Short Communication

The E211 G>A Androgen Receptor Polymorphism Is Associated with a Decreased Risk of Metastatic Prostate Cancer and Androgenetic Alopecia

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Abstract

The androgen receptor (*AR*) gene encodes a transcription factor, which mediates androgen action in target tissues, including the prostate. Prostate cancer is androgen dependent, implicating *AR* in susceptibility to this male condition. Male pattern balding, androgenetic alopecia, has recently been associated with prostate cancer, suggesting shared androgen pathways. The CAG and GGC repeats in the *AR* have been studied extensively as markers of prostate cancer susceptibility, with inconclusive findings, whereas the *AR*-E211 G>A polymorphism has been associated with androgenetic alopecia. We assessed the repeat linked single nucleotide polymorphism as a marker of risk association in prostate cancer, including androgenetic alopecia, in an Australian population-based case-control study. In 815 prostate cancer cases and 719 controls, the proportion of *A*-allele carriers was the same in each group. Overall, there was no evidence for an association between the *A* allele and risk of prostate cancer, however, the proportion of *A*-allele carriers in metastatic prostate cancer (5%) was lower than in less advanced disease (16%, $P = 0.03$). The proportion of *A*-allele carriers was 24% in nonbald men but it was lower in men with vertex alopecia alone (13%, $P = 0.001$) or in combination with frontal alopecia (7%, $P < 0.0001$). This inverse association between the *A* allele and baldness was independent of prostate cancer status (P for interaction = 0.2). These results suggest that the *AR*-E211 *A* allele, in linkage with the functional repeat sequences, is associated with a lower risk of metastatic prostate cancer and a lower risk of alopecia. (Cancer Epidemiol Biomarkers Prev 2005;14(4):993–6)

Introduction

Androgen receptor (MIM 313700), a member of the nuclear receptor superfamily, functions as a ligand-dependent transcription factor and is essential for androgen action (1). The androgen receptor (*AR*) gene, located at band q12 on the X chromosome, is highly conserved, with germline loss-of-function mutations resulting in androgen insensitivity syndrome, also known as testicular feminization (2). Three polymorphisms have been identified in the NH_2-terminal transactivation domain encoded by exon 1 and include two trinucleotide repeats and a single nucleotide polymorphism. The CAG (polyglutamine) and less polymorphic GGC (polyglycine) repeat lengths have been inversely correlated with protein level and transactivation activity of androgen receptor (3-7). The *AR*-E211 G>A (rs6152) single nucleotide polymorphism is a synonymous change as the glutamic acid remains unchanged (GenBank accession no. M27423), also known as the *Stu*I (restriction site) or E213 (SNPper database, http://snpper.chip.org) polymorphism, occurring in 13% to 20% of Caucasian populations (8, 9). It is located between the trinucleotide repeats with which it has been shown to be in partial linkage disequilibrium with both repeats (10).

Androgens regulate prostate gland growth and differentiation via the androgen receptor and androgen-responsive genes. Androgens stimulate prostate cancer growth via three mechanisms; (1) expression, activation, and up-regulation of androgen receptor activity; (2) ligand-independent activation of androgen receptor; and (3) mutations in the *AR* gene (11, 12). Unlike androgen insensitivity syndrome, mutations in prostate cancer tissue are predominantly somatic, with a number associated with the transition from androgen-dependent to androgen-independent growth (2, 13). Ethnicity is a significant risk factor for prostate cancer, with the highest incidence in African-Americans, intermediate in Caucasians, and lowest in Asians (14). Thus, the notion that the shorter more transcriptionally active CAG repeat length, as observed in most African-Americans, could indirectly predispose to prostate cancer has been tested in a number of population-based case-control studies with major inconsistencies in the results (15, 16). The less studied GGC repeat has also been inconsistently associated with prostate cancer risk (17, 18).

The involvement of androgens in androgenetic alopecia, or male pattern baldness, is well established (19). Lack of balding has been reported in eunuchs, in androgen-insensitive individuals, as well as in pseudohermaphrodites with reduced or no levels of dihydrotestosterone, the active metabolite of testosterone, whereas increased levels of the androgen receptor and aromatase (the enzyme that converts androgens to estrogens) have been associated with balding (20). In 1998,

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the involvement of genes of androgen synthesis in androgenetic alopecia was suggested, however, no association was found for the genes encoding the two isoforms of 5α-reductase (*SRD5A1* and *SRD5A2*) involved in the conversion of testosterone to dihydrotestosterone (21). In 2001, an association between *AR*-E211 G>A and androgenetic alopecia was shown (9).

In this study, we genotyped the stable dimorphic *AR*-E211 G>A, previously shown to be in partial linkage disequilibrium with the less stable functional repeat polymorphisms, in an Australian-based study of 815 prostate cancer cases and 719 control men and evaluated for association between this polymorphism and androgen-related conditions (i.e., prostate cancer and androgenetic alopecia).

Subjects and Methods

Subjects. A population-based case-control study of prostate cancer was carried out in Australia from 1994 to 1997 in the capital cities of New South Wales (Sydney), Victoria (Melbourne), and Western Australia (Perth). Eligible cases with histopathologically confirmed adenocarcinoma of the prostate with Gleason score ≥ 5 and diagnosed before age 70 were ascertained from the cancer registries. Controls were randomly selected from males on the State Electoral Rolls (registration to vote is compulsory for adult Australian citizens) and were frequency matched to the expected age distribution of the prostate cancer cases in a ratio of one control per case. A structured face-to-face interview was done with each participant and baldness (androgenetic alopecia) was recorded during the interview using an adapted Hamilton-Norwood scale, including four categories, balding, frontal balding, vertex balding, and combined frontal and vertex balding, as previously described (22). Blood samples were obtained from 745 controls (71%) and 862 cases (83%) residing in the Melbourne and Perth regions. The great majority of subjects were born in Australia, the British Isles, or Western Europe and therefore classified as Caucasian, with only 14 controls (2%) and 9 cases (1%) born in Asia. Statistical analysis was carried out on 815 cases and 719 controls for whom the androgen receptor genotype was measured. The analysis by tumor stage was done by dividing stage into three categories: stage I to II (T_1-T_2, nonmetastatic tumors confined to the prostate gland), III (T_3, nonmetastatic tumors that have grown through the prostate capsule or possibly to nearby muscles and organs like seminal vesicles), and IV (T_4 or N+ or M+, tumors that have metastasized to the regional lymph nodes or more distant parts of the body).

Genomic Analysis. The 201-base fragment including the E211 single nucleotide polymorphism was amplified using denaturing gradient gel electrophoresis primers; forward 5′-ACCTTAAAGACATCCTGAGCGA-3′ and reverse 5′-TGGACACCGACACTGCCTTA-3′, with a GC clamp (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCG-3′) added to the 5′ end of the reverse primer and a GC stretch 5′-CGCCCGC-3′ to the 5′ end of the forward primer. Amplification was done in a 25-μL reaction, containing 20 ng of genomic DNA, 20 pmol of each primer, 0.1 mmol/L of each deoxynucleotide triphosphate, 2.5 mmol/L of 10× Mg^{2+} reaction buffer, and 0.5 unit of DNA Taq Polymerase (Roche Diagnostics, Basel, Switzerland). Thermal conditions were as follows: denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute 15 seconds, followed by a final extension of 72°C for 8 minutes using an MJ Research Thermal Cycler (MJ Research, Inc., Waltham, MA). For optimal denaturing gradient gel electrophoresis analysis a heteroduplexing step was done postamplification, with denaturation at 96°C for 10 minutes followed by renaturation at 60°C for 40 minutes. Amplified products were electrophoresed in a 9% polyacrylamide gel (acrylamide/bisacrylamide, 37.5:1) containing a 40% to 80% urea and formamide (100% UF = 7 mmol/L urea/40% deionized formamide) denaturing gradient, in a Tris-acetate-EDTA buffer at 60°C and 110 V overnight using the Ingeny phorU-2 denaturing gradient gel electrophoresis system (Ingeny International, Goes, the Netherlands; www.ingeny.com). The E211 single nucleotide polymorphism was identified as a single shifted denaturing gradient gel electrophoresis band.

Statistical Analysis. Estimates and comparisons of allele frequencies were carried out using standard procedures based on exact methods. Case-control analyses were conducted using unconditional logistic regression, whereas associations of genotype with tumor stage and baldness status (four categories) were tested using polytomous logistic regression. The confidence interval (95% CI) of the odds ratio (OR) for metastatic disease was estimated using the ''bootstrap'' technique on 10,000 replicates of two thirds of the data, as small numbers made the asymptotic likelihood theory invalid (23). The likelihood ratio test was used to test whether the inclusion in the models of additional variables like age, country of birth, and family history of prostate cancer modified the estimates for the genotype. The model with alopecia as outcome was fitted separately for cases and controls and then fitted in a single model including an indicator variable to adjust for the case/control status. Finally, an interaction term was added to test whether the effect of genotype in cases differed from the effect in controls. The statistical analysis was done using the statistical package STATA (StataCorp 2003, www.stata.com). All tests were two sided.

Results

Prostate Cancer. Genotyping of the *AR*-E211 G>A variant in the population-based case-control study identified the frequency of the *A* allele to be 15% (126 of 815) in cases and 15% (107 of 719) in controls. Case-control analyses showed no overall association between prostate cancer and the *A* allele (OR, 1.05; 95% CI, 0.79-1.39). In addition, there was no association between the polymorphism and age, country of birth, or family history of prostate cancer in cases and controls

Table 1. *AR*-E211 polymorphism and prostate cancer

	Controls	Stage I-II	Stage III	Stage IV	*P**
G	612 (85%)	477 (85%)	167 (81%)	42 (95%)	0.02
A	107 (15%)	83 (15%)	40 (19%)	2 (5%)	
OR (95% CI)	References	1.00 (0.73 - 1.36)	1.37 (0.92 - 2.05)	0.27 (0.01 - 0.94)†	
P	—	>0.9	0.1	0.03	

NOTE: ORs are from polytomous logistic regression.
*Test for different ORs across the tumor stages (likelihood ratio test).
†Confidence intervals were obtained replicating two thirds of the data 10,000 times using the bootstrapping technique.

Table 2. *AR*-E211 polymorphism and androgenetic alopecia

	No balding	Frontal	Vertex	Vertex and frontal	*P**
Controls					
G	110 (81%)	211 (83%)	121 (85%)	165 (93%)	0.007
A	26 (19%)	43 (17%)	22 (15%)	13 (7%)	
OR (95% CI)	References	0.86 (0.50 - 1.48)	0.77 (0.41 - 1.44)	0.33 (0.16 - 0.68)	
P	—	0.6	0.4	0.002	
Cases					
G	98 (72%)	200 (79%)	183 (89%)	192 (94%)	<0.0001
A	38 (28%)	52 (21%)	23 (11%)	12 (6%)	
OR (95% CI)	References	0.67 (0.41 - 1.09)	0.32 (0.18 - 0.57)	0.16 (0.08 - 0.32)	
P	—	0.1	<0.0001	<0.0001	

NOTE: ORs are from polytomous logistic regression.
*Test for different ORs across the baldness categories (likelihood ratio test).

(all $P \geq 0.1$). In the cases there was no association between the polymorphism and tumor grade ($P = 0.8$). The proportion of *A*-allele carriers was lower in metastatic (5%) than in less advanced disease (15% in stage I-II and 19% in stage III tumors, $P = 0.03$). The ORs for prostate cancer by tumor stage, derived from polytomous logistic regression, are shown in Table 1. The OR for metastatic disease compared with controls was 0.27 (95% CI = 0.01-0.94) whereas the ORs for stage I to II and stage III tumors were both close to 1 (both $P \geq 0.1$). The test for null effect of the E211 genotype (i.e., all three ORs simultaneously equal to 1) was 0.05.

Androgenetic Alopecia. The pattern of balding was determined for 798 (98%) prostate cancer cases and 711 (99%) controls. The highest frequency of the *A* allele was found in nonbald men (24%) compared with men with frontal baldness (19%) or vertex baldness alone (13%) or in combination with frontal baldness (7%). Table 2 shows the effect of genotype on alopecia in terms of ORs separately for prostate cancer cases and controls. From the model obtained combining cases and controls, we observed an overall inverse association between alopecia and the *A* allele ($P < 0.0001$). This association was not different in cases and controls (P for interaction = 0.2). In particular, the *A* allele was associated with a lower risk of vertex baldness alone (OR, 0.48; 95% CI, 0.31-0.72) or in combination with frontal baldness (OR, 0.23; 95% CI, 0.14-0.37). We found no evidence of an association between frontal baldness alone and the *A* allele (OR, 0.75; 95% CI, 0.53-1.07).

Discussion

Given the *AR* gene is highly conserved, with only a single dimorphic marker reported in Caucasian populations, we investigated the role of this polymorphism on androgen-related phenotypes i.e., prostate cancer (tumor stage and grade) and risk factors for prostate cancer (age, country of birth, family history, and androgenetic alopecia) using a well-defined population-based case-control study. Although E211 G>A is in partial linkage disequilibrium with both CAG and GGC repeats (10), its utilization as a genetic marker has been limited. It is well established that nucleotide repeat sequences are highly polymorphic, reflecting a high rate of mutation (24), whereas dimorphic polymorphisms are more stable with lower rates of mutation (25), and thus ideal markers in association studies. We therefore utilized the E211 marker to ascertain association with prostate cancer and known risk factors, including androgenetic alopecia. Whereas we found no overall association between this polymorphism and prostate cancer risk, including risk factors such as age at diagnosis, family history, and country of birth, a significant association between the presence of the *A* allele and a decreased risk of metastatic prostate cancer (stage IV), as compared with less advanced disease (stages I-III), was observed. No association with tumor grade was observed. Independent of prostate cancer status, a highly significant association between the presence of the *A* allele and lack of balding, in particular vertex and full patterned baldness, was observed. As age of onset of balding was not recorded in this study, further studies are required to determine whether E211 G>A is associated with early- or late-onset alopecia.

Although E211 G>A does not result in an amino acid change and is therefore unlikely to convey a direct effect on protein expression, structure, or function, this silent marker is 401 bases downstream of the functional CAG repeat and 736 bases upstream of the functional GGC repeat, as well as being reported to be in partial linkage disequilibrium with both repeat sequences. It is therefore possible that the association between lowered risk of metastatic prostate cancer and male patterned baldness in the presence of the *A* allele may reflect the effect of the repeat status. Both CAG and GGC repeat lengths have been associated with prostate cancer risk, whereas others have failed to detect any association between these repeats and risk of prostate cancer (15-18). A number of studies associate shorter CAG repeat lengths with increased prostate cancer risk, supported by association with functionally enhanced androgen receptor activity. In our study, the *A* allele is not associated with overall prostate cancer risk, but decreases the risk of metastatic disease. Shorter CAG repeats have been reported to be more common in advanced tumors when considering tumor grade and/or stage and younger age at diagnosis. Shorter GGC repeats have also been associated with increased prostate cancer risk, whereas other studies report this risk to be age dependent. Contradictory results have also been reported for the combined effect of the CAG and GGC repeats from increasing to decreasing prostate cancer risk (reviewed in ref. 18). In androgenetic alopecia, a statistically significant association between shorter CAG repeat lengths and the presence of balding has been shown (26), whereas in a later study this significance could only be achieved in combination with the GGC repeat polymorphism (9). In these studies prostate cancer status was not considered.

Possible reasons for discrepancies across studies could be case bias due to tumor or prostate cancer risk factors, potential misclassification of controls due to bias selection procedures, and the high degree of naturally occurring variability of trinucleotide repeat sequences. In this study, we attempted to address these issues by avoiding subgroup restrictions on our cases, by recruiting controls not from a prostate cancer screening population but from the general population (via State Electoral Rolls) according to age-matching of cases, and lastly by using a more stable dimorphic polymorphism so as to

reduce the amount of naturally occurring intersample variability. The results of this approach suggest that the E211 *A* allele is a marker for decreased risk of metastatic prostate cancer and confirm that it is a marker of reduced risk of alopecia in a Caucasian-based population. Whether these associations can be attributed to linkage to one or both of the functional repeat sequences at the same loci requires further investigation.

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References

1. Gelmann EP. Molecular biology of the androgen receptor. J Clin Oncol 2002; 20:3001–15.
2. Gottlieb B, Beitel LK, Wu JH, Trifiro M. The androgen receptor gene database (ARDB): 2004 update. Hum Mutat 2004;23:527–33.
3. Chamberlain NL, Driver ED, Miesfeld RL. The length and location of the CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 1994;22:3181–6.
4. Choong CS, Kemppainen JA, Zhou ZX, Wilson EM. Reduced androgen receptor gene expression with first exon CAG repeat expansion. Mol Endocrinol 1996;10:1527–35.
5. Gao T, Marcelli M, McPhaul MJ. Transcriptional activation and transient expression of the human androgen receptor. J Steriod Biochem Mol Biol 1996;59:9–20.
6. Ding D, Xu L, Menon M, Veer Reddy GP, Barrack ER. Effect of short CAG (Glutamine) repeat on human androgen receptor function. Prostate 2004; 58:23–32.
7. Ding D, Xu L, Menon M, Veer Reddy GP, Barrack ER. Effect of GGC (Glycine) repeat length polymorphism in the human androgen receptor on androgen action. Prostate 2004;9999:1–7.
8. Lu J, Danielsen M. A *Stu*I polymorphism in the human androgen receptor gene. Clin Genet 1996;49:323–4.
9. Ellis JA, Stebbing M, Harrap SB. Polymorphism of the androgen receptor gene is associated with male pattern baldness. J Invest Dermatol 2001;116: 452–5.
10. Ross RK, Pike MC, Coetzee GA, et al. Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. Cancer Res 1998;58: 4497–504.
11. Bosland MC. The role of steroid hormones in prostate carcinogenesis. J Natl Cancer Inst Monogr 2000;27:39–66.
12. Culig Z. Role of the androgen receptor axis in prostate cancer. Urology 2003; 62:21–6.
13. DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular aspects of prostate cancer. Lancet 2003;361:955–64.
14. Hsing AW, Tsao L, Devesa SS. International trends and patterns of prostate cancer incidence and mortality. Int J Cancer 2000;85:60–7.
15. Nelson KA, Witte JS. Androgen receptor CAG repeats and prostate cancer. Am J Epidemiol 2002;155:883–90.
16. Clark PE, Irvine RA, Coetzee GA. The androgen receptor CAG repeat and prostate cancer risk. Methods Mol Med 2003;81:255–66.
17. Chang B-L, Zheng SL, Hawkins GA, et al. Polymorphic GGC repeats in the androgen receptor gene are associated with hereditary and sporadic prostate cancer risk. Hum Genet 2002;110:122–9.
18. Chen C, Lamharzi N, Weiss NS, et al. Androgen receptor polymorphism and the incidence of prostate cancer. Cancer Epidemiol Biomarkers Prev 2002;11: 1033–40.
19. Sinclair R. Male pattern androgenetic alopecia. BMJ 1998;317:865–9.
20. Ellis JA, Harrap SB. The genetics of androgenetic alopecia. Clin Dermatol 2001;19:149–54.
21. Ellis JA, Stebbing M, Harrap SB. Genetic analysis of male pattern baldness and the 5-reductase genes. J Invest Dermatol 1998;110:849–53.
22. Giles GG, Severi G, Sinclair R, et al. Androgenetic Alopecia and prostate cancer: findings from an Australian case-control study. Cancer Epidemiol Biomarkers Prev 2002;11:549–53.
23. Davison AC, Hinkley DV. Bootstrap Methods and their application. Cambridge: Cambridge University Press; 1997.
24. Chakraborty R, Kimmel M, Strivers DN, Davidson LJ, Deka R. Relative mutation rates at di-, tri-, and tetranucleotide microsatellite loci. Proc Natl Acad Sci U S A 1997;94:1041–6.
25. Gray IC, Campbell DA, Spurr NK. Single nucleotide polymorphisms as tools in human genetics. Hum Mol Genet 2000;9:2403–8.
26. Sawaya ME, Shalita AR. Androgen receptor polymorphisms (CAG repeat lengths) in androgenetic alopecia, hirsutism, and acne. J Cutaneous Med Surgery 1998;3:9–15.