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Original article

Validation of copy number variants associated with prostate cancer risk and prognosis

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Abstract

Objective: Two recent studies have reported novel heritable copy number variants on chromosomes 2p, 15q, and 12q to be associated with prostate cancer (PCa) risk in non-Hispanic Caucasians. The goal of this study was to determine whether these findings could be independently confirmed in the Caucasian population from the South Texas area.

Methods and materials: The study subjects consisted of participants of the San Antonio Biomarkers of Risk for PCa cohort and additional cases ascertained in the same metropolitan area. We genotyped all 7 of the reported copy number variants using real-time quantitative polymerase chain reaction in 1,536 (317 cases and 1,219 controls) non-Hispanic Caucasian men, and additionally, we genotyped 632 (191 cases and 441 controls) Hispanic Caucasian men for one of these variants, a deletion on 2p24.3.

Results: Association of the deletion on 2p24.3 with overall PCa risk did not meet our significance criteria but was consistent with previous reports (odds ratio, 1.40; 95% confidence interval 0.99–2.00; $P = 0.06$). Among Hispanic Caucasians, this deletion is much less prevalent (minor allele frequencies of 0.059 and 0.024 in non-Hispanic and Hispanic Caucasians, respectively) and did not show evidence of association with risk for PCa. Interestingly, among non-Hispanic Caucasians, carrying a homozygous deletion of 2p24.3 was significantly associated with high-grade PCa as defined by Gleason score sum ≥8 (odds ratio, 27.99; 95% confidence interval 1.99–392.6; $P = 0.007$ [the Fisher exact test]). The remaining 6 copy number variable regions either were not polymorphic in our cohort of non-Hispanic Caucasians or showed no evidence of association.

Conclusions: Our findings are consistent with the reported observation that a heritable deletion on 2p24.3 is associated with PCa risk in non-Hispanic Caucasians. Additionally, our observations indicate that the 2p24.3 variant is associated with risk for high-grade PCa in a recessive manner. We were unable to replicate any association with PCa for the variants on chromosomes 15q and 12q, which may be explained by regional population differences in low frequency variants and disease heterogeneity. Published by Elsevier Inc.

Keywords: Prostate; Cancer; Risk; Deletion; Prognosis

1. Introduction

Prostate cancer (PCa) is the most common nonskin cancer among American men. The majority of PCa cases, however, is indolent and may not require treatments that are associated with significant rates of voiding and sexual function complications. There is great impetus to identify markers that distinguish indolent from aggressive disease. Given that risk for PCa may be attributed to a heritable component in as many as 42% of cases [1], it may be possible to identify genetic polymorphisms that can be used

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to better predict risk and prognosis. Multiple studies have attempted to identify genetic variants that are associated with risk for PCa.

The contribution of copy number variants (CNVs) to risk for complex diseases has not yet been fully elucidated, partially because of the difficulty in identification and accurate genotyping [2]. Efforts to characterize common and rare copy number variation in various human populations are still underway. Common CNVs have been shown to be in linkage disequilibrium with adjacent single nucleotide polymorphisms (SNPs) [3–5], which indicates that some common CNVs have already been indirectly assessed for association with traits in SNP-based genome-wide association studies (GWAS). However, recurrent variants and risk-bearing alleles with low minor allele frequencies (MAF) may not be well tagged by SNPs in GWAS, and these variants would likely require direct assessment.

Few studies have investigated risk for PCa attributed to CNVs directly. Liu et al. [6] published the first genome-wide investigation of germ line CNVs and risk for PCa, in which they report a germ line deletion spanning 5,947 base pairs at 2p24.3 associated with risk for PCa and aggressive PCa among men of European descent. Demichelis et al. [7] reported 6 deletions associated with risk for PCa with a false discovery rate <0.2. Two of these variants, deletions on 15q and 12q, were further supported in this same publication with evidence from bioinformatics analysis and functional assays [7].

GWAS for any genetic variant type are subject to a range of errors and biases, and therefore, replication of association signals in independent samples are necessary to confirm true associations [8]. One of the primary purposes of the San Antonio Center for Biomarkers of Risk for Prostate Cancer (SABOR), a Clinical and Validation Center of the Early Detection Research Network of the National Cancer Institute, is to independently confirm PCa biomarkers, including genetic variants that are predictive of risk. With this motivation, we report the investigation of these reported variants in non-Hispanic and Hispanic Caucasians from San Antonio, Texas.

2. Materials and methods

2.1. Study subjects

Study subjects consisted of 1,372 (153 cases and 1,219 controls) non-Hispanic Caucasian and 516 (75 cases and 441 controls) Hispanic Caucasian participants of SABOR. SABOR is a prospective longitudinal study that examines behavioral, genetic, and other markers of risk of PCa. All study participants are screened annually for PCa using prostate-specific antigen (PSA) serum measurements, with exception to those with PSA < 1, who are screened every other year. In addition, 164 non-Hispanic Caucasian and 116 Hispanic Caucasian men were recruited from a parallel cohort study of prevalent cases in the same metropolitan area. This study consists of men who were diagnosed with PCa prior to enrollment in the study and were recruited using the same methods as SABOR (local advertisement). Age for this study was calculated in the following manner: for prevalent cases, self-reported age of diagnosis was used; for incident cases, age of diagnosis was used; noncancer (control) participants were censored at their most recent SABOR examination age. Self-reported age of diagnosis for most of the prevalent cases was confirmed through medical records. Institutional review board approval was obtained from the University of Texas Health Science Center at San Antonio. Informed consent was obtained from all participants of both cohorts. We refer to the total population as the SABOR cohort.

Liu et al. [6] defined cancer aggressiveness as meeting any of the following criteria: $T_{3/4}$, N^+, M^+, Gleason score sum ≥ 8, or PSA > 50 ng/ml. Although it would be ideal to use identical criterion to define aggressiveness, PSA serum levels at the time of diagnosis as well as staging were not available for all participants of PREF, and thus, these variables were not utilized in defining aggressiveness. Gleason scores were more broadly available, and thus, in this study, aggressive cases in this study were defined by Gleason score of ≥ 8.

2.2. CNV genotyping

Deoxyribonucleic acid was isolated from whole blood using QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, CA). For real-time quantitative polymerase chain reaction (qPCR), all primers and probes were purchased from Applied Biosystems (Valencia, CA). The primer/probe pairs were either predesigned or designed for the regions of interest using Primer Express (Applied Biosystems, Valencia, CA). qPCR primer/probe information is summarized in Table 1.

For qPCR, primers and probes for a target sequence and reference sequence (Ribonuclease P) were multiplexed in 384-well plates and all samples were run in duplicate. Fluorescence was detected using the 7900HT real-time PCR System (Applied Biosystems, Valencia, CA). Real-time PCR data were analyzed using the reference-free $\Delta\Delta$ct method implemented in CopyCaller software (Applied Biosystems, Valencia, CA). This approach estimates the mean Δct for a copy number of 1 and subsequently uses this value to calculate $\Delta\Delta$ct. We have found this method broadly consistent with the $\Delta\Delta$ct method using a reference sample. Discrete copy number calls were determined by plotting histograms of the raw calculated copy number values, which for polymorphic regions reveals nonoverlapping Gaussian distributions representing integer copy number states [9].

To confirm that CNV calls from qPCR accurately detected the 2p24.3 deletion identified by Liu et al. [6], we conducted PCR genotyping as reported by Liu et al. [6] for all homozygous deletions, 12 heterozygous deletions, and 14 wild-type individuals, including 4 individuals on the Gaussian tails where the distributions approach each other in the non-Hispanic Caucasian samples. We observed 100%