry; Riitta Salonen, M.D., Ph.D., for participation in the management of the KIHD study; and the staff of the Research Institute of Public Health, University of Kuopio (www.uku/laitokset/RIPH) and Oy Jurilab Ltd. (www.jurilab.com) for their contributions.

Manuscript received May 13, 2002; revised March 19, 2003; accepted April 22, 2003.