Gender Specificity of a Genetic Variant of Androgen Receptor and Risk of Coronary Artery Disease

Konstantinos Agiannitopoulos,¹ Angeliki Bakalgianni,¹ Eirini Marouli,¹
Ioanna Zormpa,¹ Athanasios Manginas,² Spyros Papamenzelopoulos,²
and Klea Lamnissou^{1*}

¹Department of Genetics and Biotechnology, Faculty of Biology, University of Athens, Athens, Greece

²First Cardiology Department, Onassis Cardiac Surgery Center, Athens, Greece

Background: Androgens are known to influence the risk of developing cardiovascular diseases. This study aims at investigating the possible association between G1733A polymorphism in the coding region of androgen receptor (AR) gene and premature coronary artery disease (CAD). **Methods:** A total of 460 Greek subjects were investigated for the G1733A polymorphism. The patient group consisted of 250 CAD individuals, aged less than 58 years, while 210 healthy individuals served as controls. Genotyping was performed using the PCR-RFLP method. **Results:** Significant differ-

ences in genotype distribution ($P = 0.0067$) and allele frequencies ($P = 0.0060$) have been observed between patients and controls in the women's subgroup. Conversely, the genotype/allele frequencies were similar between patients and controls in the subgroup of men. **Conclusion:** We may conclude that the G1733A polymorphism of AR gene could be a useful genetic marker for the assessment of a woman's risk for CAD in our Caucasian Greek population. J. Clin. Lab. Anal. 30:204–207, 2016. © 2015 Wiley Periodicals, Inc.

Key words: coronary artery disease; androgen receptor; polymorphism; risk factor

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality all over the world, especially in the developed countries. A common multifactorial CVD, coronary artery disease (CAD), resulting from the accumulation of atheromatous plaques within the walls of the coronary arteries, appears to depend on the interaction between environmental risk factors and multiple predisposing genes. Besides several well-known risk factors, such as dyslipidemia, hypertension, smoking, and diabetes, gender is considered to be a major risk factor for the development of CAD. Several studies indicated that the incidence of CAD is 2.0- to 4.5-fold higher in men than in women (1, 2). The role of sex hormones in CVD has been studied extensively in the last two decades, but the differences in the pattern between the two genders have not been explained properly (3, 4). It has been suggested that estrogens, the female sex hormones, have favorable effects against CVD (4, 5), while the effects of physiological androgen levels on cardiac function remain controversial. It is well documented that androgens have

several physiological effects in both sexes (6). Androgens affect the metabolism of lipoproteins, vascular endothelium, smooth muscle, and blood coagulation. The physiologic effects of androgens are mediated by androgen receptor (AR), a ligand-activated transcription factor (7), which is a member of the nuclear receptor super family.

The AR gene is located on the X-chromosome at Xq11–12 and the protein coding region comprises eight exons (8). The large exon 1 that correlates with the transactivation function of the AR contains several polymorphic loci, such as CAG and GGN repeats and a number of single-nucleotide polymorphisms (SNPs) (9–11). One of these polymorphisms, rs6152, determined as a G-to-A substitution (G1733A) in the third nucleotide position of the

*Correspondence to: Klea Lamnissou, Department of Genetics and Biotechnology, Faculty of Biology, University of Athens, Athens 15701, Greece. E-mail: klamniss@biol.uoa.gr

Received 4 December 2013; Accepted 24 November 2014

DOI 10.1002/jcla.21837

Published online in Wiley Online Library (wileyonlinelibrary.com).

211 codon presents great interest (10, 11). The functional effect of the G1733A SNP has not yet been studied extensively. However, the A allele has been associated with male pattern baldness (12), prostate cancer (13), recurrent spontaneous abortions (14, 15), and polycystic ovary syndrome (16). There are also data suggesting an association of the minor allele of rs6152 with endometrial cancer (17). Furthermore, meta-analysis results indicate that the G allele of this AR polymorphism might be a potential risk factor for androgenetic alopecia (18). It is also supported that genetic variation within the AR may influence the susceptibility of autism with a gender-dependent influenced way (19). The aim of the current case-control study was to investigate the possible association between the G1733A genetic polymorphism of AR and premature CAD in the Caucasian Greek population.

MATERIALS AND METHODS

Patients and Controls

Our study group consisted of 460 unrelated individuals; 250 CAD patients and 210 healthy controls. All individuals enrolled in the study were Greeks and informed consent was obtained from all participants. The patient group consisted of subjects aged less than 58 years, presenting symptomatic CAD, documented by coronary angiography at the "Onassis Cardiac Surgery Center" of Athens. CAD was defined with at least one main coronary vessel >50% luminal narrowing or with a history of acute myocardial infarction (MI). The control group consisted of healthy individuals with no evidence of CVD, such as history of angina pectoris or MI. They all underwent regular physical examinations and had normal coronary angiograms. All women of the control group were in menopause. Subjects were defined as hypertensive if their blood pressure was >140/90 mmHg and diabetic when fasting glucose was >126 mg/dl. Hypercholesterolemia was defined as having total cholesterol >220 mg/dl. Family history was considered positive for CAD if at least one first-degree relative was diagnosed with CAD or MI by the age of 65 years. Finally, subjects were defined as smokers if they were current or past smokers. The study conforms to the Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (July 18, 1964).

DNA Isolation and Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by a standard salting out method (20). The genotyping of the G1733A SNP was carried out using the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The PCR

amplification primers were forward (5' – CTG GAT GAG GAA CAG CAA CC – 3') and reverse (5' – CGT TGT CAG AAA TGG TCG AA – 3'). The PCR procedure was as follows: an initial denaturation step at 94°C for 2 min; then amplification for 35 cycles at 94°C for 30 s, 57°C for 40 s, and 72°C for 40 s, followed by a final extension step at 72°C for 7 min. The resulting 379-bp PCR product was digested with the restriction enzyme *StuI* at 37°C for 16 h. The restricted fragments were separated on 2% agarose gel electrophoresis and fragments were visualized by ethidium bromide staining and ultraviolet transillumination. The presence of A variant (379-bp fragment) or G variant (313-bp fragment) indicates the two alleles. Therefore, we have three genotypes in women—GG, GA, AA (they have two X chromosomes)—and only two genotypes in men—G or A (they have one X chromosome).

Statistical Analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS for windows v. 10). Data are presented as a total number, percentage, or mean. Chi-square test was used to test for independent relationships between categorical variables, such as genotype distribution. Differences in allele frequencies were tested for statistical significance at the 95% confidence interval using Fisher's exact test. A *P*-value less than 0.05 was considered statistically significant. The odds ratio (OR) was used as a measure of the strength of the association between allele frequencies and CAD.

RESULTS

The clinical characteristics of the CAD patients and controls are presented in Table 1. As seen, the mean age of the CAD patients is lower than that of the controls. In an effort to eliminate the possibility of individuals of the control group to develop CAD in the future, we used healthy individuals of higher ages. An elderly healthy group represents better a CAD-free population. CAD patients, as clearly seen in Table 1, had a significant higher

TABLE 1. Baseline Characteristics of CAD Patients and Control Subjects

	Patients <i>n</i> = 250	Controls <i>n</i> = 210	<i>P</i>
Mean age	52.8 (36–57)	67.8 (63–85)	<0.0001
Hypertension, <i>n</i> (%)	140 (56.0)	28 (14.0)	<0.0001
Diabetes, <i>n</i> (%)	65 (26.0)	10 (5.0)	<0.0001
Hypercholesterolemia, <i>n</i> (%)	151 (60.4)	27 (13.5)	<0.0001
Smoking, <i>n</i> (%)	170 (68.0)	60 (30)	<0.0001
Family history, <i>n</i> (%)	98 (39.2)	17 (8.5)	<0.0001

TABLE 2. Genotype Distribution and Allele Frequencies of the G1733A Polymorphism of AR in the Subgroup of Women

Genotype	Patients <i>n</i> = 85	Controls <i>n</i> = 105	OR (95% CI)	<i>P</i>
GG	51 (0.60)	74 (0.70)		0.0067*
GA	19 (0.22)	27 (0.26)		
AA	15 (0.18)	4 (0.04)		
Allele				
G	0.74	0.83	2.025 (1.238–3.311)	0.0060**
A	0.26	0.17		

Data are expressed as number (percentage) of each genotype.

**P*-value from χ^2 test for homogeneity.

***P*-value from Fisher's exact test for homogeneity.

TABLE 3. Genotype/Allele Frequencies of the G1733A Polymorphism of AR in the Subgroup of Men

Genotype/Allele	Patients <i>n</i> = 165	Controls <i>n</i> = 105	OR (95% CI)	<i>P</i>
G	142 (0.86)	88 (0.84)	1.193 (0.6036–2.357)	0.6037*
A	23 (0.14)	17 (0.16)		

Data are expressed as number (percentage) of each genotype.

**P*-value from Fisher's exact test for homogeneity.

prevalence of CAD risk factors, such as hypertension, hyperlipidemia, smoking, diabetes, and family history of CAD, compared with the control group. All subjects were examined for the rs6152 polymorphism. As previously mentioned the *AR* gene is X-linked, so the genotypes of men are only two—either G or A—while genotypes of women are three—GG, GA, and AA. The results are summarized separately in Tables 2 and 3 for the subgroups of women and men, respectively. Frequencies of GG, GA, and AA genotypes were 0.60, 0.22, and 0.18, respectively, for the CAD women and 0.70, 0.26, and 0.04, respectively, in the control group. Allele frequencies among patients and controls were 0.74 and 0.83, respectively, for the most frequent wild-type allele G and 0.26 and 0.17, respectively, for the mutant allele A (Fig. 1). The statistical analysis of these results showed that genotype and allele frequencies were significantly different between CAD women and controls ($P = 0.0067$ for genotype frequencies comparisons, $P = 0.0060$, OR [95% CI] = 2.025 [1.238–3.311] for allele frequencies comparisons; Table 2). In the subgroup of CAD men (Table 3, Fig. 1), the frequencies of G and A genotypes/alleles were 0.86 and 0.14 and 0.84 and 0.16, respectively, for the control group. The statistical analysis showed that there were no significant differences in genotypes/alleles frequencies between the CAD men and controls ($P = 0.6037$, OR [95% CI] = 1.193 [0.6063–2.357]).

Fig. 1. Allele frequencies of the G1733A polymorphism of AR in the subgroups of women and men.

DISCUSSION

The role of sex hormones in CVD is an emerging field of interest. Androgens have a major role in the progression of CVD, highlighting the need to better understand AR coregulators, nongenomic androgen effects, and androgen sensitivity. It is important to focus on novel therapeutic targets, by understanding how androgens enhance early plaque formation (21). Exposure to sex hormones throughout an individual's lifespan modulates many endocrine factors involved in atherosclerosis. Epidemiological, clinical, and experimental data suggested that there are gender-specific variations in CVD (4). The observation that CVD develops on average 10 years later in women compared to men has led to the hypothesis that female sex hormones protect women against atherosclerotic complications. Several studies have shown that in women, endogenous female sex hormones, especially estrogens, are cardioprotective via multiple mechanisms (22). Androgens have attracted significant interest in explaining the gender prevalence difference in CVD, although the effects of androgens on blood vessels are controversial. Several in vivo and in vitro studies have suggested that androgens increase the expression of proatherogenic factors (23–25). However, the understanding of the pathways mediating androgens' effect on atherosclerosis is still evolving. Testosterone, the main androgen hormone, has been shown to facilitate the initiation of atherosclerosis. In the earliest step of atherosclerosis, testosterone increases the cholesterol uptake and fatty streak formation from foam cells (26). This effect is mediated through AR (3), which is expressed in all cells of the vasculature as well as endothelial cells, smooth muscle cells, macrophages, platelets, and myocardial fibers (25). However, it is also suggested that low androgen levels are associated with adverse cardiovascular risk factors, such as obesity and atherogenic lipid profile. So, it is possible that low plasma testosterone may be implicated in the increased risk of CAD in men (21,27).

At the cellular and biochemical level, gender differences in regulating the physiological mechanisms are directly

influenced by the presence of genetic polymorphisms. Several AR polymorphisms have been studied for association with CVDs. The AR CAG repeat length polymorphism has been associated with severity of CAD in men (28) and postmenopausal women (29). Furthermore, the same polymorphism has been shown to be associated with HDL-cholesterol levels (30). The G1733A polymorphism of the AR gene has been studied for association with CVD in postmenopausal American women with negative results (11). In the current study, conducted in a homogeneous Greek population, we investigated the potential effect of the G1733A polymorphism of the AR gene in the occurrence of premature CAD. Several previous studies suggested that premature CAD has a strong genetic component (31, 32). Our results indicate that the presence of the A allele of G1733A polymorphism is associated with increased risk for premature CAD in women. So, the influence of the A allele may be gender dependent in the prevalence of CAD. We conclude that in our Caucasian Greek population the G1733A polymorphism of AR could be a useful genetic marker for the assessment of a woman's risk for CAD. However, the mechanisms underlying the association between G1733A and the risk of CAD require further investigation in larger sample or prospective studies.

REFERENCES

- British Heart Foundation Statistics Database, BHF. 1996;21–22.
- Lloyd-Jones DM, Larson MG, Beiser A, Levy D. Lifetime risk of developing coronary heart disease. *Lancet* 1999;353:89–92.
- Kaushik M, Sontineni SP, Hunter C. Cardiovascular disease and androgens: A review. *Int J Cardiol* 2010;142:8–14.
- Pérez-López FR, Larrad-Mur L, Kallen A, Chedraui P, Taylor HS. Gender differences in cardiovascular disease: Hormonal and biochemical influences. *Reprod Sci* 2010;17:511–531.
- Kallen AN, Pal L. Cardiovascular disease and ovarian function. *Curr Opin Obstet Gynecol* 2011;23:258–267.
- Ikeda Y, Aihara K, Yoshida S, Akaike M, Matsumoto T. Effects of androgens on cardiovascular remodeling. *J Endocrinol* 2012;214:1–10.
- Brinkmann AO, Klaasen P, Kuiper GG, et al. Structure and function of the androgen receptor. *Urol Res* 1989;17:87–93.
- La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischback KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 1991;352:77–79.
- Palvimo JJ, Kallio PJ, Ikonen T, Mehto M, Jänne OA. Dominant negative regulation of trans-activation by the rat androgen receptor: Roles of the N-terminal domain and heterodimer formation. *Mol Endocrinol* 1993;7:1399–1407.
- Lu J, Danielsen M. A Stu I polymorphism in the human androgen receptor gene (AR). *Clin Genet* 1996;49:323–324.
- Rexrode KM, Ridker PM, Hegener HH, Buring JE, Manson JE, Zee RY. Genetic variation of the androgen receptor and risk of myocardial infarction and ischemic stroke in women. *Stroke* 2008;39:1590–1592.
- Ellis JA, Stebbing M, Harrap SB. Polymorphism of the androgen receptor gene is associated with male pattern baldness. *J Invest Dermatol* 2001;116:452–455.
- Medeiros R, Vasconcelos A, Costa S, et al. Steroid hormone genotypes ARStuI and ER325 are linked to the progression of human prostate cancer. *Cancer Genet Cytogenet* 2003;141:91–96.
- Karvela M, Stefanakis N, Papadopoulou S, Tsitilou SG, Tsilivakos V, Lamnissou K. Evidence for association of the G1733A polymorphism of the androgen receptor gene with recurrent spontaneous abortions. *Fertil Steril* 2008;90:2010.e9–12.
- Jahaninejad T, Ghasemi N, Kalantar SM, Sheikhha MH, Pashaiefar H. StuI polymorphism on the androgen receptor gene is associated with recurrent spontaneous abortion. *J Assist Reprod Genet* 2013;30:437–440.
- Peng CY, Long XY, Lu GX. Association of AR rs6152G/A gene polymorphism with susceptibility to polycystic ovary syndrome in Chinese women. *Reprod Fertil Dev* 2010;22:881–885.
- Yang HP, Garcia-Closas M, Lasey JV Jr, et al. Genetic variation in the androgen receptor gene and endometrial cancer risk. *Cancer Epidemiol Biomarkers Prev* 2009;18:585–589.
- Zhuo FL, Xu W, Wang L, Wu Y, Xu ZL, Zhao JX. Androgen receptor gene polymorphisms and risk for androgenetic alopecia: A meta-analysis. *Clin Exp Dermatol* 2012;37:104–111.
- Henningsson S, Jonsson I, Ljunggren E, et al. Possible association between the androgen receptor gene and autism spectrum disorder. *Psychoneuroendocrinology* 2009;34:752–761.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- Liu, PY, Death, AK, Handelsman, DJ. Androgens and cardiovascular disease. *Endocr Rev* 2003;24:313–340.
- Mendelson ME, Karas RH. The protective effects of estrogens on the cardiovascular system. *N Engl J Med* 1999;340:1801–1811.
- McCrohon JA, Death AK, Nakhla S, et al. Androgen receptor expression is greater in macrophages from male than from female donors. A sex difference with implications for atherogenesis. *Circulation* 2000; 101:224–226.
- Ng MK, Liu PY, Williams AJ, et al. Prospective studies of effect of androgens on serum inflammatory markers in men. *Arterioscler Thromb Vasc Biol* 2002;22:1136–1141.
- Nheu L, Nazareth L, Xu GY, et al. Physiological effects of androgens on human vascular endothelial and smooth muscle cells in culture. *Steroids* 2011;76:1590–1596.
- Rosano GM, Sheiban I, Massaro R, et al. Low testosterone levels are associated with coronary artery disease in male patients with angina. *Int J Impot Res* 2007;19:176–182.
- Schwarcz MD, Frishman WH. Testosterone and coronary artery disease. *Cardiol Rev* 2010;18:251–257.
- Alevizaki M, Cimponeriu AT, Garofallaki M, et al. The androgen receptor gene CAG polymorphism is associated with the severity of coronary artery disease in men. *Clin Endocrinol (Oxf)* 2003;59:749–755.
- Saltiki K, Cimponeriu A, Garofalaki M, et al. Severity of coronary artery disease in postmenopausal women: association with the androgen receptor gene (CAG)_n repeat polymorphism. *Menopause* 2011;18:1225–1231.
- Herberger M, Muntwyler J, Funke H, et al. The CAG repeat polymorphism in the androgen receptor gene is associated with HDL-cholesterol but not with coronary atherosclerosis or myocardial infarction. *Clin Chem* 2005;51:1110–1115.
- Mannucci PM, Asselta R, Duga S, et al. The association of factor V Leiden with myocardial infarction is replicated in 1880 patients with premature disease. *J Thromb Haemost* 2010;8:2116–2121.
- Brown BD, Nsengimana J, Barrett JH, et al. An evaluation of inflammatory gene polymorphisms in sibships discordant for premature coronary artery disease: The GRACE-IMMUNE study. *BMC Med* 2010. doi 10.1186/1741.7015.8.5.