Androgen receptor polymorphism dependent variation in prostate specific antigen concentrations of European men.

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Running title
Androgen receptor dependent variation in PSA

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Conflict of interests
None to declare
Abstract

Background

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

Methods

Men without PCa history (n=1744) were selected from the European Male Ageing Study (EMAS) cohort of 40-80 year old men from 8 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 cut-off concentrations for further investigation of PCa.

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%CI 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 PCa, and thereby an increased risk of being referred for further examination on suspicion of PCa.

Impact

Serum PSA as a clinical marker could be improved by adjustment for AR-genotype.
Introduction

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

Androgens bind to the ligand binding domain (LBD) of the cytosolic AR. The activated AR then translocates into the nucleus where it, after forming a homodimer, binds to androgen response elements (AREs) in promoters and enhancers of androgen dependent genes and subsequently 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). 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).

Since disruption of the architecture of the prostate occurs in prostate cancer (PCa), 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 PCa. However, PSA is not a specific PCa 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’s 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, while Caucasian populations place intermediate (10, 11). This pattern is inversely mirroring the risk of PCa, as African American men have the highest risk, Asian men a low risk, and Caucasians an intermediate risk of being diagnosed with the disease (12). Extensive research regarding the association between the CAG stretch and PCa in different populations has over the years resulted in numerous reports, overall showing inconclusive results (13-15). In a study of men without histological evidence of PCa but with PSA above 4ng/mL, African-American men had significantly higher PSA levels when compared to 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 PCa (http://androgendb.mcgill.ca/). One synonymous variant, E211 (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).

Since 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 PCa, and PSA transcription is initiated by the AR, genetic variants in the AR gene may have a significant impact on the chance to be diagnosed with PCa. 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 PCa and the impact of testosterone concentration as an interacting parameter. Additionally, we wished to explore if there was a link between the AR genotype and PCa risk.
Material and Methods

Subjects

The European Male Ageing Study (EMAS) is a prospective study in which baseline information for 3369 men from the general population, aged 40-79 years in 8 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 if they at the time of the follow-up were being treated for an enlarged prostate and if the enlargement was benign or cancerous. The follow-up questionnaire also included questions about previous cancer diagnoses. The self-reported prostate and PCa 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 currently be treated for PCa, those with a previous PCa diagnosis, and those who did not report their prostatic health status (Figure 1) were excluded from the tests on association between genotype and PSA analysis but those who reported a previous or current PCa 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, 1804 had been genotyped regarding 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 1687 of the 1804 men. The most common CAG-allele was 21CAG with a frequency of 16% (Figure 2A). The mean and the median length was 22CAG, consistent with previous studies on European populations (25).

The SNP genotyping was successful in all 5 loci in 1582 of the 1804 men (88%) and of these men, 1573 (99%) belonged to one of the two most common haplotypes. The SNPs were all in strong linkage disequilibrium (LD) ($r^2 > 0.96$ for all pairwise comparisons). Since 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 PCa risk have been found to differ between populations, in order 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 1 data from the 1000 genomes project (26) (http://www.ensembl.org/).

Hormone and PSA assessment

The testosterone and 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 et al. (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 ln-transformed PSA >3SD away from mean (n=9).

Statistics

The data on PSA concentration are given as means and 95% confidence intervals (95% CI). The CAG length was divided into three groups of similar sizes containing short (≤20CAG, n=520), average length (21-23CAG, n=592) and long (≥24CAG, n=575) alleles. The CAG-groups and the SNPs were tested independently, but they were also combined into 6 groups, rs1204038G or rs1204038A combined with short, average or long CAG-groups.

Primarily, using the T-test, we investigated whether the SNP alleles differed by the CAG repeat number. Thereafter, the association between PSA levels and genotype was investigated in a univariate linear regression model. PSA values were ln-transformed in order to obtain a normal distribution of residuals. Means and 95% CI are presented as back-transformed values. Since the PSA measurements were decentralized, all analyses included center as covariate.

Subsequently, the impact of AR genotype on the risk of having PSA above the commonly used cut off values for referring patients for urological examination, 3ng/mL or 4ng/mL, was
investigated using logistic regression. The results of these analyses are presented as Odds ratios (OR) and 95%CI. Age was not included in the final statistical model as the age did not differ between the genotype groups. However, to assure that results were not due to an uneven distribution of age in the carriers of the different genotypes, the analyses were also performed with age as covariate (Supplemental Table 1-4). Additionally, since PSA levels may not only depend on AR genotype but also on testosterone levels, potential differences in ln-transformed free and total testosterone in 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 (Supplemental Table 1-4).

Finally, using the information from the follow-up questionnaire, the ORs for ever having been diagnosed with PCa for different genotypes was 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 PCa. Among the 2736 men with follow-up data available, 932 were excluded due to lack of DNA for genetic analysis or lack of baseline PSA data.

In order to test the robustness of the association between genotype and PSA levels in relation to previous risk of being diagnosed with PCa, thereby ensuring that the difference in PSA levels was not due to a larger number of men with PCa being excluded from one group, the analysis of the association between genotype and PSA was repeated for men younger than 50 years (n=440), since no men in this age span had been diagnosed with PCa.
Statistical analyses were conducted using the SPSS 20 software (SPSS, Inc., Chicago).
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 i.e. 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; Figure 2B; 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, Table 2). The tendency was the same in all centers except in Spain (Table S2). Calculations were also performed for the other tested SNPs (Table S5). The PSA concentrations did not differ between the three CAG-groups (p=0.520 with outliers, p=0.277 without outliers).

For the combined 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.

OR for PSA above the clinical cut off 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 (Table S3). Results for the other tested SNPs were similar (Table S6). No difference in OR was seen for the three CAG groups (Table 3).

For the combination of the SNP and CAG the OR for having PSA>3 ng/mL was statistically significantly increased for carriers of the A-allele and short CAG.

For PSA>4ng/mL, the OR was higher in all centers although not statistically significantly so, for carriers of A except for Spain (Table S4). The results for the other genotyped SNPs were similar (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 was found. When outliers were removed, the OR for carriers of the A-allele with average CAG-length remained significantly increased (Table 4).

**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 to 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).
**Genotype in relation to the risk of having been diagnosed with PCa**

Of the men who were eligible for this study, 60 had reported previous or current PCa diagnosis. The age and center adjusted OR of having been diagnosed with PCa 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 PCa and few of these men had PSA above 3 (n=9) or 4 (n=4) ng/mL. Although not statistically significant, the association regarding PSA levels for the A-allele carriers as compared to G-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 1000 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% while the A-allele was present in 14% of the participants. The frequency of the alleles differed between the super-populations from 1000 genomes where G was present in 100% of the ASN population but fairly uncommon (9%) in the 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 (\( r^2=0.97 \)).

**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 rs1204038. The same men, representing 16% of Caucasians, had one third of the OR of being previously diagnosed with PCa when compared to the carriers of the G-allele.

Measurements of PSA levels in serum are widely used as a selection procedure in PCa diagnosis prior to referral for urological examination, transrectal ultrasound and biopsy. Since 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 over-diagnosis and over-treatment 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 PCa diagnosis, carriers of the rs1204038 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 PCa 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 PCa history.

Our finding is in agreement with two previous studies, which reported lower risk of metastatic PCa (19) and an overall decreased risk of PCa (34) in men with the rs6152 A-allele, which is strongly linked with the A-allele of the SNP used in current study. Other studies did not find this association with PCa (35, 36), but these study populations were of multi-ethnic 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 PCa, 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 sub-populations. 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 LD between SNPs in the AR suggests that rs1204038 is a good predictor of the closely located SNP rs6152 (E211). 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 e.g. 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 E211, 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 cut off 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 PCa-risk or changes in PSA-levels, some groups have aimed at constructing genetic risk adjusted PSA cut-off 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) while 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 PCa-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 aggressive PCa 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 PCa. 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 PCa information was self-reported, which did not allow for a more in depth analysis of the clinical features of the PCa 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 PCa needs to be validated. In addition, the measurements of PSA concentrations were non-centralized. 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 non-significant 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 PCa by use of PSA measurements could benefit from being adjusted to the $AR$ rs1204038 variant, since 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 PCa diagnosis.
References


42. Luboldt H-J, Schindler JF, Rübben H. Age-Specific Reference Ranges for Prostate-Specific Antigen as a Marker for Prostate Cancer. EAU-EBU Update Series. 2007;5:38-48.


Table 1. The methods with which the PSA was measured in each center as well as the different levels

<table>
<thead>
<tr>
<th>Center</th>
<th>Method of measurement</th>
<th>PSA at baseline (ng/mL)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florence, Italy</td>
<td>Electrochemiluminescence Immunoassay</td>
<td>2.02 (1.27-2.76)</td>
<td>432 (99.8)</td>
</tr>
<tr>
<td>Leuven, Belgium</td>
<td>Immunoassay – Sandwich – Electro Chemical Luminescence (ECL)</td>
<td>1.77 (1.48-2.05)</td>
<td>442 (98)</td>
</tr>
<tr>
<td>Lodz, Poland</td>
<td>Microparticle Enzyme Immunoassay (MEIA) (Abbott AxSYM System)</td>
<td>1.66 (1.41-1.92)</td>
<td>408 (100)</td>
</tr>
<tr>
<td>Malmö, Sweden</td>
<td>Sandwich Assay</td>
<td>2.07 (1.76-2.39)</td>
<td>408 (99.8)</td>
</tr>
<tr>
<td>Manchester, England</td>
<td>Electrochemiluminescence immunoassay</td>
<td>1.80 (1.57-2.02)</td>
<td>342 (86.4)</td>
</tr>
<tr>
<td>Santiago, Spain</td>
<td>Quimioluminescence</td>
<td>2.20 (1.52-2.89)</td>
<td>404 (99.5)</td>
</tr>
<tr>
<td>Szeged, Hungary</td>
<td>Immulite 1000 CLIA (3rd generation)</td>
<td>NA</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tartu, Estonia</td>
<td>CLIA</td>
<td>1.73 (1.51-1.94)</td>
<td>430 (98.9)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1.89 (1.72-2.06)</td>
<td>2866 (85.1)</td>
</tr>
</tbody>
</table>

NA, not analysed.
Table 2. Comparison of mean PSA concentrations between the rs1204038 genotype and odds ratios for having PSA above clinically applied cut-off values in men with no PCa.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Crude PSA (ng/mL)</th>
<th>PSA &gt;3ng/mL</th>
<th>PSA &gt;4ng/mL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (95% CI)</td>
<td>p^B</td>
<td>OR (95% CI)^A</td>
</tr>
<tr>
<td>Outliers included</td>
<td>G</td>
<td>1459</td>
<td>1.48 (1.38-1.58)</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>278</td>
<td>1.76 (1.49-2.04)</td>
<td>0.045*</td>
</tr>
<tr>
<td>Outliers removed</td>
<td>G</td>
<td>1454</td>
<td>1.46 (1.38-1.55)</td>
<td>Ref</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>274</td>
<td>1.72 (1.48-1.95)</td>
<td>0.007*</td>
</tr>
</tbody>
</table>

^A Adjusted for center
^B Calculated using ln-transformed values, adjusting for center
* Significant at the 0.05 level
Table 3. Comparison of adjusted PSA between CAG lengths and odds ratios of having PSA >3 and >4 ng/mL.

<table>
<thead>
<tr>
<th>CAG</th>
<th>n</th>
<th>Mean (95%CI)</th>
<th>p&lt;sup&gt;B&lt;/sup&gt;</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>Outliers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>included</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;21CAG</td>
<td>520</td>
<td>1.51 (1.35-1.68)</td>
<td>Ref</td>
<td>Ref</td>
<td>62</td>
<td>Ref</td>
<td>Ref</td>
<td>32</td>
<td>Ref</td>
</tr>
<tr>
<td>21-23CAG</td>
<td>592</td>
<td>1.56 (1.42-1.70)</td>
<td>0.682</td>
<td>0.89 (0.61-1.30)</td>
<td>63</td>
<td>0.550</td>
<td>1.24 (0.77-2.00)</td>
<td>43</td>
<td>0.378</td>
</tr>
<tr>
<td>&gt;23CAG</td>
<td>575</td>
<td>1.46 (1.29-1.64)</td>
<td>0.496</td>
<td>0.77 (0.52-1.14)</td>
<td>53</td>
<td>0.186</td>
<td>0.80 (0.47-1.35)</td>
<td>27</td>
<td>0.398</td>
</tr>
<tr>
<td>Outliers</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>removed</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;21CAG</td>
<td>517</td>
<td>1.48 (1.33-1.63)</td>
<td>Ref</td>
<td>Ref</td>
<td>61</td>
<td>Ref</td>
<td>Ref</td>
<td>31</td>
<td>Ref</td>
</tr>
<tr>
<td>21-23CAG</td>
<td>589</td>
<td>1.57 (1.43-1.71)</td>
<td>0.385</td>
<td>0.91 (0.62-1.32)</td>
<td>63</td>
<td>0.605</td>
<td>1.28 (0.79-2.07)</td>
<td>43</td>
<td>0.319</td>
</tr>
<tr>
<td>&gt;23CAG</td>
<td>572</td>
<td>1.41 (1.28-1.54)</td>
<td>0.501</td>
<td>0.77 (0.52-1.13)</td>
<td>52</td>
<td>0.181</td>
<td>0.79 (0.46-1.35)</td>
<td>26</td>
<td>0.385</td>
</tr>
</tbody>
</table>

<sup>A</sup> Adjusted for center  
<sup>B</sup> Calculated using ln-transformed values, adjusting for center
Table 4. Comparison of adjusted PSA between CAG categories in combination with rs1204038 genotype and odds ratios of having PSA above clinically accepted cut-off 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>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95%CI)</td>
<td>OR (95% CI)</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(95% CI)</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>CAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>&lt;21CAG</td>
<td>1.43 (1.26-1.60)</td>
<td>Ref</td>
</tr>
<tr>
<td>G</td>
<td>21-23CAG</td>
<td>1.55 (1.39-1.71)</td>
<td>0.388</td>
</tr>
<tr>
<td>G</td>
<td>&gt;23CAG</td>
<td>1.46 (1.28-1.64)</td>
<td>0.979</td>
</tr>
<tr>
<td>A</td>
<td>&lt;21CAG</td>
<td>1.77 (1.36-2.17)</td>
<td>0.121</td>
</tr>
<tr>
<td>A</td>
<td>21-23CAG</td>
<td>1.61 (1.32-1.91)</td>
<td>0.295</td>
</tr>
<tr>
<td>A</td>
<td>&gt;23CAG</td>
<td>1.68 (0.53-2.82)</td>
<td>0.269</td>
</tr>
</tbody>
</table>

Outliers included

| G         | <21CAG           | 1.43 (1.26-1.60) | Ref | 37 | Ref | 18 | Reference |
| G         | 21-23CAG         | 1.55 (1.39-1.71) | 0.388 | 50 | 0.652 | 31 | 0.231 |
| G         | >23CAG           | 1.46 (1.28-1.64) | 0.979 | 51 | 0.754 | 27 | 0.887 |
| A         | <21CAG           | 1.77 (1.36-2.17) | 0.121 | 25 | 0.011* | 14 | 0.036* |
| A         | 21-23CAG         | 1.61 (1.32-1.91) | 0.295 | 13 | 0.755 | 12 | 0.036* |
| A         | >23CAG           | 1.68 (0.53-2.82) | 0.247 | 1 | 0.524 | 0 | B |

Outliers removed

| G         | <21CAG           | 1.43 (1.26-1.60) | Ref | 37 | Ref | 18 | Reference |
| G         | 21-23CAG         | 1.55 (1.39-1.71) | 0.358 | 50 | 0.661 | 31 | 0.236 |
| G         | >23CAG           | 1.40 (1.27-1.54) | 0.985 | 50 | 0.684 | 26 | 0.991 |
| A         | <21CAG           | 1.64 (1.34-1.94) | 0.112 | 24 | 0.017* | 13 | 0.061 |
| A         | 21-23CAG         | 1.64 (1.34-1.94)* | 0.041* | 13 | 0.735 | 12 | 0.034* |
| A         | >23CAG           | 1.68 (0.53-2.82) | 0.247 | 1 | 0.524 | 0 | B |

A Adjusted for center
B No participants in this category presented with PSA > 4ng/mL
* Significant at the 0.05 level
Legends to figures

Figure 1
Flowchart for inclusion in current study

Figure 2
The CAG distribution in A) pooled carriers of the G and A-allele of rs1204038, and B) in carriers of the G and the A-allele of rs1204038, respectively.
Figure 1
Figure 2A

Figure 2B
Androgen receptor polymorphism dependent variation in prostate specific antigen concentrations of European men


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