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Fine mapping of 11q13.5 identifies regions associated with prostate cancer and prostate cancer death ☆

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Abstract ***Background:*** Chromosomal region 11q13–14 associates with prostate cancer (PrCa). Previously, we identified a rare intronic mutation on *EMSY* (11q13.5) that increases the risk of aggressive PrCa and associates with familial PrCa. Here, we further study the genetic structure and variants of the PrCa susceptibility region 11q13.5.
Methods: This study included 2716 unselected hospital-based PrCa cases, 1318 cases of a screening trial and 908 controls of Finnish origin. We imputed single nucleotide polymorphisms (SNPs) and structural variants from the 1000 Genomes Project and validated the associations of the variants in two PrCa patient sets by genotyping. Genetic structure was studied with haplotype analysis.
Results: Two independent regions at 11q13.5 were associated with PrCa risk. The most significant association was at *EMSY* (rs10899221, odds ratio (OR) 1.29–1.40, $P = 3.5 \times 10^{-4}$–0.002) near the previously identified mutation. Correlated intronic SNPs rs10899221 and rs72944758 formed with other *EMSY* variants common and rare haplotypes that were associated with increased risk ($P = 4.0 \times 10^{-4}$) and decreased risk ($P = 0.01$) of PrCa, respectively. The other associated region was intergenic. Among the six validated variants, rs12277366 was

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significant in both patient sets (OR 1.15–1.17, $P = 0.01$). Haplotypes associated with an increased risk ($P = 0.02$) and a decreased risk ($P = 0.02$) were identified. In addition, the intergenic region was strongly associated with PrCa death, with the most significant association at rs12277366 (OR = 0.72, $P = 4.8 \times 10^{-5}$).

Conclusions: These findings indicate that 11q13.5 contributes to PrCa predisposition with complex genetic structure and is associated with PrCa death.

1. Introduction

Hereditary factors increase the risk of prostate cancer (PrCa), with a heritability estimate of 16–45%.[1] Genome-wide association studies (GWAS) and fine-mapping studies have identified multiple single nucleotide polymorphisms (SNPs) that associate with PrCa predisposition. However, GWAS SNPs only account for a portion of the estimated heritability.[2,3]

Chromosomal region 11q is linked with hereditary PrCa in the Finnish population.[4] The most significant linkage signal was detected between 11q13.4 and 11q22 (D11S1314 and D11S898), with the highest peak at 11q14.1 (D11S901). An association of 11q13 with PrCa was further supported by GWAS studies that identified two correlated SNPs at 11q13.2 (rs7931342 and rs10896449).[5,6] We previously screened genetic variants in exons and exon–intron boundaries of *EMSY* (*C11orf30*, 11q13.4–11q13.5) in Finnish PrCa patients.[7] EMSY interacts with BRCA2 and chromatin remodelling proteins[8] and regulates the transcription of interferon-stimulated genes.[9] Our study identified a rare intronic mutation IVS6-43A>G (rs200331695) that increases the risk of aggressive PrCa and associates with familial PrCa in the Finnish population.[7] No causative genetic variants known to contribute to cancer development have been identified in the 11q13–14 locus.

In this study, we fine mapped the chromosomal region 11q13.5 around the previously identified mutation to characterise the genomic structure of 11q13.5 and to identify the PrCa predisposing genetic variants in this region.

2. Patients and methods

2.1. Study population

The clinical characteristics of PrCa patients are summarised in Table 1. A total of 2716 unselected hospital-based patients of Finnish origin were identified from the Pirkanmaa Hospital District. Furthermore, the study included 1318 PrCa patients diagnosed in the Finnish component of the European Randomized Study of Screening for Prostate Cancer described in detail elsewhere.[10] The patients were from the screening arm of the prostate-specific antigen (PSA) screening trial. A total of 908 control samples originated from anonymous healthy Finnish male blood donors provided by the Finnish Red Cross. Full written informed consent concerning the samples and patient information was obtained from the patients. The study was performed with the appropriate research permissions from the Ethics Committee of the Tampere University Hospital, Finland, and the Ministry of Social Affairs and Health in Finland.

2.2. Genotyping and statistical methods

Genotyping is described in Supplementary Methods. A total of 31 tagging SNPs (tagSNPs) were determined covering region 76154846–76569208 at 11q13.5 (Supplementary Methods and Supplementary Table 1) and were genotyped from 1811 unselected hospital-based PrCa patients and 908 controls. Prior imputation samples with genotype call rates <90% (11/2719) and tagSNPs, which deviated from Hardy–Weinberg equilibrium (HWE) in the controls (1/31), were excluded from the study.

Imputation was performed using IMPUTE2 v2.2.3[11,12] with default settings and a 1000 Genomes cosmopolitan reference panel.[13] Imputed variants with expected minor allele frequency (MAF) $\geqslant$1% and an info score $\geqslant$0.8 and variants with MAF <1% and an info score $\geqslant$0.9 were included in the association analysis. Five genetic models of association (additive, dominant, recessive, general and heterozygote) were tested using a Frequentist test, which accounts for imputation uncertainty, with the score method implemented in SNPTEST v2.3.0.[14] Variants deviating from HWE in the controls were excluded from the analysis (1/605).

Validated variants were tested for HWE in the controls and for an association with a risk of PrCa using Cochran–Armitage trend test. We used a case–case design to test the association of the validated variants with clinicopathological features of unselected PrCa [diagnosis age, Gleason score (aggressive cancer), PrCa death, PSA level and progression after prostatectomy, radiation therapy and hormone therapy]. We calculated the P values adjusted for correlated tests (P_{ACT}) to account for multiple testing.[15] Statistical analyses were performed using PLINK v1.07[16] and R v2.11.1.[17] Pairwise measures of linkage disequilibria (LD) between SNPs (r^2), haplotype-block structure based on Gabriel's definition[18] and haplotype associations were calculated