Germ-Line Alterations in MSR1 Gene and Prostate Cancer Risk

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ABSTRACT

Purpose: The MSR1 gene maps to 8p22–23, a novel susceptibility locus for hereditary prostate cancer (HPC). Mutations in MSR1 have been reported to associate with prostate cancer (PRCA) risk. Here we report a follow-up study from Finland to evaluate the association between PRCA and MSR1 gene.

Experimental Design: The youngest affected patient from each of 120 HPC families was initially used for the screening of MSR1 mutations by single-strand conformational polymorphism analysis. Selected variants of MSR1 gene were then screened in 537 unselected PRCA cases and in 480 controls.

Results: Among 120 HPC families, five MSR1 sequence variants were identified. The carrier frequencies of the R293X, P275A, and -14743A>G variants were compared between the probands with HPC, unselected PRCA cases, and healthy male blood donors. No significant differences were observed. The odds ratios for R293X, P275A, and -14743A>G mutations were also calculated to estimate the PRCA risk. No significantly elevated or lowered risks for PRCA among these three variants were detected. However, the mean age at diagnosis of the R293X mutation carriers among HPC probands was significantly lower compared with noncarriers (55.4 versus 65.4 years; t test, P = 0.04). The same trend was observed among unselected PRCA cases (65.7 versus 68.7 years; t test, P = 0.37).

Conclusions: Our results do not support a major role for the MSR1 gene in the causation of hereditary or unselected PRCA but suggest a possible modifying role in cancer predisposition.

INTRODUCTION

A positive family history is among the strongest epidemiological risk factors for PRCA.2 Although an increasing number of PRCA susceptibility loci have been reported, including HPC1 (1q24–25), PCAP (1q42–43), HPCX (Xq27–28), CAPB (1p36), HPC20 (20q13), HPC2 (17p11), and 16q23 (1), only two genes have been identified from these loci: ELAC2 from HPC2-locus (2) and RNASEL from HPC1-locus (3). Some of the subsequent studies have been able to confirm the role of these genes (4, 5), whereas others have not (6, 7). Therefore, additional studies using larger cohorts are needed to fully evaluate the role of these two susceptibility genes in PRCA risk. In addition, more statistical power can also be generated with meta-analyses, as has been done recently with ELAC2 (8). Xu et al. (9) reported recently evidence of linkage to a new locus at 8p22–23 in 159 pedigrees affected with HPC. The same group identified both germ-line mutations and common sequence variants from MSR1 gene located in 8p22 (10, 11). Wiklund et al. (12) confirmed the linkage to 8p22–23 in Swedish HPC material. The MSR1 gene encodes the SR-A, which include three different isoforms (I, II, and III) generated by alternative splicing (13, 14). The functional SR-A protein is a trimeric molecule composed of three identical protein chains. SR-AI and SR-AII consists of six domains: cytoplasmic, membrane spanning, spacer, -helical coiled-coil, collagen-like, and isoform-specific COOH-terminal domain (15). SR-A types I and II are functional integral membrane glycoproteins that bind diverse array of macromolecules (16, 17). Type III protein also contains six domains, but it is a nonfunctional protein acting as a dominant-negative isoform by blocking modified low-density lipoprotein uptake (14). Putative biological roles of SR-A include macrophage-host cell interactions, macrophage adhesion to substratum, endocytosis of lipoproteins, and clearance and detoxification of microbial products (17). Here, we report a study from Finland to additionally evaluate the association between PRCA and MSR1 gene.

MATERIALS AND METHODS

Families with HPC. A detailed description of the original collection of families, confirmation of diagnosis, and prostate specific antigen testing for unaffected males in the HPC families is described elsewhere (18). In this study we used two different cohorts of families: the first cohort included 68 fami-
lies and the second included 52 families. Families in the first cohort had either three or more affected members or two affected members with at least the index patient diagnosed with PRCA \( \leq 60 \) years of age. The mean age at diagnosis for the index patients was 62.2 years (range, 44–81 years), and the mean number of affected family members was 3.2 (range, 2–6). The second cohort of families had only two affected members with ages at diagnosis >60 years. The mean age at diagnosis for the index patients in this cohort was 69.0 years (range, 61–86). In both family cohorts the affected persons were first- or second-degree relatives. The youngest affected patient from each of 120 HPC families was initially used for the screening of \( \text{MSR1} \) mutations by SSCP analysis. Selected variants of \( \text{MSR1} \) gene were assayed in all of the other available affected and unaffected members of the mutation-positive families.

**Patients with PRCA and Controls.** There were 634 consecutive patients diagnosed with PRCA in the Pirkanmaa Hospital District with a population of \( \approx 450,000 \) during 1999–2000. We had samples from 85% of these patients, which results in an unselected, population-based collection of patients. The mean age at diagnosis for the patients with unselected PRCA was 68.6 years (range, 47–90). Of the patients with unselected PRCA, 12% (66 of 537) reported a positive family history of PRCA. The controls consisted of DNA samples from anonymous male blood donors obtained from the Blood Center of the Finnish Red Cross in Tampere.

Written informed consent was obtained from all of the living patients and also, for families with HPC, from the unaffected members. The research protocols were approved by the Ethical Committee of the Tampere University Hospital (93175, 95062, and 99228), and the National Human Genome Research Institute (HG-0158). Permission for collection of families, in the entirety of Finland, was granted by the Ministry of Social Affairs and Health (59/08/95).

**Mutation Screening with SSCP Analysis.** SSCP analysis of the entire coding sequence of the \( \text{MSR1} \) gene was performed using primer sequences that were designed to include all of the intron-exon boundaries (GenBank accession nos. NM_138715, NM_002445, and NM_138716\(^3\)). On request, all of the primers are available from the authors. The 15-\( \mu \)l reaction mixture contained 1.5 mmM MgCl\(_2\); 20 \( \mu \)M each of dATP, dCTP, dGTP, and dTTP; 0.5 \( \mu \)Ci of \( ^{33}\)P-dCTP (Amersham Pharmacia, Uppsala, Sweden); 0.6 \( \mu \)M of each primer; 1.0 unit AmpliTaqGold; the reaction buffer provided by the supplier (PE Biosystems, Foster City, CA); and 25 ng of the genomic DNA. For exon 10 the PCR reaction mixture contained 5% DMSO. Annealing temperature of 55°C was used for exons 1–4, 9, and 11; temperature of 51°C was used for exons 5–8, and exon 10 needed temperature of 53°C. Radiolabeled PCR products were mixed with 95% formamide dye, denatured at 95°C for 5 min, and chilled on ice. The \( ^{33}\)P-labeled PCR products were electrophoresed at 800 V for 12 h at room temperature, in 0.5× mutation-detection-enhancement gel (FMC BioProducts, Rockland, ME) with 1% glycerol in 0.5× Tris-borate EDTA. After electrophoresis, gels were dried and exposed to Kodak BioMax maximum resolution films for 6 h. All of the samples in which variant bands were detected, as well as two to three normal bands per exon, were analyzed by sequencing using an automated ABI Prism 310 Genetic Analyzer (PE Biosystems, Foster City, CA). Variants were identified using Sequencher software version 3.0 (Gene Codes Corporation, Ann Arbor, MI).

**Minisequencing and SSCP for Large-Scale Population Screening of Identified Variants.** The frequencies of P275A and R293X variants were determined in the entire set of 1137 samples by SSCP analysis as described above. R26H mutation was screened in 239 patients with unselected PRCA and in 240 controls by minisequencing (19). PCR for minisequencing was performed with 100 ng of DNA, 0.2 \( \mu \)M each primer, 0.2 \( \mu \)M each dNTP, 1.5 mmM MgCl\(_2\), and 1.0 unit of AmpliTaqGold (PE Biosystems), in a final volume of 50 \( \mu \)l. The single nucleotide polymorphism in the promoter region was screened in 239 patients with unselected PRCA and in 192 controls by SSCP as described above.

**Statistical Analyses.** Association of the \( \text{MSR1} \) genotypes with HPC and unselected PRCA was tested by logistic-regresion analysis, by use of the SPSS statistical software package (SPSS 11.0). Association with demographic, clinical, and pathological features of the disease was tested by the Mann-Whitney test, Pearson \( \chi ^2 \) test, and Fisher’s exact test by use of the SPSS statistical software package (SPSS 11.0).

**RESULTS AND DISCUSSION**

Among 120 HPC families we identified five sequence variants, including one nonsense mutation at codon 293 (R293X), two missense mutations (R26H and P275A), and two sequence variants (A>G in promoter region and variation in number of T nucleotides in intron 9; Table 1). R293X, P275A, and SNP in the promoter region were also reported by Xu et al.

### Table 1 Summary of \( \text{MSR1} \) germ-line variants found in 120 patients with HPC in the SSCP analysis

<table>
<thead>
<tr>
<th>Mutation(^a)</th>
<th>Amino acid change</th>
<th>Exon/intron</th>
<th>Domain</th>
<th>Number of families</th>
</tr>
</thead>
<tbody>
<tr>
<td>-14743A&gt;G</td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>77G&gt;A</td>
<td>R26H</td>
<td>Exon 2</td>
<td>Cytoplasmic</td>
<td>1</td>
</tr>
<tr>
<td>823C&gt;G</td>
<td>P275A</td>
<td>Exon 6</td>
<td>Collagen-like</td>
<td>3</td>
</tr>
<tr>
<td>877C&gt;T</td>
<td>R293X</td>
<td>Exon 6</td>
<td>Collagen-like</td>
<td>3</td>
</tr>
<tr>
<td>1034-10 variation in n. of T nucleotides(^b)</td>
<td>–</td>
<td>Intron 9</td>
<td>–</td>
<td>17</td>
</tr>
</tbody>
</table>

\(^a\) Numbering is according to the cDNA (NM_138715) starting at the A in the start codon.

\(^b\) Min. number of T nucleotides = 18; max. = 29; most frequently 21 T nucleotides.
Table 2  Association of the variants of the MSR1 gene with patients with unselected PRCA or HPC

<table>
<thead>
<tr>
<th>Sample and mutation</th>
<th>No. of carriers/total (frequency)</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R293X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5/480 (1.0%)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with unselected PRCA</td>
<td>6/537 (1.1%)</td>
<td>1.07</td>
<td>0.33–3.54</td>
<td>0.91</td>
</tr>
<tr>
<td>Patients with HPC</td>
<td>3/120 (2.5%)</td>
<td>2.44</td>
<td>0.57–10.34</td>
<td>0.23</td>
</tr>
<tr>
<td>P275A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>20/480 (4.2%)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with unselected PRCA</td>
<td>21/537 (3.9%)</td>
<td>0.94</td>
<td>0.50–1.75</td>
<td>0.84</td>
</tr>
<tr>
<td>Patients with HPC</td>
<td>3/120 (2.5%)</td>
<td>0.59</td>
<td>0.17–2.02</td>
<td>0.40</td>
</tr>
<tr>
<td>14743 A&gt;G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>14/192 (7.3%)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with unselected PRCA</td>
<td>24/239 (10.0%)</td>
<td>1.42</td>
<td>0.71–2.83</td>
<td>0.32</td>
</tr>
<tr>
<td>Patients with HPC</td>
<td>15/120 (12.5%)</td>
<td>1.82</td>
<td>0.84–3.91</td>
<td>0.13</td>
</tr>
</tbody>
</table>

(10, 11). R293X mutation deletes most of the ligand-binding domain and the entire COOH-terminal cysteine-rich domain. P275A changes the first Gly-Xaa-Yaa repeat in the collagen-like domain. To investigate the possible segregation of the variants R293X, R26H, and P275A, we sequenced all of the available affected and unaffected male relatives from the mutation-positive families. R26H was found only in 1 family including 3 affected men. The proband and his 50-year-old unaffected brother carried the mutation. The affected father could not be genotyped, but his affected brother did not carry the variant. In addition, R26H mutation was screened in 239 patients with unselected PRCA and in 240 healthy male blood donors. No mutation carriers were found. Thus, R26H is a very rare novel mutation. The SNP in promoter region (−14743A>G) was found in 12.5% (15 of 120) of the HPC families. We screened the variant from all of the other available affected male relatives from these families. There was no clear evidence of cosegregation; 19 of 32 of the screened affectedcs carried the variant. In addition, 10.0% (24 of 239) of the unselected PRCA patients and 7.3% (14 of 192) of the control individuals carried the variant. The carrier frequencies did not differ significantly between the three different sample groups (Pearson χ² test, P = 0.30).

R293X and P275A were both found in three probands from different families. One family having 3 affected members carried both mutations but in different individuals. In this family, there was 1 affected (the proband) and 2 unaffected R293X carriers, and 1 unaffected P275A carrier. The second affected did not carry either of the variants, and the third affected could not be genotyped. The 2 other R293X mutation-positive families had only 2 affecteds; in both families they both carried the mutation. Two of the P275A-positive families had 2 affected relatives; in both families they both carried the mutation. Carrier frequencies of R293X and P275A variants were compared between patients and control subjects. The carrier frequencies for R293X were 2.5%, 1.1%, and 1.0%, in the 120 probands with HPC, 537 unselected PRCA cases, and 480 healthy blood donors, respectively. There was no statistically significant difference in the carrier frequencies between the different sample groups (Pearson χ² test, P = 0.41), although the percentage was highest among HPC patients. The carrier frequencies for P275A were 2.5%, 3.9%, and 4.2% in the probands with HPC, unselected PRCA cases, and healthy blood donors, respectively. No significant difference was observed in the carrier frequencies between the different sample groups (Pearson χ² test, P = 0.70). We also calculated the odds ratios for R293X, P275A, and −14743A>G mutations to estimate the PRCA risk (Table 2.). There were no significant elevated or lowered risks for PRCA among these three variants.

The mean age at diagnosis of the R293X mutation carriers among HPC probands was significantly lower compared with noncarriers (55.3 versus 65.4 years; t test, P = 0.04; Table 3). Difference in ages at diagnosis in these groups can be because of small number of mutation carriers (n = 3). However, the same trend was observed among unselected PRCA cases (65.7 versus 68.7 years; t test, P = 0.37). Although the exact role of MSR1 in prostate carcinogenesis is unknown, some processes involving macrophages have been implicated in the development of PRCA (20). In addition, the degree of macrophage infiltration has been shown to associate with PRCA prognosis (21). These reports support our finding that MSR1 might modify age at diagnosis. No other statistically significant associations of the R293X, P275A, or −14743A>G variant with demographic, clinical, or pathological features of the disease were observed (Tables 3 and 4.). In the study from the United States, R293X was observed in 3.2% (6 of 190) of families (all 6 were of European descent; Ref. 10). Each family had at least three first-degree relatives affected with PRCA. In the same study, the carrier frequency of R293X was 2.5% (8 of 317) among individuals with non-HPC (affected men either without a family history of PRCA or with one affected first degree relative). In the present study, the detected R293X frequency of 2.5% among our HPC probands is the same as the frequency in the non-HPC cases in the United States study. It should be noticed that the sample grouping criteria and definition were different in these two studies, because 54% of our HPC families had only two affected relatives. In addition, R293X was observed in 1.5% of men exposed to asbestos in the study of Xu et al. (10). This group was analyzed to determine the frequency in the general United States population. Interestingly, we detected similar frequency of 1.0% among our population controls, which were anonymous male blood donors.
The carrier frequencies for P275A in the United States studies were 15.8% (30 of 190), 9.6% (29 of 301), and 16.4% (41 of 250) in the HPC families, non-HPC patients, and unaffected controls, respectively (10, 11). These frequencies were much higher compared with our results. This suggests that P275A is a more common variant in the heterogeneous United States population compared with the Finnish population, which is known to be historically isolated and genetically homogeneous (22). P275A did not show cosegregation in the United States families either.

Our study is the first reported follow-up study to investigate the role of MSR1 in PRCA causation. The initial reports by Xu et al. (10, 11) found that germ-line mutations and sequence variants of the MSR1 gene are associated with PRCA risk. Our results do not support a major role for the MSR1 gene in the causation of hereditary or unselected PRCA. However, R293X mutation might influence disease onset by lowering the age at diagnosis. Consistent with the results, our recent genome wide linkage study in Finnish HPC families found no evidence for linkage on chromosome 8p (23). Therefore, it is not surprising that we did not detect any significant association between MSR1 and PRCA. The present results warrant additional studies of the role that MSR1 variants have as risk factors for HPC and unselected PRCA in other populations.

ACKNOWLEDGMENTS

We thank Minna Sjöblom and Riitta Vaalavuo for excellent technical assistance. We also thank all of the participating patients and families for their cooperation.

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