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Title:
PROGNOSTIC MARKERS FOR PROSTATE CANCER RECURRANCE

Abstract:
Objective. The relationship between inherited genetic variations in 5α-reductase type 1 (SRD5A1) and type 2 (SRD5A2) genes and the risk of biochemical recurrence after radical prostatectomy (RP) in prostate cancer (PCa) remains a fairly unexplored area of research. Patients and Methods. We studied 526 men with organ-confined and locally advanced PCa with a median follow-up time of 7.4 years. We investigated the effects of allelic variants of SRD5A1 and SRD5A2 genes and haplotype-tagging single nucleotide polymorphisms (htSNPs; n=19) on recurrence-free survival after RP using Kaplan-Meier plots, the log-rank test, and Cox proportional hazard models. Results. Upon adjusting for known prognostic clinical and pathological factors, eight htSNPs were shown to be independent predictors of recurrence. The SRD5A1 rs166050 polymorphism was associated with an increased recurrence risk of HR=1.83 (95% CI, 1.04-3.21; P=0.035), while the rs518673 in SRD5A1 was associated with a decreased risk (HR=0.59, 95% CI, 0.41-0.85; P=0.004). The SRD5A2 gene was strongly associated with the risk of relapse with six polymorphisms being positively associated with recurrence including the known SRD5A2 V89L (rs523349) (HR=2.14, 95% CI, 1.23-3.70; P=0.007) and a protective htSNP rs12470143 with a HR of 0.66, (95% CI, 0.46-0.95; P=0.023). By combining SRD5A1 (rs518673T) and SRD5A2 (rs12470143 A), the protective effect was shown to be additive with the maximum protection conferred by 3 or 4 alleles (HR=0.33, 95% CI, 0.17-0.63; P=0.001). Conclusion. Germline polymorphisms in 5α-reductase genes are independent prognostic genetic biomarkers that predict PCa biochemical recurrence after radical prostatectomy and may represent useful molecular tools for a genotype-tailored clinical approach.

Figure 1

[Continued on next page]

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Prognostic Markers for Prostate Cancer Recurrence

Field of the invention

[0001] The present invention relates to markers for the prognosis of recurrence of prostate cancer.

Background of the invention

[0002] Prostate Cancer (PCa) is a major public health concern since it is the sixth most common cancer in the world and the second leading cause of cancer death in North American men. Of the several known risk factors, the most important are age, ethnicity, dietary and genetic factors. Patients with localized (clinical stage T1-T2) and locally advanced (T3) PCa are frequently treated with radical prostatectomy (RP), a potentially curative procedure. It is estimated that over 30% of men undergoing RP will have disease relapse, also referred to as biochemical recurrence (BCR) as the first clinical indication of rising serum level of PSA.

[0003] Currently, the Tumor/Nodes/Metastasis (TNM) staging system, the Gleason score, and pre-treatment serum prostate-specific antigen (PSA) are the most important factors influencing both the likelihood of more extensive disease and the probability of subsequent relapse following RP. Indeed, these tools include both nomograms and risk tables incorporating clinical variables that can predict, although still imperfectly, the likelihood of tumor recurrence and provide causal prognosis information to guide clinicians in their therapeutic decisions. The risk of disease progression greatly differs between individuals and the heterogeneity in clinical behaviour further emphasizes the need to find novel markers of progression. Even if cases of PCa are considered localized at the time of diagnosis, the rate of BCR after RP is still significant and occur most often in the 5 years after surgery. The persistence of tumor cells in a state of either complete or near dormancy prior to metastatic progression is likely accountable for disease recurrence while these residual cells are most probably responsive to hormones.
Androgen hormones, such as testosterone (T) and 5a-dihydrotestosterone (DHT), have been clearly implicated in development of PCa. Circulating T, secreted by the testis, and adrenal steroid precursors (dihydroepiandrosterone; DHEA and DHEA-sulfate; DHEA-S) are among factors influencing androgen levels in the prostate and other tissues.\(^9\)\(^-\)\(^11\) Prostate cells, as well as a number of peripheral tissues, contain a variety of steroidogenic enzymes required for the local formation of active androgens from adrenal precursors.\(^12\)\(^-\)\(^14\)\(^15\) Namely, the conversion of T by 5a-reductases (SRD5A1 and SRD5A2) leads to DHT, the more potent androgen receptor (AR) agonist in target cells. SRD5A2 is the major 5a-reductase enzyme expressed in the prostate compared to SRD5A1.\(^16\) However, while the expression of SRD5A2 decreases in prostate cancer cells, SRD5A1 is increased in tumoral tissues.\(^16\)\(^-\)\(^20\) This imbalance in the expression of SRD5A genes in PCa tumors illustrate the complex relation between 5a-reductases, DHT synthesis and PCa progression.

Androgen deprivation therapy (ADT) is the standard of care for metastatic PCa and is also used to treat asymptomatic patients with PSA recurrence after failed primary therapy (RP), further reinforcing the initial androgen dependency of these cells.\(^21\)\(^-\)\(^22\) Finasteride, a 5a-reductase type 2 inhibitor currently used in the clinic, has been recently shown to be an effective chemopreventive medication reducing by almost 25% the risk of PCa incidence.\(^23\) Additionally, data from clinical studies were recently used to model a risk-adapted PSA-based chemoprevention strategy.\(^24\) Despite this well recognized hormonal dependence of prostate cancer cells in the early cancer stage, very few studies have investigated the associations between polymorphisms in the androgen biosynthesis pathway and clinical outcome after surgical procedure.\(^25\)\(^-\)\(^32\) To date, common polymorphisms such as those in sex-steroids biosynthesis pathways have been extensively studied in relation to risk of PCa.\(^19\)\(^,\)\(^33\)\(^-\)\(^43\) However, almost all of these studies did not address the association between polymorphisms in genes regulating hormonal exposure with PCa recurrence and survival, and were not designed to do so. Long-term longitudinal studies are thus still required to systematically evaluate the impact of a patient's genetic profile on risk of recurrence.
[0006] We hypothesize that variations in \textit{SRD5A} genes may alter systemic androgen availability and affect the tumoral microenvironment exposure to hormones, which could modify the risk of PCa recurrence after RP. This is based on the fact that 5a-reductases have a well-characterized physiological role in DHT biosynthesis, are associated with PCa risk and more recently, with cancer progression\textsuperscript{26-28} and represent molecular targets in PCa prevention trials \textsuperscript{23-24}. In this study, we performed a detailed genetic analysis of the \textit{SRD5A1} and \textit{SRD5A2} genes in relation to PCa progression. We also aimed at validating the association of the known \textit{SRD5A2} V89L polymorphism associated with BCR by other groups.\textsuperscript{26-28} One particular feature of our study is that the association of inherited variations with the risk of BCR was determined after RP as the sole initial curative intent in a cohort of 526 men with clinically localized and locally advanced PCa.

**Summary of the invention**

[0007] There is therefore provided an \textit{in-vitro} method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of: a) obtaining a nucleic acid from a nucleic acid-containing sample (particularly a non-tumor or a tumor sample) from said human subject; and b) determining the individual's genetic variations (or haplotypes) in \textit{SRD5A1} or \textit{SRD5A2} gene in comparison to normal sequence of said genes; whereby the presence of at least one genetic variation in \textit{SRD5A1} or \textit{SRD5A2} in said subject's nucleic acid is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur.

[0008] The invention further provides the method as defined herein wherein step b) further comprises the step of: b') identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being selected from the group consisting of: rs518673; rs166050; rs2470143; rs2208532; rs2300702; rs4952197 and rs676033 or any of their associated variants as listed in Table 3; whereby the presence of at least one of said markers in said subject's sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur.
The present invention also provides a method for adapting a course of treatment of prostate cancer in a human subject after the subject has undergone radical prostatectomy, comprising the steps of: a) providing a prognosis or predicting the likelihood of a human subject to develop prostate cancer recurrence in accordance with the method as defined herein; and b) adapting a course of treatment according to whether said subject has an increased or decreased likelihood that the cancer will recur.

The invention further provides a kit for predicting the likelihood of a human subject to develop prostate cancer and/or recurrence by detecting a SNP in a reference sequence selected from the group consisting of: rs518673; rs166050; rs12470143; rs2208532; rs2300702; rs4952197 and rs676033 or their associated SNPs; said kit comprising reagents for determining the individual's genetic variations (or haplotypes) in SRD5A1 or SRD5A2 gene.

**Detailed description of the invention**

**Description of the figures**

**Figure 1** shows the risk of recurrence associated with known clinical and pathological prognostic variables (A) and SRD5A genes (B). Boxes represent hazard ratios (HR) and their 95% CI. PSA categories are in ng/ml. Reference categories (HR: 1.00) are: PSA at diagnosis ≤ 10ng/ml, pG ≤ 6, and pT ≤ T2b. Genetic linkage between htSNPs tested for each SRD5A gene is represented in the triangles on the left in panel B; and

**Figure 2** illustrates Kaplan-Meier estimates of recurrence-free survival for A) SRD5A1, B) SRD5A2 and C) both genes. Only positive htSNPs in multivariate analysis are represented. Values for log-rank P values (LR) are shown in each frame. Numbers (0 to 4) in panel C indicate the number of protective alleles for both genes. SRD5A1 protective allele is rs518673T and SRD5A2 protective allele is rs12470143A.

**Definitions and abbreviations**

ADT: androgen-deprivation therapy; AR: androgen receptor; BCR: biochemical recurrence; DHT: 5α-dihydrotestosterone; HR: hazard ratio; htSNP: haplotype-tagging SNP;
PCa: prostate cancer; PSA: prostate-specific antigen; SRD5A1: 5α-reductase type 1; SRD5A2: 5α-reductase type 2; RP: radical prostatectomy; SNP: Single nucleotide polymorphism; T: Testosterone.

**Detailed description of particular embodiments**

[0014] In accordance with the method of the invention, it will be well recognized by persons skilled in the art that genetic variations are assessed in comparison to the gene sequence identified for the normal gene. Such gene sequence for each of these enzymes in their normal state can be found at:

- for **SRD5A1**: Ensembl accession number ENSG00000145545 and can be consulted from the NCBI Internet site at the reference number: gene ID 6715.
- for **SRD5A2**: Ensembl accession number ENSG0000049319 and can be consulted from the NCBI internet site at the reference number: gene ID 6716.

[0015] In accordance with particular aspects of the present invention, step b) of the method may further comprise: b”) contacting the subject’s nucleic acid with a reagent that specifically binds to at least one of said single nucleotide polymorphism (SNP); and c) detecting the binding of reagent to at least one of said SNP, whereby the binding of said reagent to at least one SNP is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will recur.

[0016] The invention also provides the method as defined herein, wherein the SNP is found in reference sequences (rs) selected from the group consisting of: rs166050; rs2208532; rs2300702; rs4952197; and rs676033, or any of their associated SNPs whereby the presence of said SNP is an indication of an increased likelihood that the prostate cancer will recur. Particularly, the SNP is found in reference sequences (rs) selected from the group consisting of: rs2208532 or rs676033, whereby the presence of said SNP is an indication of an increased likelihood that the prostate cancer will recur. More particularly,
the SNP is found in rs2208532 or any of its associated SNPs and the presence of said SNP is an indication of an increased likelihood that the prostate cancer will recur.

[0017] The invention also provides the method as defined herein, wherein the SNP is found in reference sequences (rs) selected from the group consisting of: rs518673 or rs12470143, whereby the presence of said SNP is an indication of a decreased likelihood that the prostate cancer will recur. Particularly, the presence of SNP found in rs518673 or any of its associated SNPs is an indication of a decreased likelihood that the prostate cancer will recur. More particularly, the presence of both rs518673T and rs12470143A or any of their associated SNPs is still a further indication of a decreased likelihood that the prostate cancer will recur.

[0018] The invention also provides the method as defined herein wherein in step a), the nucleic acid is DNA or RNA. In particular embodiment of the invention, the DNA is extracted from a non-tumor or a tumor sample from said human subject to be utilized directly for identification of the individual's genetic variations. Particularly, examples of nucleic acid detection methods are: direct sequencing or pyrosequencing, massively parallel sequencing, high-throughput sequencing (a.k.a next generation sequencing), high performance liquid chromatography (HPLC) fragment analysis, capillarity electrophoresis and quantitative PGR (as, for example, detection by Taqman® probe, Scorpions™ ARMS Primer or SYBR Green). In one aspect, the amplification of the DNA is carried out by means of PGR. Several methods for detecting and analyzing the PGR amplification products have been previously disclosed. The general principles and conditions for amplification and detection of genetic variations, such as using PGR, are well known for the skilled person in the art.

[0019] Alternatively, other methods of nucleic acid detection such as hybridization carried out using appropriately labeled probe, detection using microarrays e.g. chips containing many oligonucleotides for hybridization (as, for example, those produced by Affymetrix Corp.) or probe-less technologies and cleavage-based methods may be used. Preferably, amplification of the DNA can be carried out using primers that are specific to the marker,
and the amplified primer extension products can be detected with the use of nucleic acid probe. More particularly, the DNA is amplified by PGR prior to incubation with the probe and the amplified primer extension products can be detected using procedure and equipment for detection of the label.

[0020] The invention also provides the method as defined herein, wherein the subject's tumor sample is from a biopsy. The invention also provides the method as defined herein, wherein the subject's non-tumor sample is selected from the group consisting of: tissue or biological fluid. Particularly, the tissue is a lymph node, hair or a buccal smear. Still particularly, the biological fluid is sputum, saliva, blood, seaim urine, semen or plasma.

[0021] The invention also provides the method as defined herein, wherein, when the likelihood of recurrence is increased, the subject is prescribed 5a-reductase inhibitors therapy.

[0022] The invention also provides the kit as defined herein, comprising PGR primer-probe set, wherein the primers are selected from the group consisting of: SEQ ID Nos. 1 to 38; and the probe is selected from the group consisting of: SEQ ID Nos. 39 to 57.

Examples

Patients and Methods

Clinical Data and Outcome Collections

[0023] The study cohort, mostly composed of Caucasians, included 526 men who underwent RP at l'Hotel-Dieu de Quebec Hospital (QC, Canada) between February 1999 and December 2002. Each participant provided written consent before surgery for the analysis of their genome and the research protocol was approved by the research ethical committee at the Centre Hospitalier Universitaire de Quebec (CHUQ, QC, Canada). All patients were followed postoperatively with serial PSA measurements and detailed clinical information was available.

DNA Isolation and Genetic Analysis
Polymorphisms studied were chosen according to one or more of the following criteria: i) to be likely functional (with supportive data in the literature), ii) to have previously been associated with PCa risk, aggressiveness, age at onset, BCR or ADT efficiency, and iii) to explain most of the haplotype diversity in the CEU (Utah residents with Northern and Western European Ancestry) Hapmap population. For both SRD5A genes, a region covering all the exons, introns and 5 kb of the 5' and 3' sections of the genes was screened using a haplotype tagging SNPs (htSNPs) strategy to maximize coverage, using HapMap Phase 2 (www.hapmap.org/cgi-perl/gbrowse/hapmap3r2_B36) and data from the CEU unrelated subjects based on a $r^2 \geq 0.80$ and a minimum minor allele frequency $>0.05$.

Peripheral blood was collected on the morning of a preoperative clinic visit and kept frozen at -80°C until analysis. Genomic DNA was purified using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Mississauga, ON, Canada) and stored at -20°C, PGR amplifications were performed using Sequenom iPLEX matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry by the sequencing service of McGill University and Genome Quebec INNOVATION center (QC, Canada). For oligos sequence, see Table 1. Negative controls were present for every aim of analyses and quality controls (random replicates of known genotypes) were successfully performed in 5% of the study cohort.
Table 1. Oligonucleotides used for genotyping analysis.

<table>
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<th>Gene</th>
<th>SNP</th>
<th>Forward Primer Sequence</th>
<th>SEQ ID No.</th>
<th>Reverse Primer Sequence</th>
<th>SEQ ID No.</th>
<th>Probe Sequence</th>
<th>SEQ ID No.</th>
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<td>ACGTTGGATGCCGCTTTCTCATGTTGTCG</td>
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<td>38</td>
<td>TAGATCTACTACATCAGAAG</td>
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</table>
Statistical Analysis

[0026] Based on our sample size, we were able to detect a hazard ratio of 1.50, for a minor allele with frequency of 5%, with over 80% statistical power. Allelic frequencies and Hardy-Weinberg equilibrium were computed with PLINK (version 1.07), a free open-source whole genome association analysis toolset. To analyse their association with BCR, each SNP was considered using 3 models since the function of most htSNP remain unknown. The first model tested, named the genomic model, considered the SNP as a categorical variable with a common allele homozygote (reference; based on the frequent allele reported in the Hapmap project), heterozygote and a minor allele homozygote. The second model, named the dominant model, considered the SNP with only 2 categories: one with a common allele homozygote (reference) and one with at least one minor allele. Finally, the third one referred to as the recessive model, also considered the SNP with only 2 categories: one with at least one common allele (reference) and one minor allele homozygote. Cox regression was performed on each SNP considering the 3 above mentioned models with adjustment for confounding variables namely PSA level at diagnosis, age at diagnosis, smoking status, pathological Gleason grade, pathological stage and neoadjuvant ADT. All co-variables were treated as categorical, and for PSA level, Gleason scores and stage, they were used as described by the well-recognized D'Amico risk classification. Smoking status and neoadjuvant ADT were classified as "positive" or "negative" while age was classified as <65 and ≥65 years old. The censoring variable was BCR, which was defined as 1) two consecutive PSA values ≥ 0.3 µg/L, 2) one PSA value ≥ 0.3 µg/L followed by ADT, 3) a last-recorded PSA value ≥ 0.3 µg/L, and 4) the initiation of ADT or radiation therapy by the patient's physician. Kaplan-Meier analyses were also processed for every SNP (log rank), while only results for SNPs which were significantly associated with BCR in Cox regression multivariate analysis are shown. For Kaplan-Meier and Cox regression, statistical analyses were performed using PASW statistics 17 (SPSS Inc., Chicago, IL) and R version 2.10.0 (http://www.r-project.org/).

[0027] Haplotypes were inferred using Phase v2.1.1 program, and their relative frequency, as well as pairwise linkage disequilibrium between SNPs, were determined with
HAPLOVIEW 4.1. Univariate and multivariate analysis were performed with or without minor haplotypes (frequency <5%), without significant impact on the P values - therefore, only results without minor haplotypes are shown.

**Results**

Clinical and pathological characteristics of the study cohort are shown in **Table 2**. All 526 patients had initially RP as curative intent enabling a precise pathological evaluation. The actual median follow-up time of the cohort is 7.4 years (range: 0.5 to 10.2 years). The cohort had mainly organ-confined and locally advanced tumors, as PCa cases were composed mainly of pT2 (60%) and pT3 (37%) pathological tumor stages (**Table 2**).

Overall, 130 cancer cases experienced BCR (25%), which was our primary outcome variable, with a median time to relapse of 2.1 years.

**Table 2.** Clinical and Pathological Characteristics of the Study Cohort.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>%</th>
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<td>Age at diagnosis, years (n = 526)</td>
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<td></td>
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<tr>
<td>Mean</td>
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<td></td>
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<tr>
<td>Standard deviation</td>
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<tr>
<td>Range</td>
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<td>Smoking status (n = 523)</td>
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</tr>
<tr>
<td>No</td>
<td>438</td>
<td>84</td>
</tr>
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<td>Yes</td>
<td>85</td>
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<td>PSA at diagnosis (ng/mL) (n = 521)</td>
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<td>69</td>
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<td>&gt;10-20</td>
<td>103</td>
<td>20</td>
</tr>
<tr>
<td>&gt;20</td>
<td>56</td>
<td>11</td>
</tr>
<tr>
<td>Pathological Gleason score (n = 509)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pG ≤ 6</td>
<td>158</td>
<td>31</td>
</tr>
<tr>
<td>pG = 7</td>
<td>244</td>
<td>48</td>
</tr>
<tr>
<td>pG ≥ 8</td>
<td>107</td>
<td>21</td>
</tr>
<tr>
<td>Pathological T stage (n = 522)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT ≤ T2</td>
<td>314</td>
<td>60</td>
</tr>
<tr>
<td>pT = T3</td>
<td>195</td>
<td>37</td>
</tr>
<tr>
<td>pT = T4</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Neoadjuvant hormonotherapy (n = 526)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31</td>
<td>6</td>
</tr>
<tr>
<td>No</td>
<td>495</td>
<td>94</td>
</tr>
</tbody>
</table>
Biochemical recurrence (n=526)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>130</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>396</td>
<td>75</td>
</tr>
</tbody>
</table>

PSA, prostate-specific antigen; n, number of patients for which the information was available.

[0029] Risk of recurrence associated with known clinical and pathological prognostic variables are shown in Figure 1A. As expected, the risk of recurrence increase with higher PSA values at diagnosis with a risk of relapse HR=1.5 (p=0.081) and HR=2.1 (p=0.003) with PSA values of 10-20 ng/mL and ≥ 20 ng/mL, respectively. Gleason scores of 7 and ≥ 8 are also positively associated with relapse with HR of 2.6 (p=0.002) and 5.4 (p<0.001), respectively. Pathological stage was not associated with biochemical recurrence risk (Figure 1A).

[0030] A total of 19 htSNPs of which 2 functional coding SNPs, distributed across the two SRD5A genes, were studied herein. The htSNPs strategy allowed us to study 89 genetic variations in both genes (Table 3). htSNPs were selected with a strategy to maximize gene coverage and to reflect adequately the Caucasian haplotype genetic diversity.

Table 3. List of htSNPs included in this study and their associated SNPs
Associated SNPs are polymorphisms in strong linkage with the htsNP with a r²≥0.80. The list of associated SNPs is derived from the analysis of a region covering approximately 250 kb for each SRD5A genes.

[0031] After analyses with a Cox regression multivariate model, adjusted for all clinical and pathological factors known to affect BCR, 8 htsSNPs were positively associated (P<0.05) with the risk of relapse. Their relative frequencies in cancer patients with and without relapse, and the corresponding hazard ratios (95% CI) are displayed in Figure 1 and Table 4. Genetic linkage between the 19 htsSNPs tested in both SRD5A genes is also represented.

[0032] Among 11 htsSNPs in SRD5A1, one SNP showed a P<0.05 by log-rank test for recurrence-free survival and corresponds to a protective allele (rs5 18673T). Another risk allele for biochemical recurrence (rsI66050C) was almost significant (log-rank test=P<0.051) (Figure 2). The SRD5A1 rsI66050 gene polymorphism was associated with an increased recurrence risk of HR=1.83, 95% CI, 1.04-3.21; P=0.035, while the rs518673 in SRD5A1 was associated with a decreased recurrence risk (HR=0.59, 95% CI, 0.41-0.85; P=0.004). Haplotype analyses further revealed five common SRD5A1 haplotypes (H) with a prevalence of > 5% (Table 5). SRD5A1 H2 was significantly associated with the risk of BCR with a HR of 0.64 (0.44-0.94; P=0.023) but did not remained significant in the multivariate model (HR=0.66; CI 95%; 0.46-0.95; p=0.073).
We found 6 htSNPs for the SRD5A2 gene to be associated with recurrence-free survival. The SDR5A2 protective htSNP rs12470143 was significant with a HR of 0.66 (0.46-0.95; P=0.023). A significant association was observed with the non-synonymous SNP V89L (rs523349) with a HR of 2.14 (95% CI, 1.23-3.70; P=0.007) while no association was seen with the other known coding variation A49T (rs9282858) (HR=0.81, 95% CI, 0.36-1.85; P=0.62) (Table 4). The other 4 risk alleles for recurrence were rs2208532, rs2300702, rs4952197, rs676033 with HR of 1.68, 1.88, 1.55 and 1.90, respectively (Table 4). In our Caucasian population, significant linkage disequilibrium was noted between rs676033 and rs523349 (r²=0.90; Figure 1). Genetic linkage was moderate between rs4952197 and rs676033 (r²=0.69) and between rs49522197 and rs523349 (r²=0.77). The genetic associations between other positive polymorphisms associated with BCR was below 50%. However, genetic linkage between these genetic variations was not ascertained in other populations (such as Asians, African-Americans, Hispanics) and remains to be defined.

For the SRD5A2 gene, we found 4 haplotypes. Of those, H2 and H3 were significantly associated with the risk of BCR but only H3 remained an independent predictor of recurrence in adjusted Cox proportional hazards analysis (adjusted HR=1.63; 1.11-2.39; P=0.013; Table 5).

Protective alleles in both SDR5A genes were significant in the dominant model while risk alleles were significant using the recessive model. Because of the absence of linkage between most htSNPs, the protection conferred by these alleles was not modified by other variants in that population.
Table 4. Genotypes frequencies of SNPs in 5a-reductase genes and their association with BCR.

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNPs</th>
<th>Genotypes</th>
<th>Genotypes by categories</th>
<th>Genomic Model</th>
<th>Secondary Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No BCR</td>
<td>BCR</td>
<td>HR</td>
</tr>
<tr>
<td>SRD5A1</td>
<td>rs518673</td>
<td>CT</td>
<td>43/189/159</td>
<td>9/50/70</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td></td>
<td></td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>rs166050</td>
<td>TC</td>
<td>20/152/222</td>
<td>14/46/70</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td></td>
<td></td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>rs2208532</td>
<td>AG</td>
<td>76/178/140</td>
<td>38/55/37</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td></td>
<td></td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>rs523349</td>
<td>GC</td>
<td>23/139/223</td>
<td>15/47/68</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td></td>
<td></td>
<td>2.10</td>
</tr>
<tr>
<td></td>
<td>rs676033</td>
<td>GA</td>
<td>31/145/218</td>
<td>17/47/66</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td></td>
<td></td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>rs1247014</td>
<td>QA</td>
<td>90/182/122</td>
<td>22/58/50</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td></td>
<td></td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>rs2300702</td>
<td>CG</td>
<td>71/173/146</td>
<td>29/57/43</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td></td>
<td></td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>rs4952197</td>
<td>GA</td>
<td>20/124/248</td>
<td>11/45/74</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td></td>
<td></td>
<td>2.11</td>
</tr>
</tbody>
</table>

1 Genotypes shown are for heterozygotes and minor allele homozygotes, as homozygotes for the frequent alleles are the reference (hazard ratio set at 1.00). 2 Number of genotypes are as follow: minor allele homozygote/heterozygote/frequent allele homozygote. HR: hazard ratio, CI: confidence intervals. HR: hazard ratio; 95% CI: 95% confidence interval; BCR: biochemical recurrence.
<table>
<thead>
<tr>
<th>Genes</th>
<th>H</th>
<th>Haplotype sequences</th>
<th>Freq (%)</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRD5A1</td>
<td>1</td>
<td>CACATCT</td>
<td>25.0</td>
<td>1.00</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>TGTGTCT</td>
<td>21.5</td>
<td>0.64</td>
<td>(0.44 - 0.94)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>TACATACT</td>
<td>20.3</td>
<td>0.74</td>
<td>(0.51 - 1.07)</td>
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<tr>
<td></td>
<td>4</td>
<td>TGGGTCT</td>
<td>13.1</td>
<td>1.16</td>
<td>(0.79 - 1.69)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>TGTAGAT</td>
<td>10.4</td>
<td>0.77</td>
<td>(0.49 - 1.23)</td>
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<tr>
<td>SRD5A2</td>
<td>1</td>
<td>AAAGTACGGGCA</td>
<td>35.5</td>
<td>1.00</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>GAAATGAAACCA</td>
<td>21.0</td>
<td>1.42</td>
<td>(1.02 - 1.97)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>GAAGTGCAGGGCA</td>
<td>13.7</td>
<td>1.46</td>
<td>(1.00 - 2.11)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>GCGGACAGGGCG</td>
<td>12.0</td>
<td>1.01</td>
<td>(0.85 - 1.56)</td>
</tr>
</tbody>
</table>

SRD5A2 SNPs: rs12470143, rs2281546, rs3754838, rs4952222, rs7562326, rs2208532, rs2300702, rs4952197, rs676033, rs523349, rs9282858 and rs9332975. SRD5A1 SNPs: rs168050, rs501999, rs518673, rs3822430, rs500182, rs6192120 and rs4702378.

H: haplotype; Freq: frequencies.
We then investigated the combined effects of protective alleles in both SRD5A genes (Table 6). By combining SRD5A1 (rs518673 T) and SRD5A2 (rs12470143 A), the protective effect was shown to be additive in Kaplan-Meier analysis with the maximum protection conferred by 3 or 4 alleles (Figure 2C) and remained an independent predictor of recurrence in Cox proportional hazards analysis (HR=0.33, 0.17-0.63; P=0.001 (Table 6). An allele dosage effect was observed with each additional allele diminishing by 26% the risk of recurrence of BCR (P=0.003).

Table 6. Combined protective effects of SRD5A1 and SRD5A2 htSNPs.

<table>
<thead>
<tr>
<th>Protective alleles (n)</th>
<th>Cases (n)</th>
<th>Freq (%)</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
<th>L-R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>0</td>
<td>82</td>
<td>15.6</td>
<td>1.00</td>
<td>Reference</td>
<td>0.008</td>
</tr>
<tr>
<td>1 or 2</td>
<td>350</td>
<td>66.5</td>
<td>0.57</td>
<td>(0.38 - 0.86)</td>
<td>0.001</td>
</tr>
<tr>
<td>3 or 4</td>
<td>88</td>
<td>16.7</td>
<td>0.35</td>
<td>(0.19 - 0.66)</td>
<td>0.001</td>
</tr>
<tr>
<td>Continuous1</td>
<td>520</td>
<td>n/a</td>
<td>0.75</td>
<td>(0.63 - 0.89)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

1Number of protective alleles was considered as a continuous variable in univariate and multivariate analysis.

n: number, L-R: log rank P value.
SRD5A1 protective allele is rs518673T.
SRD5A2 protective allele is rs12470143A.

Using Chi-square likelihood ratio test, no htSNPs were associated with PSA at diagnosis, pathological Gleason score or pathological stage (P > 0.05). Only the SRD5A2 rs2208532 was significantly associated with the pathological stage (P=0.048; minor allele homozygote being over-represented in tumors with pT ≥ T3a). htSNPs associated with BCR were shown to be the third most important predictors of recurrence after the Gleason score and PSA values.

Discussion

One of the most significant challenges in oncology remains our inability to predict PCa disease recurrence and clinical outcome due to a lack of key prognostic markers. The prediction of recurrence following radical prostatectomy is clearly important for personalized treatments and follow-up strategies due to striking heterogeneity in prostate cancer clinical behaviour. Inherited variations in sex-steroid biosynthesis enzymes are
attracting candidates as novel predictive markers since variation in these biotransformation pathways may modify exposure of any residual cancer cells to active androgens produced from adrenal precursors still available after RP in men with PCs. It is likely that alteration in hormonal exposure of the tumor microenvironment may ultimately also affect the risk of recurrence.

[0039] Here, in a cohort of 526 PCs cases, significant associations of BCR with multiple genetic polymorphisms in both SRD5A genes were observed. Of the 19 hSNPs tested, 8 inherited variations (42%) were shown to affect the risk of recurrence after RP either by conferring protection or an elevated risk, independently of known clinical and pathological predictors of prostate cancer recurrence. One of the best characterized SNP in the SRD5A2 gene is the functional V89L (rs523349) polymorphism associated with a decreased in enzymatic activity. This polymorphism has been associated with an aggressive form of prostate cancer in a recent large case-control study with prostate cancer risk in some studies, and with conflicting results with biochemical recurrence. The clinical utility of this marker must be further evaluated before any clinical implementation and risk-adapted PCs treatment strategies with these molecular markers. One important finding of our study is a significant association between the V89L (rs523349) polymorphism and the risk of BCR after prostatectomy. A 2.14-fold risk of BCR for homozygotes of the minor C allele was observed. This result is in agreement with Shibata and colleagues and with a recent large study that associated this SNP with an aggressive form of PCs. However, for the SRD5A2 A49T variant (rs9282858), a SNP associated in some studies with the risk of PCs, no association was observed with recurrence-free survival after RP indicating that this SNP would have no obvious role in PCs recurrence. This result is consistent with the latest meta-analysis investigating this SNP, showing no association with the risk of PCs.

[0040] Our study is the first to show strong positive associations of multiple independent SRD5A2 genetic variations with BCR. These findings suggest that the 5α-reductase type 2 germline variations play a critical role in prostate cancer recurrence after RP. Most variations represent independent risk alleles for BCR with one variant (rs1247043) associated with significant protection. Our data also argue for a significant role of SRD5A1
in the risk of recurrence after RP. Variations in this gene have been previously associated
with the risk of PCa.\textsuperscript{12-18} Two SNPs were positively associated with BCR; the rs166050
associated with an increased recurrence risk while the rs5 18673 conferred protection. By
combining the number of protective alleles in both genes, namely \textit{SRD5A1} (rs518673T)
and \textit{SRD5A2} (rs12470 143A), this independent protective effect was shown to be additive
and maximal in patients carrying 3 to 4 of these alleles.

[0041] Strengths of our study includes the large sample size combined with a significant
median follow-up time of 7.4 years that provided information on potential confounders.
Limitations include the limited number of some clinically relevant events such as
metastasis, hormone-resistance, or death related to the localized features of the tumors, that
prevented us from looking at the association between molecular signature in \textit{SRD5A} genes
and risk for these events.

[0042] Findings are remarkable for the fact that they complement the evidence on somatic
and germline genetic changes to predict disease recurrence.\textsuperscript{31-54,56} Additional investigations
are required to characterize the underlying biological mechanisms driving the positive
associations of inherited germline variations in the 5a-reductase pathway with BCR. At the
time of biochemical relapse and PSA elevation, the disease is particularly androgen-
dependent for growth and progression. We can only speculate that these genetic variations
influence active androgen formation and exposure of disseminated cancer cells remaining
after RP, potentially driving more hormone-dependent cells into cell replication, and
subsequently leading to inter-individual differences in recurrence.

[0043] In conclusion, our data reveal that multiple genetic markers in \textit{SRD5A} genes
contribute to biochemical recurrence risk after radical prostatectomy. These markers appear
independent of current predictors of recurrence such as Gleason score and PSA level and
predict risk better than the pathological stage. These findings may ultimately help refine
our ability to identify individuals at low or high risk of cancer relapse after RP, beyond
known prognostic variables, and for whom a more personalized approach might optimize
outcome, especially in the era of 5a-reductase inhibitors therapy.
References


21
CLAIMS

1. An in-vitro method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of:
   a) obtaining a nucleic acid from a nucleic acid containing non-tumor or tumor-sample from said human subject; and
   b) determining the individual's genetic variations (or haplotypes) in SRD5A1 or SRD5A2 gene;

   whereby the presence of at least one genetic variation in SRD5A1 or SRD5A2 in said subject's nucleic acid is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur.

2. The method of claim 1, wherein step b) comprises the step of:
   b') identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being selected from the group consisting of: rs518673; rs166050; rs12470143; rs2208532; rs2300702; rs4952197 and rs676033 or any of their associated variants as listed in Table 3;

   whereby the presence of at least one of said markers in said subject's sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur.

3. The method of claim 1 or 2, wherein step b) further comprises:
   b'”) contacting the subject's nucleic acid with a reagent that specifically binds to at least one of said single nucleotide polymorphism (SNP); and
   c) detecting the binding of reagent to at least one of said SNP,

   whereby the binding of said reagent to at least one SNP is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur.
4. The method of claim 3, wherein said SNP is found in reference sequences (rs) selected from the group consisting of: rs166050; rs2208532; rs2300702; rs4952197; and rs676033, or any of their associated SNPs whereby the presence of said SNP is an indication of an increased likelihood that the prostate cancer will develop or recur.

5. The method of claim 4, wherein said SNP is found in reference sequences (rs) selected from the group consisting of: rs2208532 or rs676033, whereby the presence of said SNP is an indication of an increased likelihood that the prostate cancer will develop or recur.

6. The method of claim 5, wherein said SNP is found in rs2208532 or any of its associated SNPs and the presence of said SNP is an indication of an increased likelihood that the prostate cancer will develop or recur.

7. The method of claim 3, wherein said SNP is found in reference sequences (rs) selected from the group consisting of: rs518673 or rs12470143, whereby the presence of said SNP is an indication of a decreased likelihood that the prostate cancer will develop or recur.

8. The method of claim 7, wherein said presence of SNP found in rs518673 or any of its associated SNP is an indication of a decreased likelihood that the prostate cancer will develop or recur.

9. The method of claim 7, wherein the presence of both rs518673T and rs12470143A or any of their associated SNPs is still a further indication of a decreased likelihood that the prostate cancer will develop or recur.

10. The method of claim 1, wherein in step a) said nucleic acid is DNA or RNA.

11. The method of claim 10, wherein said genetic variation of DNA or RNA is detected with the use of a nucleic acid probe.
12. The method of claim 11, wherein said DNA is amplified by PGR prior to incubation with the probe.

13. The method of claim 1, wherein the subject's nucleic acid-containing sample is a tumor or a non-tumor sample.

14. The method of claim 13, wherein the tumor sample originates from a biopsy.

15. The method of claim 13, wherein the subject's non-tumor sample is selected from the group consisting of: tissue or biological fluid.

16. The method of claim 15, wherein the tissue is selected from the group consisting of: lymph node, hair and buccal smear.

17. The method of claim 15, wherein the biological fluid is selected from the group consisting of: sputum, saliva, blood, seaim, urine, semen and plasma.

18. A method for adapting a course of treatment of prostate cancer in a human subject after the subject has undergone radical prostatectomy, comprising the steps of:
   a) providing a prognosis or predicting the likelihood of a human subject to develop prostate cancer recurrence in accordance with any one of claims 1 to 14; and
   b) adapting a course of treatment according to whether said subject has an increased or decreased likelihood that the cancer will recur.

19. The method according to claim 18, wherein, when the likelihood of recurrence is increased, the subject is prescribed 5a-reductase inhibitors therapy.

20. A kit for predicting the likelihood of a human subject to develop prostate cancer recurrence by detecting a SNP in a reference sequence selected from the group consisting of: rs518673; rs66050; rs12470143; rs2208532; rs2300702; rs4952197 and rs676033 or
their associated SNPs; said kit comprising reagents for determining the individual's genetic variations (or haplotypes) in SRD5A1 or SRD5A2 gene.

21. The kit of claim 20, comprising PGR primer-probe set, wherein the primer is selected from the group consisting of: SEQ ID Nos. 1 to 38; and the probe is selected from the group consisting of: SEQ ID Nos. 39 to 57.

22. The method of claim 10, wherein said genetic variation of DNA or RNA is detected with the use of a probeless methodology selected from the group consisting of: direct sequencing or pyrosequencing, massively parallel sequencing, high-throughput sequencing high performance liquid chromatography (HPLC) fragment analysis, and capillarity electrophoresis.

23. The method of claim 2, further comprising the step of: identifying the presence of rs523349 (V89L); whereby the presence of rs523349 in said subject's sample is an indication that said subject has an increased likelihood that the prostate cancer will develop or recur.
FIGURES

A) [Graph showing PSA and pG/pT categories with P values]

\[ \begin{align*}
\text{PSA} &> 10 - 20 & 0.081 \\
&> 20 & 0.003 \\
\text{pG} &\geq 7 & 0.002 \\
&\geq 8 & < 0.001 \\
\text{pT} &= T2c \\
&\geq T3a & -
\end{align*} \]

B) [Graph showing HR and number of rs for SRD5A1 and SRD5A2 with P values]

\[ \begin{align*}
\text{SRD5A1} &166050 & 0.035 \\
&501999 & - \\
&518673 & 0.004 \\
&3822430 & - \\
&500182 & - \\
&8192120 & - \\
&4702378 & - \\
&12470143 & 0.024 \\
&2281546 & - \\
&3754838 & - \\
&4952222 & - \\
&7562326 & - \\
&2208532 & 0.008 \\
&2300702 & 0.044 \\
&4952197 & 0.047 \\
&676033 & 0.017 \\
&523349 & 0.007 \\
&9282858 & - \\
&9332975 & -
\end{align*} \]

Figure 1
C) Combined effects of rs518673 (SRD5A1) and rs12470143 (SRD5A2)

Figure 2
### INTERNATIONAL SEARCH REPORT

**International application No.**

PCT/CA20 11/050326

#### A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C12Q 1/68** (2006.01), **C40B 30/00** (2006.01), **C40B 30/04** (2006.01), **C40B 40/06** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC.

#### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (2006.01): **C12Q 1/68, C40B 30/00, C40B 30/04, C40B 40/06**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

CL: TXTEN, EPDOC, MEDLINE, Canadian Patent Database

Keywords: alpha reductase, steroid dehydrogenase, SRD5A, prostate cancer, polymorphism, claimed rs#s

#### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>NAM, R. K. et al. (Jan. 20(1)) UROLOGY 57(1):199-204, ISSN 1527-9995 <em>whole document, especially abstract</em></td>
<td>1, 3, 10-19, 22</td>
</tr>
<tr>
<td>X</td>
<td>W09937986 A2 (REICHARDT J. K. et al.) 29 July 1999 (29-07-1999) <em>whole document, especially example 2 (pages 27-28) and claims 2-5</em></td>
<td>1, 3, 10-19, 22</td>
</tr>
</tbody>
</table>

[ ] Further documents are listed in the continuation of Box C. [X] See patent family annex

- * Special categories of cited documents
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish thepublication date of another invention or other special reasons (as specified)
  - "O" document referring to an oral disclosure, e.g. exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

- **"I"** interim document published after the international filing date or priority date and not so closely related to the application that it can be considered to be an inventive step under Article 54

- **"X"** documents of particular relevance to the claimed invention but not considered to be an inventive step under Article 54

- **"Y"** document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other relevant documents, such combination being obvious to a person skilled in the art

- **"Z"** document member of the same patent family

### Date of the actual completion of the international search

12 July 2011 (12-07-2011)

### Date of mailing of the international search report

13 September 2011 (13-09-2011)

## Name and mailing address of the ISA/CA

**Canadian Intellectual Property Office**

Place du Portage 1, C1 14 - 1st Floor, Box PCT

50 Victoria Street

Gatineau, Quebec K1A 0C9

Facsimile No.: 001-819-993-2476

Authorized officer

**Rebecca Minaker 819-994-9333**
INTERNATIONAL SEARCH REPORT

Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim Nos. : 18-19
   because they relate to subject matter not required to be searched by this Authority, namely:
   Claims 18-19 are directed to a method for treatment of the human or animal body by surgery or therapy which the international Search Authority is not required to search. However, this Authority has earned out a search based on the uses of the methods defined in claims 18-19.

2. [ ] Claim Nos. :
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim Nos. :
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Refer to Extra sheets 1-2

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Patent Document</th>
<th>Publication Date</th>
<th>Patent Family Member(s)</th>
<th>Publication Date</th>
</tr>
</thead>
</table>
Group 1: Claims 1-3 and 7-23 (all partially)

An in-vitro method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of obtaining a nucleic acid from a nucleic acid containing sample from said human subject and identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being rs518673 or any of its associated variants as listed in Table 3, whereby the presence of said SNP in said subject's sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur, and to methods for adapting a course of treatment, and to kits related thereto.

Group 2: Claims 1-4 and 10-23 (all partially)

An in-vitro method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of obtaining a nucleic acid from a nucleic acid containing sample from said human subject and identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being rs166050 or any of its associated variants as listed in Table 3, whereby the presence of said SNP in said subject's sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur, and to methods for adapting a course of treatment, and to kits related thereto.

Group 3: Claims 1-3, 7 and 9-23 (all partially)

An in-vitro method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of obtaining a nucleic acid from a nucleic acid containing sample from said human subject and identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being rs12470143 or any of its associated variants as listed in Table 3, whereby the presence of said SNP in said subject's sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur, and to methods for adapting a course of treatment, and to kits related thereto.

Group 4: Claims 1-6 and 10-23 (all partially)

An in-vitro method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of obtaining a nucleic acid from a nucleic acid containing sample from said human subject and identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being rs2208532 or any of its associated variants as listed in Table 3, whereby the presence of said SNP in said subject's sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur, and to methods for adapting a course of treatment, and to kits related thereto.

Group 5: Claims 1-4, and 10-23 (all partially)

An in-vitro method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of obtaining a nucleic acid from a nucleic acid containing sample from said human subject and identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being rs2300702 or any of its associated variants as listed in Table 3, whereby the presence of said SNP in said subject's sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur, and to methods for adapting a course of treatment, and to kits related thereto.

-Continued on next page-
Group 6: Claims 1-4, and 10-23 (all partially)

An in-vitro method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of obtaining a nucleic acid from a nucleic acid containing sample from said human subject and identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being rs4952197 or any of its associated variants as listed in Table 3, whereby the presence of said SNP in said subject’s sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur, and to methods for adapting a course of treatment, and to kits related thereto.

Group 7: Claims 1-5 and 10-23 (all partially)

An in-vitro method for providing a diagnosis, prognosis or predicting the likelihood of a human subject to develop prostate cancer or a recurrence thereof, said method comprising the steps of obtaining a nucleic acid from a nucleic acid containing sample from said human subject and identifying at least one single nucleotide polymorphism (SNP) in said nucleic acids, said SNP being rs676033 or any of its associated variants as listed in Table 3, whereby the presence of said SNP in said subject's sample is an indication that said subject has an increased or a decreased likelihood that the prostate cancer will develop or recur, and to methods for adapting a course of treatment, and to kits related thereto.

The requirements of unity of invention are not fulfilled in that there is no unique technical relationship among the inventions as they do not involve one or more of the same or corresponding technical features. The expression "special technical features" means those features which define a contribution which each of the claimed inventions considered as a whole makes over the prior art.

The unifying feature of the groups appears to relate to single nucleotide polymorphisms (SNPs) in the SRD5A1 or SRD5A2 genes which are indicative that a subject has an increased or a decreased likelihood that prostate cancer will develop or recur. This inventive concept is not new. For example, Nam et al. (Urology (2001) 57(1): 199-204) disclose that men who have the V allele of the V89L (rs 523349) polymorphism of the SRD5A2 gene have a twofold increase in the risk of prostate cancer development and an additional twofold increase in the risk of progression compared with men with the L/L genotype.