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The association between inflammation-related genes and serum androgen levels in men: The Prostate, Lung, Colorectal, and Ovarian Study

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Abstract

BACKGROUND—Androgens and inflammation have been implicated in the etiology of several cancers, including prostate cancer. Serum androgens have been shown to correlate with markers of inflammation and expression of inflammation-related genes.

METHODS—In this report, we evaluated associations between 9,932 single nucleotide polymorphisms (SNPs) marking common genetic variants in 774 inflammation-related genes and four serum androgen levels (total testosterone [T], bioavailable T [BioT]; 5α-androstane-3α, 17β-diol glucuronide [3αdiol G], and 4-Androstene-3,17-dione [androstenedione]), in 560 healthy men (median age 64 years) drawn from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Baseline serum androgens were measured by radioimmunoassay. Genotypes were determined as part of the Cancer Genetic Markers of Susceptibility Study genome-wide scan. SNP-hormone associations were evaluated using linear regression of hormones adjusted for age. Gene-based p-values were generated using an adaptive rank truncated product method.

RESULTS—Suggestive associations were observed for two inflammation-related genes and circulating androgen levels (false discovery rate [FDR] q-value<0.1) in both SNP and gene-based tests. Specifically, T was associated with common variants in *MMP2* and *CD14*, with the most significant SNPs being rs893226G>T in *MMP2* and rs3822356T>C in *CD14* (FDR q-value=0.09 for both SNPs). Other genes implicated in either SNP or gene-based tests were *IK* with T and BioT, *PRG2* with T, and *TNFSF9* with androstenedione.

CONCLUSIONS—These results suggest possible cross-talk between androgen levels and inflammation pathways, but larger studies are needed to confirm these findings and to further clarify the interrelationship between inflammation and androgens and their effects on cancer risk.

Keywords

Inflammation; Androgens; Genes; Testosterone; Polymorphism; Single Nucleotide

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INTRODUCTION

The interplay between androgens and inflammation has been shown to contribute to carcinogenesis in several tumor types [1]. In animal models, treatment with androgens lowers circulating levels of pro-inflammatory cytokines and increases levels of anti-inflammatory cytokines [2]. In humans, reduced circulating androgen levels have been reported in subjects with chronic inflammatory conditions such as rheumatoid arthritis or systemic lupus erythematosus [3] and acute systemic inflammatory conditions such as sepsis [4]. While expression of some inflammation-related genes is known to be under androgenic control [5–8], less is known about the correlation between variation in inflammation genes and levels of circulating androgens. Therefore, we evaluated the association between serum androgen levels and common genetic variants across genes previously implicated in inflammation pathways. Our study evaluated a set of 9,932 common single nucleotide polymorphisms (SNPs) drawn from 774 inflammation-related genes genotyped in the Cancer Genetic Markers of Susceptibility (CGEMS) study in relation to measured serum androgen levels in 560 healthy men from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial [9].

MATERIALS AND METHODS

Study Population

The PLCO Trial was designed to determine the effect of cancer screening on mortality and has been described in detail previously [10, 11]. Briefly, from 1993 to 2001, 155,000 men and women aged 55–74 with no history of prostate, lung, colorectal or ovarian cancer were enrolled from 10 study centers across the US after written informed consent. Subjects were randomized into either the cancer-screening or control arm. The study protocol was approved by institutional review boards at each study center and at the National Cancer Institute. Men randomized into the prostate cancer screening arm were offered screening by prostate specific antigen (PSA) and digital rectal examination (DRE) at baseline and annually for five and three years, respectively. At baseline, subjects completed a questionnaire describing demographic characteristics and medical history. Blood samples were collected and stored at baseline and subsequent clinical exams. During annual follow-up, men with suspicious screening findings (PSA>4.0 ng/mL or DRE indicating suspicious prostate findings) were referred to their primary physician for diagnostic tests. Prostate cancer was confirmed by medical record review for these men as well as any who reported cancer events during follow-up. Additional case ascertainment was done through linkage with the National Death Index and cancer registries, if available.

A nested case-control sample (matched on calendar year of entry, age ±5 yrs, and number of years of follow-up) was selected from subjects in the prostate cancer screening arm using incidence-density sampling as part of the National Cancer Institute's CGEMS study [9]. The purpose of the CGEMS study is to determine the contribution of common genomic variation to risk of prostate cancer. From 1,105 non-Hispanic white subjects without prostate cancer in the CGEMS study, we excluded subjects from Hawaii because of small numbers (N=11) and 7 subjects with <95% completion for the inflammation-related SNPs included in this study, leaving 1,087 subjects with genetic data. Of these, 560 had available measurements for total testosterone (T), 5α-androstane-3α, 17β-diol glucuronide (3αdiol G), 4-Androstene-3,17-dione (androstenedione) and sex-hormone binding globulin (SHBG) that was measured as part of an ancillary PLCO study [12] and were included in this analysis.

Selection of Genes and SNPs

As part of the CGEMS study, 561,494 tag SNPs were genotyped using the HumanHap300 and HumanHap240 chips by Illumina Corporation (San Diego, CA). Genotyping quality control measures have been described previously [9]. A comprehensive list of 784 inflammation-related genes was compiled from existing databases of inflammation-related gene pathways (SuperArray [now SA Biosciences] http://www.sabiosciences.com/Cytokines_Inflammation.php; and Biocarta). To incorporate potential regulatory regions, SNPs genotyped within the regions extending from 20kb upstream to 10kb downstream of the gene boundaries for these 784 genes were selected for this study (NCBI Build 36.3). In total, 9,932 SNPs with a minor allele frequency (MAF) greater than 0.05 from 774 genes were available for analysis (10 genes did not have tag SNPs with MAF greater than 0.05). Percent of non-missing genotypes per SNP was greater than 90% for all SNPs. There was evidence of departure from fitness for Hardy-Weinberg proportion for 12 autosomal SNPs ($P<1\times10^{-4}$) in our study population, but these SNPs remained in the analysis for completeness. Of the 774 genes, 758 had more than one SNP and were included in exploratory gene-based tests.

Serum androgens and SHBG

Measurement of serum androgens and SHBG has been described previously [12] and is summarized here. Using baseline non-fasting blood samples, androstenedione and 3αdiol G were measured by direct double-antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX). T was measured by direct radioimmunoassay (Immunotech, Marseille, France). SHBG was measured by sandwich immunoradiometric assay (CIS-Bio, Gif-sur-Yvette, France). Average coefficients of variation for the assays were 14%, 11%, 14% and 18% for androstenedione, 3αdiol G, T, and SHBG, respectively. T (nmol/L) and SHBG (nmol/L) were used to calculate bioavailable testosterone (BioT) using the validated formula described previously [13].

Statistical analysis

Mean (SD) and median (interquartile range) were calculated for the serum androgens as well as age, body mass index (BMI; kg/m^2), and baseline PSA level (ng/mL). Androgens were natural log-transformed and included individually in linear models of the SNP (coded as counts of the minor allele) and age at entry (5-year intervals: 55–59, 60–64, 65–69, and 70–74) to generate beta estimates, standard errors, and two-sided p-values for each of the 9,932 SNPs. Lack of substantial population stratification within the non-Hispanic whites in CGEMS has been previously confirmed [14], so no population stratification principal components were included. Additional adjustment for BMI did not materially change effect estimates and so is not reported herein. We plotted nominal $-\log_{10}$ p-values by chromosomal position for each of the androgens to visually inspect single-SNP results. For the lead SNPs, defined as those SNPs with a false discovery rate (FDR) q-value less than 0.1, we used the Kruskal-Wallis test to indicate significant differences in androgen levels (in the original scale) by genotype.

Since inflammation genes were the biologic unit of interest, we also conducted gene-based tests using an adaptive rank truncated product (ARTP) method [15] to combine the SNP-level p-values into a summary gene-based p-value. This method is useful for detecting genes with multiple weak SNP-associations that may not be apparent in single-SNP analyses. Briefly, 10,000 permutation datasets were created for the 9,932 SNPs for each of the four serum androgens included in this study. The ARTP statistic was used to summarize the observed and permuted p-values for the SNPs within 758 genes using a set of j truncation points with the jth truncated point value equal to $j_{max}\{1,[n/20]\}$, where j=1, 2,.., 5, n denotes the number of SNPs in the gene and [n/20] represents the largest integer that is less than or

equal to [n/20]. To improve the accuracy of the estimates for small p-values, we used 40,000 permutations for the top twenty genes and 1 million permutations for the top two genes.

SNP- and gene-based tests were adjusted for multiple tests using the Benjamini and Hochberg [16] step-up false discovery rate (FDR)-controlling procedure (alpha=0.05) to estimate FDR q-values as implemented in the R *multtest* package (available from http://cran.r-project.org/web/packages/multtest/index.html). The FDR q-value for a test is the expected proportion of false positives among values at least as extreme as the tested value. FDR q-values indicate which tests have a low expected proportion of false positives after taking into consideration the other tests performed and can be roughly equated to a p-value adjusted for multiple testing.

RESULTS

Selected study characteristics for the sample of 560 healthy men in this report are included in Table I, including age, BMI, PSA, and serum androgen levels. The sample of men with androgen measurements did not differ from the men without measurements with respect to age, BMI or PSA (T-test p-values=0.16, 0.45, and 0.96. respectively).

None of the 9,932 inflammation-related SNPs examined was significantly associated with serum androgen levels after FDR-adjustment for multiple testing for all SNPs (FDR-adjusted q-value<0.05). The lowest p-values were seen for SNPs on chromosome 5 and 16 for T and BioT as well as on chromosome 19 for androstenedione (Supplementary Figure I). Associations for three SNPs with FDR-q-values<0.1 are provided in Table II. Two SNP markers, rs893226:G>T near the matrix metallopeptidase 2 (*MMP2*) gene and rs3822356:T>C near the CD14 molecule (*CD14*) gene were associated with increased serum T concentrations (nominal $P=1.5\times10^{-5}$ and 1.7×10^{-5}, respecitvely; FDR q-value=0.09 for both SNPs). In addition, the SNP marker, rs348373:C>T near the tumor necrosis factor (ligand) superfamily, member 9 (*TNFSF9*) gene was associated with increased serum androstenedione (nominal $P=4.9\times10^{-6}$; FDR q-value=0.05). MAFs for the three SNPs were 0.38, 0.24, and 0.11, respectively, and did not differ among men with and without androgen measurements (p-value>0.1 for all SNPs). The three SNPs were consistent with Hardy-Weinberg proportion. On the original scale, median serum T was 14.9, 16.0, and 19.5 nmol/L among men with GG, GT, and TT genotypes for the rs893226 SNP (nominal $P=1\times10^{-4}$) and 15.2, 16.8, and 19.9 nmol/L among men with TT, TC, and CC genotypes for the rs3822356 SNP (nominal $P=7\times10^{-4}$). Median androstenedione levels were 1.2, 1.4, and 1.5 ng/dL among men with CC, CT, and TT genotypes for the rs348373 SNP (nominal $P=2\times10^{-4}$), respectively.

None of the 758 genes examined was significantly associated with androgen levels in gene-based tests after adjustment for multiple testing (FDR-adjusted P<0.05). The nominal gene-based p-values for each of the 758 genes in association with the four androgens are presented in Supplementary Table I. Supporting the SNP results, the *MMP2* and, *CD14* genes showed suggestive associations with T levels (FDR q-value=0.06 for both genes) (Table III). Other suggestive gene-based associations were found between T and IK cytokine (*IK*) as well as the bone marrow proteoglycan 2 (*PRG2*) gene. *MMP2*, *CD14*, and *IK* were also associated with BioT with borderline significance.

DISCUSSION

In this exploratory study of inflammation-related genes and serum androgen levels, we evaluated 9,932 inflammation-related SNPs; three were of marginal significance in association with serum androgens, with two SNPs (one in *MMP2* [rs893226] and the other

in *CD14* [rs3822356]) associated with T levels. Gene-based analyses also showed borderline significant associations between T and the *MMP2*, and *CD14* genes. Other borderline associations were identified in SNP or gene-based tests, but not in both, including associations between a *TNFSF9* SNP and androstenedione as well as an association between T and the *IK* and *PGR2* genes. These preliminary results suggest possible cross-talk between the inflammation and androgen pathways, two important pathways that have been linked to both cancer development and progression [1].

Of interest is the observation that three of the genes identified here (*CD14*, *TNFSF9*, and *MMP2*) are involved in tumor necrosis factor (TNF) signaling, which has been implicated in cross-talk with androgen signaling. For example, TNF-α and other pro-inflammatory cytokines can inhibit Leydig cell steroidogenesis [4] and stimulate aromatase activity [17], the enzyme responsible for conversion of T to estradiol. *CD14* codes for a glycoprotein expressed on the surface of monocytes, macrophages, and neutrophils that binds to lipopolysaccharide (LPS) on bacterial cells to stimulate the synthesis and release of proinflammatory cytokines such as TNF-α [18]. The *TNFSF9* gene product, a transmembrane cytokine within the TNF superfamily, appears to be involved in sustained TNF production after LPS stimulation [19]. TNF-α, in turn, has been shown to influence transcription of the *MMP2* gene in laboratory studies [20, 22]. *MMP2* codes for an enzyme that degrades extracellular matrix as part of normal physiological processes [23] as well as progression of several solid tumors [23, 24]. Interestingly, the *MMP2* gene appears to be under partial androgenic control since binding sites for the androgen receptor have recently been identified in the promoter of the gene [5].

While the androgens examined in this report are key metabolites of the androgen metabolic pathway [25], our preliminary observations were seen with T or BioT (the estimated fraction of serum T that is not bound to SHBG) rather than 3αdiol G, the latter of which is commonly considered a surrogate for tissue-androgenic activity. Consequently, we infer that the lack of association between inflammation-related genes and serum 3αdiol G suggests that if variation in inflammation-related genes influences androgen pathways, it is more likely related to synthesis or secretion of androgens rather than intraprostatic androgen metabolism. This hypothesis, however, needs confirmation in larger studies.

Our study has several strengths. First, we had high-quality genotype data on tag SNPs covering a substantial proportion of common genetic variation [9]. Second, we used validated assays to determine serum androgen levels in a population of men that were screened regularly for prostate cancer. Third, we confirmed no strong confounding by BMI. Lastly, we considered gene-based tests in addition to SNP-based results, which allowed us to detect signal for genes like *PRG2* that had several SNPs with associations of borderline significance. Limitations of the study should also be noted. First, due to small sample size ($N<600$), we had limited power to detect modest associations for SNPs. Coverage of the genes was not complete and specifically did not target known functional elements in these genes. The SNPs used in this study were tag SNPs included on a genome-wide panel because they are 1) common and 2) represent genome-wide variation based on linkage disequilibrium patterns. While the probability of false positives was minimized by adjusting for multiple testing (FDR-adjusted q-value<0.1), our preliminary results require further studies to confirm the findings.

CONCLUSIONS

Our results suggest candidate gene associations between serum androgens and variation in several inflammation-related genes. Larger studies are needed to confirm these associations

and to better understand mechanisms of cross-talk between androgen and inflammation pathways and their joint effects on cancer risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

3αdiol G	5α-androstane-3α, 17β-diol glucuronide
androstenedione	4-Androstene-3,17-dione
ARTP	adaptive rank truncated product
BioT	bioavailable testosterone
BMI	body mass index
CGEMS	Cancer Genetic Markers of Susceptibility
DRE	digital rectal exam
FDR	false discovery rate
IK	IK cytokine
LPS	lipopolysaccharide
MAF	minor allele frequency
MMP2	matrix metallopeptidase 2
PLCO	Prostate, Lung, Colorectal, and Ovarian
SNPs	single nucleotide polymorphisms
PRG2	bone marrow proteoglycan 2
PSA	prostate specific antigen
SHBG	sex hormone binding globulin
T	total testosterone
TNF	tumor necrosis factor
TNFSF9	tumor necrosis factor (ligand) superfamily, member 9

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Table I

Selected baseline study characteristics for 560 healthy men from the Prostate, Lung, Colorectal, and Ovarian study

Characteristic	Mean (SD)	Median (Interquartile Range)
Age (y)	64.2 (4.9)	64.0 (7.0)
BMI (kg/m^2)	27.6 (3.9)	27.2 (4.9)
PSA level (ng/mL)	1.6 (1.5)	1.1 (1.2)
T (nmol/L)	17.6 (8.5)	16.0 (9.7)
Biovailable T	4.8 (1.9)	4.6 (2.3)
3αdiol G (ng/dL)	8.2 (5.7)	6.8 (6.4)
Androstenedione (ng/dL)	1.3 (0.4)	1.2 (0.6)
Sex hormone-binding globulin (nmol/L)	48.8 (23.0)	43.8 (26.0)

3αdiol G, 5α-androstane-3α, 17β-diol glucuronide; Androstenedione, 4-Androstene-3,17-dione; PSA, prostate-specific antigen; T, total testosterone

Table II

Promising associations between serum androgens and inflammation-related SNPs in 560 healthy men (FDR-adjusted q-value <0.1)

Androgen	dbSNP ID	Minor/Major Allele	MAF	Cytoband	Genomic position	SNP Function	Gene	Beta*	SE	Nominal P-value	FDR-adjusted q-value
T	rs893226	T/G	0.38	16q12.2	54060397	intergenic	*MMP2*	0.13	0.03	1.5×10^{-5}	0.09
	rs3822356	C/T	0.24	5q31.3	140002620	intronic	*CD14*	0.15	0.03	1.7×10^{-5}	0.09
Androstenedione	rs348373	T/C	0.11	19p13.3	6494342	intergenic	*TNFSF9*	0.16	0.03	4.9×10^{-6}	0.05

MAF, minor allele frequency; 4-Androstene-3,17-dione; FDR, false discovery rate; SNP, single nucleotide polymorphism; T, total testosterone

*adjusted for age (55–59, 60–64, 65–69, and 70–74); additive coding used for SNPs with minor allele as coded allele; beta is for natural log transformed serum sex hormones

Table III

Gene-based FDR- q-values for serum androgens and the top inflammation genes in 560 healthy men (FDR-adjusted q-value < 0.1)*

Gene Symbol	Cytoband	T	3αdiol G	Androstene-dione	Bioavailable T	SNPs Included in Gene-based Test
MMP2†	16q12.2	0.06	0.91	0.85	0.09	rs837531, rs1816595, rs12924764, rs11643163, rs7187242, rs11859163, rs893226, rs1005913, rs1347653, rs16955194, rs243866, rs17301608, rs1053605, rs9302671, rs243842, rs183112, rs1992116, rs243840, rs11639960, rs243834, rs1861320, rs8054459
CD14	5q31.3	0.06	0.80	0.99	0.09	rs778583, rs778584, rs7721577, rs2569188, rs2569193, rs12517200, rs3822356, rs753279, rs1583005
IK	5q31.3	0.06	0.80	0.99	0.09	rs2569188, rs2569193, rs12517200, rs3822356, rs753279, rs1583005, rs2286394
PRG2†	11q12	0.06	0.92	0.87	0.16	rs10400305, rs3741089, rs490358, rs10792094, rs555097, rs3851114, rs3741085, rs10792095

3αdiol G, 5α-androstane-3α, 17β-diol glucuronide; 4-Androstene-3,17-dione; FDR, false discovery rate; SNPs, single nucleotide polymorphisms; T, total testosterone

*Global gene-based P-values generated across single nucleotide polymorphisms within 20kb upstream or 10kb downstream of the gene boundaries using an adaptive rank truncated product method with 40,000 bootstrap samples; P-values are for association with natural log-transformed androgens after adjustment for FDR using the method by Benjamini and Hochberg[16]

†Gene-based P-values for these genes used 1 million bootstrap samples