Androgen Receptor Polymorphism-Dependent Variation in Prostate-Specific Antigen Concentrations of European Men

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Abstract

Background: Androgens acting via the androgen receptor (AR) stimulate production of PSA, which is a clinical marker of prostate cancer. Because genetic variants in the AR may have a significant impact on the risk of being diagnosed with prostate cancer, the aim was to investigate whether AR variants were associated with the risk of having PSA above clinically used cutoff thresholds of 3 or 4 ng/mL in men without prostate cancer.

Methods: Men without prostate cancer history (n = 1,744) were selected from the European Male Ageing Study cohort of 40 to 79-year-old men from eight different European centers. Using linear and logistic regression models, with age and center as covariates, we investigated whether AR variants (CAG repeat-length and/or SNP genotype) were associated with having serum PSA concentrations above 3 or 4 ng/mL, which often are set as cutoff concentrations for further investigation of prostate cancer.

Results: Carriers of the SNP rs1204038 A-allele (16% of the men) were more likely to have PSA>3 and 4 ng/mL (OR; 95% confidence intervals, 1.65; 1.13–2.40 and 1.87; 1.18–2.96, respectively) than G-allele carriers. They also had shorter CAG repeats (median 20 vs. 23, P < 0.0005), but CAG repeat length per se did not affect the PSA concentrations.

Conclusion: The A-allele of the SNP rs1204038 gives a 65% higher risk of having PSA above 3 ng/mL than the G-allele in men without prostate cancer, and thereby an increased risk of being referred for further examination on suspicion of prostate cancer.

Impact: Serum PSA as a clinical marker could be improved by adjustment for AR-genotype. Cancer Epidemiol Biomarkers Prev; 23(10); 1–9. ©2014 AACR.

Introduction

The androgen receptor (AR) gene, located on chromosome Xq11-q12 encodes for a nuclear receptor (AR) that mediates the effects of testosterone and 5α-DHT by acting as a ligand-dependent transcription factor (1).

Androgens bind to the ligand-binding domain of the cytosolic AR. The activated AR then translocates into the nucleus where it, after forming a homodimer, binds to androgen response elements (ARE) in promoters and activates transcription of these genes. One gene that has been found to have several AREs and is activated by androgens is Kallikrein-related peptidase 3, KLK3 (2), encoding for the serine protease commonly known as prostate specific antigen (PSA) (3), which often are set as cutoff concentrations for further investigation of prostate cancer.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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PSA (3). In normal physiology, PSA has an important role in the seminal coagulum where it digests semenogelins after ejaculation, resulting in the liquefaction of semen and the release of motile spermatozoa (4).

Because disruption of the architecture of the prostate occurs in prostate cancer, with subsequent leakage of PSA into the circulation, PSA is commonly used as a marker for this disease and in the follow-up of patients treated with androgen ablation for prostate cancer. However, PSA is not a specific prostate cancer marker, as also ethnicity, age, inflammation, benign prostate hyperplasia as well as genetic factors are known to influence the concentration of circulating PSA (5); estimates of heritability show that approximately 45% of the variability in total PSA can be explained by inherited factors (6).

The AR’s transcriptional efficiency rests on the available amount of androgen as well as on the constitution of the receptor itself. Two repetitive sequences in the transactivation domain of the AR gene, generally designated the CAG and GGN repeat encoding for a polyglutamine and a polyglycine tract, respectively, are involved in the fine-tuning of the AR function (7). The repetitive CAG-region in the AR varies in number in the general population, ranging from approximately 10 to 30 repeats (8). Extreme lengths (>40 base triplets) causes the late-onset neuromuscular disorder spinal bulbar muscular atrophy (SBMA), also known as Kennedy disease (9). Men affected by SBMA show symptoms of mild androgen insensitivity, often presented as hypogonadism, gynecomastia, and a reduction in sperm production as the disease progresses.

Within the normal CAG length range, the CAG repeat distribution has been found to differ between populations so that African Americans generally tend to have shorter alleles than Asian populations, whereas Caucasian populations place intermediate (10, 11). This pattern is inversely mirroring the risk of prostate cancer, as African American men have the highest risk, Asian men a low risk, and Caucasians an intermediate risk of being diagnosed with prostate cancer (12). Extensive research about the association between the CAG stretch and prostate cancer in different populations has over the years resulted in numerous reports, overall showing inconclusive results (13–15). In a study of men without histologic evidence of prostate cancer but with PSA above 4 ng/mL, African American men had significantly higher PSA levels when compared with White men (16).

Missense mutations in the AR in 46XY individuals lead to androgen insensitivity, manifesting from female phenotype in its most severe form to subfertility in phenotypic males in its mildest form (17). Some AR mutations, however, most of them somatic, have also been found in prostate cancer (http://androgendb.mcgill.ca/). One synonymous variant, E213 (rs6152G>A), is located between the two polymorphic CAG and GGN tracts. This variant has been linked to androgenetic alopecia in Caucasian populations, where the minor allele (rs6152A) in these populations was associated with a decreased risk (18–20). In a previous EMAS analysis, carriers of the rs6152A variant were also found to have significantly lower estradiol levels than those with rs6152G (21).

Because the levels of serum PSA are widely used in population screening for the selection of men to be referred for urological examination on the suspicion of prostate cancer, and PSA transcription is initiated by the AR, genetic variants in the AR may have a significant impact on the chance to be diagnosed with prostate cancer. The objective of the current study was, therefore, to investigate the effects of AR haplotypes as well as CAG repeat length on PSA concentrations in serum of men without previous diagnosis of prostate cancer and the impact of testosterone concentration as an interacting parameter. In addition, we wished to explore whether there was a link between the AR genotype and prostate cancer risk.

Materials and Methods

Subjects

The European Male Ageing Study (EMAS) is a prospective study in which baseline information for 3,369 men from the general population, ages 40 to 79 years in eight European centers in Italy, the United Kingdom, Poland, Sweden, Belgium, Spain, Estonia, and Hungary was collected during the period 2003–2005 (22). After a median of 4.3 years after enrollment, a follow-up was done (23) and a postal questionnaire sent out. This questionnaire included questions about the prostate where the men were asked whether they at the time of the follow-up were being treated for an enlarged prostate and whether the enlargement was benign or cancerous. The follow-up questionnaire also included questions about previous cancer diagnoses. The self-reported prostate and prostate cancer status assessed from this questionnaire was used as criteria for the inclusion in current study.

The 633 men in EMAS who did not participate in the follow-up were excluded. Using the data collected from the follow-up questionnaire, all men who claimed to be currently being treated for prostate cancer, those with a previous prostate cancer diagnosis, and those who did not report their prostate health status (Fig. 1) were excluded from the tests on association between genotype and PSA analysis but those who reported a previous or current prostate cancer were included in a separate analysis on AR variants in relation to risk of this malignancy. Furthermore, all men who had not been genotyped for variants in the AR or did not have their baseline PSA measured were excluded (all participants from the Hungarian center), leaving a final inclusion frequency of 64%.

All men participated after informed consent (22). The study was approved by the ethical committee boards of the respective countries.

Genotyping

Of the included men, 1,804 had been genotyped about the AR’s CAG repeat as well as five SNPs: rs6152, rs1204038, rs2255702, rs7061037, and rs5918760, located...
within the AR (21, 24). The CAG length was successfully genotyped in 1,687 of the 1,804 men. The most common CAG-allele was 21CAG with a frequency of 16% (Fig. 2A). The mean and the median length was 22CAG, consistent with previous studies on European populations (25).

The SNP genotyping was successful in all five loci in 1,582 of the 1,804 men (88%) and of these men, 1,573 (99%) belonged to one of the two most common haplotypes. The SNPs were all in strong linkage disequilibrium ($r^2 > 0.96$ for all pairwise comparisons). Because rs1204038 had the highest genotyping frequency, with 99.6% of the men with available DNA successfully genotyped, its two alleles, G and A, with allele frequencies of 84% and 16%, respectively, were used in all calculations. The strongest linkage of this SNP in the EMAS cohort was with the exonic SNP rs6152 ($r^2 = 0.99$).

As frequencies of variants in the AR as well as PSA levels and prostate cancer risk have been found to differ between populations, to confirm that our cohort is representative for the European men, the genotype data for the men in the EMAS were compared with distribution of the same SNPs in the phase I data from the 1,000 genomes project (ref. 26; http://www.ensembl.org/).

**Hormone and PSA assessment**

The testosterone and sex hormone binding globulin (SHBG) analyses were centralized and the methods used for measuring hormone concentrations have previously been described (21).

In brief, serum was separated from fasting morning blood samples (22). The serum samples were then assayed for SHBG using electrochemoluminescence immunoassays (27). Each sample was also assayed for total testosterone using gas chromatography-mass spectrometry as described before (28, 29). Free T levels were calculated according to the formula by Vermeulen and colleagues (30).

Serum PSA was measured at each center according to the local standard procedure (Table 1). All PSA analyses were conducted both with and without outliers defined as In-transformed PSA $>3SD$ away from mean ($n = 9$).
Concentrations. The analyses on SNP genotype and PSA concentration was not dependent on testosterone; it was also assessed to ensure that the effect of genotype on PSA concentration was not dependent on testosterone levels (total or free) in relation to PSA concentration. The interaction between genotype (one of the three above mentioned categorizations) and testosterone levels, potential differences in ln-transformed PSA distribution of age in the carriers of the different genotypes were investigated. The interaction between genotype (one of the three above mentioned categorizations) and testosterone levels (total or free) in relation to PSA concentration was also assessed to ensure that the effect of genotype on PSA concentration was not dependent on testosterone concentrations. The analyses on SNP genotype and PSA levels were also performed using testosterone as a covariate (Supplementary Tables S1–S4).

Finally, using the information from the follow-up questionnaire, the ORs for ever having been diagnosed with prostate cancer for different genotypes were calculated by means of logistic regression analysis with center and age as covariates. This was done to deduce if carriers of the variant with increased PSA levels were more prone to be diagnosed with prostate cancer. Among the 2,736 men with follow-up data available, 932 were excluded because of lack of DNA for genetic analysis or lack of baseline PSA data.

To test the robustness of the association between genotype and PSA levels in relation to previous risk of being diagnosed with prostate cancer, thereby ensuring that the difference in PSA levels was not due to a larger number of men with prostate cancer being excluded from one group, the analysis of the association between genotype and PSA was repeated for men younger than 50 years (n = 440), because no men in this age span had been diagnosed with prostate cancer.

Statistical analyses were conducted using the SPSS 20 software (SPSS, Inc.).

### Results

#### CAG repeat lengths in relation to SNP genotype

A significant difference in the CAG-allele distribution was found between the G and the A-allele in all centers, as well as in the total EMAS-group, that is, carriers of rs1204038G had longer CAG repeats than rs1204038A (mean, 95% CI, 22.5, 22.4–22.7 vs. 19.9, 19.7–20.2; P < 0.0005; Fig. 2B and Supplementary Table S1).

#### PSA in relation to genotype

When PSA concentrations in carriers of the two alleles were compared, men with the A-allele had 14% higher PSA concentration than carriers of the G-allele (P = 0.045 including outliers, and P = 0.007 excluding outliers).
although not statistically significantly so, for carriers of having PSA increased for carriers of the groups (Table 3). No difference in OR was seen for the three other tested SNPs were similar (Supplementary Table S6). No difference in OR was seen in each center (Supplementary Table S3). Results for except for Spain (Supplementary Table S4). The results for the other genotyped SNPs were similar (Supplementary Table S7). When the centers were pooled, the OR was significantly increased for men with the A-allele. This result was independent of CAG length, although no subjects with high PSA and long CAG were found. When outliers were removed, the OR for carriers of the A-allele with average CAG-length remained significantly increased (Table 4).

### Table 2. Comparison of mean PSA concentrations between the rs1204038 genotype and ORs for having PSA above clinically applied cutoff values in men with no prostate cancer

<table>
<thead>
<tr>
<th>Allele</th>
<th>Crude PSA (ng/mL)</th>
<th>Mean (95% CI)</th>
<th>PSA &gt; 3 ng/mL</th>
<th>OR (95% CI)</th>
<th>n</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>PSA &gt; 3 ng/mL</td>
<td></td>
<td></td>
<td></td>
<td>PSA &gt; 4 ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outliers included</td>
<td>G</td>
<td>1,459</td>
<td>1.48 (1.38–1.58)</td>
<td>Ref.</td>
<td>143</td>
<td>Ref.</td>
<td>81</td>
<td>Ref.</td>
<td>1.90 (1.21–2.99)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>278</td>
<td>1.76 (1.49–2.04)</td>
<td>0.045c</td>
<td>42</td>
<td>0.008c</td>
<td>81</td>
<td>Ref.</td>
<td>1.87 (1.18–2.96)</td>
</tr>
<tr>
<td>Outliers removed</td>
<td>G</td>
<td>1,454</td>
<td>1.46 (1.38–1.55)</td>
<td>Ref.</td>
<td>142</td>
<td>Ref.</td>
<td>80</td>
<td>Ref.</td>
<td>1.90 (1.21–2.99)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>274</td>
<td>1.72 (1.48–1.95)</td>
<td>0.007c</td>
<td>41</td>
<td>0.009c</td>
<td>80</td>
<td>Ref.</td>
<td>1.87 (1.18–2.96)</td>
</tr>
</tbody>
</table>

*Calculated using ln-transformed values, adjusting for center.
*Adjusted for center.
*Significant at the 0.05 level.

### OR for PSA above the clinical cutoff level

With carriers of the G-allele as reference, the OR of having PSA >3 or 4 ng/mL was significantly increased in carriers of the A-allele (Table 2). The same tendency was seen in each center (Supplementary Table S3). Results for the other tested SNPs were similar (Supplementary Table S6). No difference in OR was seen for the three CAG groups (Table 3).

For the combination of the SNP and CAG repeats, using the G-allele in combination with short CAG as reference, men with the A-allele and average CAG had 15% higher PSA concentration than the reference (P = 0.041). The remaining combinations did not differ significantly from the reference.

### Interaction of genotype and testosterone in relation to PSA levels

The levels of ln-transformed free and total testosterone did not differ significantly between the two alleles of rs1204038, neither before nor after adjustment for age. When the shorter CAG-repeat group was compared with the middle and the longer CAG-group, the shorter CAG-allele group had significantly lower levels of ln-transformed free testosterone than the longer CAG repeat group both before (P = 0.032) and after (P = 0.027) adjustment for age. The same tendency was seen for ln-transformed total testosterone, but not significantly so.

However, the interaction analysis between the different genotype groups and free or total testosterone did not reveal significant associations in relation to the PSA level (data not shown).

### Table 3. Comparison of adjusted PSA between CAG lengths and ORs for having PSA >3 and >4 ng/mL

<table>
<thead>
<tr>
<th>CAG</th>
<th>n</th>
<th>Crude PSA (ng/mL)</th>
<th>Mean (95% CI)</th>
<th>PSA &gt; 3 ng/mL</th>
<th>OR (95% CI)</th>
<th>n</th>
<th>P</th>
<th>PSA &gt; 4 ng/mL</th>
<th>OR (95% CI)</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>PSA &gt; 3 ng/mL</td>
<td></td>
<td></td>
<td></td>
<td>PSA &gt; 4 ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outliers included</td>
<td>&lt;21CAG</td>
<td>520</td>
<td>1.51 (1.35–1.68)</td>
<td>Ref.</td>
<td>62</td>
<td>Ref.</td>
<td>32</td>
<td>Ref.</td>
<td>1.24 (0.77–2.00)</td>
<td>43</td>
<td>0.378</td>
</tr>
<tr>
<td></td>
<td>21–23CAG</td>
<td>592</td>
<td>1.56 (1.42–1.70)</td>
<td>0.682</td>
<td>63</td>
<td>0.550</td>
<td>32</td>
<td>Ref.</td>
<td>1.24 (0.77–2.00)</td>
<td>43</td>
<td>0.378</td>
</tr>
<tr>
<td></td>
<td>&gt;23CAG</td>
<td>575</td>
<td>1.46 (1.29–1.64)</td>
<td>0.96</td>
<td>53</td>
<td>0.186</td>
<td>32</td>
<td>Ref.</td>
<td>1.24 (0.77–2.00)</td>
<td>43</td>
<td>0.378</td>
</tr>
<tr>
<td>Outliers removed</td>
<td>&lt;21CAG</td>
<td>517</td>
<td>1.48 (1.33–1.63)</td>
<td>Ref.</td>
<td>61</td>
<td>Ref.</td>
<td>31</td>
<td>Ref.</td>
<td>1.28 (0.79–2.07)</td>
<td>43</td>
<td>0.319</td>
</tr>
<tr>
<td></td>
<td>21–23CAG</td>
<td>589</td>
<td>1.57 (1.43–1.71)</td>
<td>0.385</td>
<td>63</td>
<td>0.605</td>
<td>31</td>
<td>Ref.</td>
<td>1.28 (0.79–2.07)</td>
<td>43</td>
<td>0.319</td>
</tr>
<tr>
<td></td>
<td>&gt;23CAG</td>
<td>572</td>
<td>1.41 (1.28–1.54)</td>
<td>0.501</td>
<td>52</td>
<td>0.181</td>
<td>31</td>
<td>Ref.</td>
<td>1.28 (0.79–2.07)</td>
<td>43</td>
<td>0.319</td>
</tr>
</tbody>
</table>

*Calculated using ln-transformed values, adjusting for center.
*Adjusted for center.
Cancer Epidemiology, Biomarkers & Prevention
Cancer Epidemiol Biomarkers Prev; 23(10) October 2014

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Table 4. Comparison of adjusted PSA between CAG categories in combination with rs1204038 genotype and ORs of having PSA above clinically accepted cutoff points

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Crude PSA (ng/mL)</th>
<th>PSA &gt; 3 ng/mL</th>
<th>PSA &gt; 4 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95%CI) P</td>
<td>OR (95% CI)</td>
<td>n P</td>
</tr>
<tr>
<td>Outliers included G</td>
<td>&lt;21CAG</td>
<td>1.43 (1.26-1.60) Ref.</td>
<td>Ref. 37 Ref. 18 Ref.</td>
</tr>
<tr>
<td>G</td>
<td>21-23CAG</td>
<td>1.55 (1.39-1.71)      0.388</td>
<td>1.11 (0.71-1.74) 50 0.652</td>
</tr>
<tr>
<td>G</td>
<td>&gt;23CAG</td>
<td>1.46 (1.28-1.64)      0.979</td>
<td>0.93 (0.60-1.46) 51 0.754</td>
</tr>
<tr>
<td>A</td>
<td>&lt;21CAG</td>
<td>1.40 (1.36-2.17) 0.121</td>
<td>2.05 (1.18-3.57) 25 0.011b 2.19 (1.05-4.55b 14 0.036b</td>
</tr>
<tr>
<td>A</td>
<td>21-23CAG</td>
<td>1.61 (1.32-1.91) 0.295</td>
<td>1.11 (0.57-2.18) 13 0.755</td>
</tr>
<tr>
<td>A</td>
<td>&gt;23CAG</td>
<td>1.68 (0.53-2.82) 0.269</td>
<td>2.05 (0.23-18.32) 1 0.520</td>
</tr>
<tr>
<td>Outliers removed G</td>
<td>&lt;21CAG</td>
<td>1.43 (1.26-1.60) Ref.</td>
<td>Ref. 37 Ref. 18 Ref.</td>
</tr>
<tr>
<td>G</td>
<td>21-23CAG</td>
<td>1.55 (1.39-1.71) 0.358</td>
<td>1.11 (0.71-1.74) 50 0.661</td>
</tr>
<tr>
<td>G</td>
<td>&gt;23CAG</td>
<td>1.40 (1.27-1.54) 0.985</td>
<td>0.91 (0.58-1.43) 50 0.684</td>
</tr>
<tr>
<td>A</td>
<td>&lt;21CAG</td>
<td>1.64 (1.34-1.94) 0.112</td>
<td>1.98 (1.13-3.47) 24 0.017a 2.04 (0.97-4.31) 13 0.061</td>
</tr>
<tr>
<td>A</td>
<td>21-23CAG</td>
<td>1.64 (1.34-1.94) 0.041b 1.12 (0.57-2.20) 13 0.735</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>&gt;23CAG</td>
<td>1.68 (0.53-2.82) 0.247</td>
<td>2.04 (0.23-18.18) 1 0.524</td>
</tr>
</tbody>
</table>

Discussion

The main finding of the current study was the almost doubled OR of having serum PSA levels above 3 ng/mL or 4 ng/mL in European men with the A-allele in the AR SNP rs120438. The same men, representing 16% of Caucasians, had one third of the OR of being previously diagnosed with prostate cancer when compared with the carriers of the G-allele.

Measurements of PSA levels in serum are widely used as a selection procedure in prostate cancer diagnosis before referral for urological examination, transrectal ultrasound, and biopsy. Because prostate tumors often remain unnoticed during a long time period, PSA in the circulation may influence the chance of being diagnosed with this disease or not. Early diagnosis and treatment may be beneficial in terms of higher chance of successful treatment and survival but with the risk of overdagnosis and overtreatment of clinically irrelevant tumors which may have negative implications in relation to life quality and financial costs (31–33).

According to our findings in subjects without a prostate cancer diagnosis, carriers of the rs120438 A-allele may be at higher probability of being referred for urological examination due to higher PSA levels. Although we found that the history of previous prostate cancer was more common in counterparts with the G-allele, the trend of higher PSA levels in carriers of the A-allele was even observed in the youngest age group, which did not include any subjects with a prostate cancer history.

Our finding is in agreement with two previous studies, which reported lower risk of metastatic prostate cancer (19) and an overall decreased risk of prostate cancer (34) in men with the rs6152 A-allele, which is strongly linked with the A-allele of the SNP used in current study. Other

Genotype in relation to the risk of having been diagnosed with prostate cancer

Of the men who were eligible for this study, 60 had reported previous or current prostate cancer diagnosis. The age and center adjusted OR of having been diagnosed with prostate cancer for carriers of the A-allele was significantly decreased (OR, 95% CI, 0.27, 0.08–0.88) when compared with carriers of the G-allele.

Among men being younger than 50 years at baseline, none of the subjects were diagnosed with prostate cancer and few of these men had PSA above 3 (n = 9) or 4 (n = 4) ng/mL. Although not statistically significant, the association about PSA levels for the A-allele carriers was similar to those found in the entire cohort; OR for having PSA >3 ng/mL being 3.24 (95% CI, 0.74–14.15, P = 0.118) and >4 ng/mL: 4.47 (95% CI, 0.59–34.10, P = 0.149) both with and without outliers.

Comparison of genotype distribution with 1,000 genomes

In the 580 X-chromosomes from the EUR super-population, only the two haplotypes found in EMAS were present at a frequency >1%. The allele distribution of rs1204038 mirrored that of EMAS, as the G-allele was present in 86%, whereas the A-allele was present in 14% of the participants. The frequency of the alleles differed between the super-populations from 1,000 genomes where G was present in 100% of the East-Asian ASN population but fairly uncommon (9%) in the African AFR super-population.

The $r^2$ value for the linkage between rs1204038 and rs6152, the two SNPs in which the $r^2$ was the highest in EMAS, also differed for the super-populations but in the EUR the $r^2$ was similar to the $r^2$ found in EMAS ($r^2 = 0.97$).
studies did not find this association with prostate cancer (35, 36), but these study populations were of multiethnic origin, which may have blurred the results.

A previous study on the relationship between CAG-length and PSA levels reported that shorter CAG-alleles were associated with high PSA concentrations in serum of older men (37), as well as in semen of young men (38). Another study on elderly men without prostate cancer, but with urinary tract symptoms, did not find this pattern when dividing CAG-length into quartiles (39). The large number of participants from several European countries included in current study allowed the CAG distribution of the different haplotypes to be independently tested in a number of subpopulations. In the present study, the CAG length per se did not significantly affect the results, but carriers of the rs1204038A allele had shorter CAG stretches than those with the G-allele, indicating that some of the effects found in previous association studies between CAG-length and PSA could be due to an overrepresentation of carriers of the A-allele in subjects with short CAG repeat.

The underlying molecular mechanism of our finding is still unknown, but the high linkage disequilibrium between SNPs in the AR suggests that rs1204038 is a good predictor of the closely located SNP rs6152 (E213). The G to A substitution in this SNP does not change the amino acid sequence, but new data indicating that synonymous SNPs can affect RNA levels by changing, for example, splicing, protein expression levels and folding, have accumulated and the list of synonymous mutations linked to human diseases is constantly expanding (40, 41). Therefore, it cannot be excluded that E213, the only common SNP in the protein coding region of the AR, would somehow affect AR function, possibly in combination with the CAG tract or even through linkage with currently undiscovered regulatory elements outside of the gene.

The PSA screening procedure has been criticized for having low specificity, leading to unnecessary prostate biopsies. In some clinical units, age-specific cutoff levels are applied, but whether this routine is of relevance is debated (42). This has encouraged searches for genetic variants that can be used to adjust or enhance the PSA test and thereby personalizing the result. By creating a risk profile containing a combination of SNPs identified through GWAS studies associated with prostate cancer-risk or changes in PSA-levels, some groups have aimed at constructing genetic risk adjusted PSA cutoff levels (43, 44). Others have combined the risk profiles with the measured PSA in the selection of men that should be referred to biopsy (45–50). However, the results have been promising but not convincing. Some did not find that the genetic profile enhanced the outcome at all (46), whereas others reported an improvement, but often not large enough to alone support genetic testing as a clinical strategy (45, 47, 48, 50). These studies shared certain SNPs, while other SNPs were study specific. With the large amount of SNPs that are found to be associated with prostate cancer risk discovered every year through GWAS studies, and the many SNPs that are found to affect the normal PSA level in a man, it is possible that the optimal combination of SNPs that should be included to construct the genetic risk profile has not been discovered. As the minor allele of the SNP used in the current study is associated with both a decreased risk of prostate cancer diagnosis and higher PSA levels, it is possible that its inclusion could improve the performance of the genetic models for PSA adjustment.

The strength of this study is that a large and general population-based cohort of subjects was utilized, including subjects from several countries, not recruited for a study on prostate cancer. A limitation of this study was the relatively high number of excluded subjects. However, we have no reason to believe that this should lead to any significant “selection bias” that would influence the results of this study. Another limiting factor was that the prostate cancer information was self-reported, which did not allow for a more in-depth analysis of the clinical features of the prostate cancer cases. There is a risk that symptom-free men could have a latent tumor. This would, however dilute the results rather than give false-positive results. In addition, the retrospective nature of the study gives a risk of recall bias, and therefore the association with prostate cancer needs to be validated. In addition, the measurements of PSA concentrations were noncentralized. However, to minimize this effect, center was included as a covariate in the assessment of associations between genotype and PSA. The analyses were also performed in each center separately and even though this resulted in a loss of power, the trend was similar at each location except for in Spain. However, suggested by the highly nonsignificant $P$ values, this deviation from the trend is most likely resulting from the smaller sample size.

In summary, the current study indicates that screening for prostate cancer by use of PSA measurements could benefit from being adjusted to the AR rs1204038 variant, because carriers of the A-allele in this position have 65% higher OR of presenting with PSA concentrations higher than 3 ng/mL than carriers of the G-allele, but a lower risk of prostate cancer diagnosis.

Disclosure of Potential Conflicts of Interest

A. Giwercman receives honoraria from speakers’ bureau from pharmaceutical companies for lecturing and is a consultant/advisory board member for Bayer AG. F.C.W. Wu reports receiving commercial research grant from Eli Lily, receives honoraria from speakers’ bureau from Besins Healthcare, Bayer Schering, and is a consultant/advisory board member for Besins Healthcare. No potential conflicts of interest were disclosed by the other authors.

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Research.
References


Androgen Receptor Polymorphism-Dependent Variation in Prostate-Specific Antigen Concentrations of European Men

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