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14. ABSTRACT

Individual copy number variations in the genome may play a substantial role in influencing trait variation, yet due to technical limitations they have been understudied. We have performed the first genome-wide association of copy number variants and risk for prostate cancer in Mexican Americans. We found a highly protective deletion on 8q24 which is present in Mexican Americans but extremely rare in Caucasians. Due to the strong effect of this deletion, this discovery has implications for prostate cancer risk assessment and for understanding the etiology of prostate cancer. This variant warrants further study. We have also identified a rare 900 bp deletion in the PTEN gene to be associated with increased risk for prostate cancer. These data support our hypothesis that heritable structural variation may affect risk for PCa and/or its progression. Moreover, these variants may be unique to ethnic population and underscores the need to investigate genetic risk in multiple populations. As genes are identified from these studies, they may prove to be both useful biomarkers for early diagnosis and/or excellent therapeutic targets for both prevention and treatment of prostate cancer.

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INTRODUCTION

Prostate cancer is known to have a strong genetic component. Thus, the identification of the heritable genetic alteration(s) that precedes or increases susceptibility to somatic cancerous changes in the prostate could likely lead to improved identification of high risk individuals for early screening and possibly to new treatment strategies. Recently, it has become apparent that structural variation comprises similar diversity of human genomes as SNPs and may play a significant role in disease susceptibility and resistance. The goal of this research project is to screen the entire autosomal genome for copy number variation (CNVs) in constitutional DNA to assess their role in risk of development of prostate cancer and then evaluate any direct effect on the prostate. Growing evidence from our study and others indicates that these genetic factors may be rare and numerous and, importantly, unique to the different ethnic populations. The differences in these genetic risk factors may partially explain the ethnic disparities in incidence of prostate cancer. This study is one of few examining genetic risk factors among the Mexican American population.

BODY

We have genotyped prostate cancer cases and elderly hyper-normal controls of Mexican American descent from the San Antonio Biomarkers or Risk (SABOR) cohort for ~750,000 markers across the genome using the Illumina OmniExpress assay. The average age of cases genotyped was 60.36 ± 6.36 years and the average age of controls was 70.89 ± 5.93 years. The groups did not differ in admixture estimates based upon the individual measures that were previously calculated for this cohort.[1] We identified 462 CNVs which were polymorphic in at least 2 individuals. 7 of 8 CNVs which were nominally associated (<0.05) with prostate cancer status were genotyped in additional cases and controls. A rare non-recurrent 8486 base pair deletion on 8q24 (distinct from the MYC locus) was associated with decreased prostate cancer risk in 989 Mexican American men (Odds ratio 0.20, p=0.02). Only 3 of 1530 Caucasian subjects carried the deletion, indicating that this deletion is not likely to affect risk in the Caucasian population. (Figure 1)

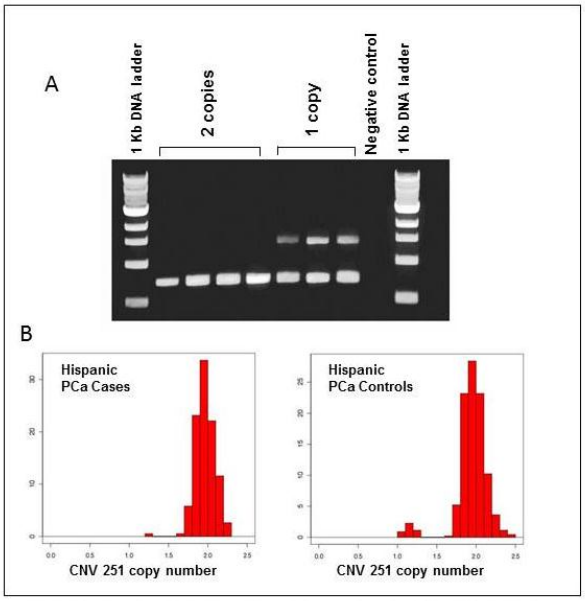

Figure 1. Genotyping of CNV 251 deletion in San Antonio Cohorts. A) Gel electrophoresis of PCR products, generated with nested primers, displaying genotype states of the deletion. Negative control indicates no DNA. B) Histogram of calculated copy number values from qPCR assays. Gaussian distributions are present representing integer copy number values 0, 1 and 2.

The deleted sequence contains a putative conserved transcription factor binding site for NKX3.1. NKX3.1 is an androgen regulated homeobox gene involved in prostate cancer development, is required for stem cell maintenance, and marks the luminal epithelial cell that is the cell of origin for prostate cancer[2]. Unfortunately NKX3.1 is not expressed by lymphocytes so functional analyses as described in Task 2 would not be informative for this CNV. In addition, the deletion variant appears to reduce risk for PCa, so follow-up as described in Task 3 in which prostate tumors were to be examined is not relevant for this CNV. Other assays will be needed to determine the functional significance of this variant.

During this past year we have taken further steps to identify CNVs in our cohort. Using the newly released program IMPUTE2.2[3], the 1000 Genomes project reference panel, and our SNP genotyping data, we have imputed known CNVs in our samples. We used allelic association in the program PLINK to test for association with cancer status. In total, 3725 CNVs with minor allele frequency (MAF) >0.01 were identified and tested. None of the 760 common CNVs with MAF >0.1 were significantly associated. Among the rare CNVs that were nominally associated with prostate cancer status, the most significant to fall within a gene is a 900 bp deletion in the gene PTEN. Using the imputed genotypes, 9 of 96 cases and 0 of 92 controls carried the variant (p=0.00328). We sequenced the 9 samples bearing the deletion to identify the precise breakpoints and determined the proximal breakpoint to be 57-59 bp from exon2 of PTEN. We then genotyped all 626 Mexican American SABOR samples (433 controls, 193 cases) using qPCR. The original genotype imputations were confirmed and 17 additional subjects were identified as bearing the CNV. In total, 12 cases and 14 controls carried the deletion (OR 2.44 95%CI:1.08-5.51, p=0.032, logistic regression adjusting for age). Notably, the controls bearing the deletion were significantly younger than controls not harboring the variant (53.3 yr vs. 60.2, respectively; p=0.002) which leaves the possibility of conversion to PCa early for these men. We are in the process of conducting a survival analysis for this variant with PCa status as the outcome variable. In addition, we can now proceed with Task 3 to examine prostate tumors of bearers of this variant (and 2 others) that appear to increase risk for PCa.

Another critical step in the identification and reporting of genetic variation in relation to PCa is to replicate our discoveries in other cohorts. As mentioned, few genetic studies are being conducted among the Mexican American population. We are reaching out to colleagues to identify such cohorts and carry out replication studies. We are also applying multiple methods to conduct rare variant burden testing in our cohort. The area of rare variant analyses is one of intense research which we are involved in and following.

Replication work of reported heritable CNVRs and risk for PCa

In addition to performing CNV discovery in the SABOR cohort, we continue to utilize this resource to examine and validate CNVs that have been reported by other research groups. During this project year, Demichelis et al. reported on the identification of rare CNVs at 15q21.3 and 12q21.31 that were associated with prostate cancer risk among men of Caucasian origin.[4] As before, we tested whether this finding could be confirmed in the Hispanic Caucasian subjects of the SABOR and PREF. In total, we tested 6 of the reported CNVs and none were associated with prostate cancer in the SABOR. Three of these rare variants were nonpolymorphic in this population.

KEY RESEARCH ACCOMPLISHMENTS and REPORTABLE OUTCOMES

- Blackburn A., Gelfond J., Goring H.H., Beuten Y., Thompson I., Leach RJ, and Lehman DM. (2009) Identification of Copy Number Variable Regions (CNVRs) Associated with Risk of Prostate Cancer in Mexican-Americans. Abstract presented at 59th Annual meeting of the American Society of Human Genetics, Honolulu HI, October 2009
- Lehman DM. Identification of Copy Number Variable Regions (CNVRs) Associated with Risk of Prostate Cancer in Mexican-Americans, DOD IMPaCT conference, Health Disparities, Department of Defense, Orlando, F,L March 2011 (Invited Speaker)

- Blackburn A, Gelfond J, Yao L, Thompson IA, Leach RJ, Lehman DM (2011). A heritable deletion on 8q24 lowers risk for prostate cancer in Mexican Americans. Abstract presented at the Cancer Therapy and Research Center Annual Symposium, UT Health Science Center, San Antonio TX
- August Blackburn, Jonathan Gelfond, Yao Li, Iriscilla Ayala, Ian Thompson, Robin J. Leach, Donna M. Lehman (2012). A Heritable Deletion on 8q24 Lowers Risk for Prostate Cancer in Mexican Americans. Platform presentation at Texas Genetic Society annual meeting, March 22-24, San Antonio TX

CONCLUSION

We have performed the first genome-wide association of copy number variants and risk for prostate cancer in Mexican Americans. We found a highly protective deletion on 8q24 which is present in Mexican Americans but extremely rare in Caucasians. Due to the strong effect of this deletion, this discovery has implications for prostate cancer risk assessment and for understanding the etiology of prostate cancer. This variant warrants further study. We have also identified a rare 900 bp deletion in the PTEN gene to be associated with increased risk for prostate cancer. These data support our hypothesis that heritable structural variation may affect risk for PCa and/or its progression. Moreover, these variants may be unique to ethnic population and underscores the need to investigate genetic risk in multiple populations. As genes are identified from these studies, they may prove to be both useful biomarkers for early diagnosis and/or excellent therapeutic targets for both prevention and treatment of prostate cancer.

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