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Genetic basis of prostate cancer: Association studies

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Summary. Prostate cancer (PCa) is the most common malignancy in men. This paper reviews the results of previous work of the study group PROSTATSERBIA. In the candidate gene association study at the beginning of our research, we tested the association between several single nucleotide polymorphisms (SNPs) in the NOS3 gene and PCa risk and/or progression. In a population-based case-control study, we explored the possible association between PCa risk and seven SNPs identified by genome-wide association analyses (GWASs) in two chromosomal regions (8q24 and 17q12). For the first time in a European population, microRNA genetic variants and genetic variants in RNA-induced silencing complex (RISC) genes have been analyzed for their potential association with PCa.

Keywords: association studies, genetic variants, prostate cancer.

Prostate cancer (PCa) is the most common malignancy in men and a major cause of cancer deaths (Attard et al. 2016). PCa incidence has rapidly increased throughout southeastern Europe, including Serbia. In Serbian men, PCa is the third leading cause of cancer deaths (Znaor et al. 2013). Despite a significant morbidity and mortality to a lesser extent, the etiology of prostate cancer remains largely unknown (Menegaux et al. 2014).

Standard prognostic parameters for PCa diagnosis and treatment are the serum level of prostate specific antigen (PSA), the clinical stage of the disease according to tumor/node/metastasis (TNM) classification and the Gleason score (Humphrey et al. 2004). In recent decades, thanks to the use of PSA screening, PCa is frequently diagnosed in its initial stages. PCa screening and early detection can lead to the diagnosis and overtreatment of clinically insignificant disease with long-term effects on patient quality of life. These data are one of the reasons for the great interest of the scientific community in the molecular basis of PCa, particularly the discovery of new biomarkers that may be utilized in the prediction of PCa incidence, outcomes and response to therapy (Sharma et al. 2015).

The most common genetic variants in the human genome are single nucleotide polymorphisms (SNPs). Numerous SNPs as genetic susceptibility markers have been identified from family-based studies, candidate gene association studies and genome-wide association studies (GWASs) (Rebbeck 2017). This paper reviews the results of previous work of the study group PROSTATSERBIA (Single nucleotide polymorphisms and prostate cancer risk in Serbian population), a member of The Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium. In the last ten years, we have collected peripheral blood samples from 400 patients diagnosed with PCa and 400 patients with benign prostatic hyperplasia (BPH), and have had access to samples of buccal swabs of healthy controls from Serbian population (400). Using the abovementioned collection of samples, we performed a number of association studies. Association studies compare the frequency of genetic variants in patients with the disease (cases) versus healthy volunteers (controls) (Ahmed and Eeles 2016).This case-control studies were approved by the Ethical Committees of the Clinical Center "Dr Dragiša Mišović" and Clinical Center "Zvezdara" in Belgrade, Serbia.

Numerous studies showed that the production of endogenous nitric oxide (NO) is associated with apoptosis of tumorigenic cells (El-Sehemy et al. 2016) and involved in the regulation of tumor angiogenesis (Amankwah et al.

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2012). Our initial research was related to the endothelial nitric oxide synthase 3 (NOS3) gene as the candidate gene for PCa. This type of association study is known as a candidate gene association study (Ahmed and Eeles 2016). Several SNPs have been identified in the NOS3 gene. Two common polymorphisms are -786T>C (rs2070744) and 894G>T (rs1799983), but -1468T>A, -922G>A, -764A>G, -714G>T, -690C>T, -649G>A and 4a4b polymorphism (27-bp repeat, intron 4) are also reported. The results of association studies have shown that several polymorphisms in the NOS3 gene are associated with PCa (Lee et al. 2009).

A common single nucleotide genetic variant located in exon 7 (894G>T) of the NOS3 gene results in amino acid substitution at the position 298 (Glu298Asp), which is implicated in low NOS3 levels due to reduced protein stability (Tesauro et al. 2000). Marangoni et al. (2008) identified -786T>C polymorphism as the most important promoter alteration of the NOS3 gene that may affect PCa progression, but not its occurrence. More recent studies reported a significant difference in both genotype distribution and allele frequency between PCa patients and healthy controls, showing that patients with the NOS3 CC genotype have an increased risk for PCa (Safarinejad et al. 2013).

For 786T>C polymorphism, we established that the C allele is associated with the absence of metastases. It was found that, compared with NOS3 690C>T variant CC genotype, CT and TT genotypes confer a decreased risk of developing metastases (dominant model, P = 0.049; OR, 0.50; 95% CI, 0.25-1.00) and show an association with a low clinical tumor stage, compared to stages T3 and T4 (dominant model, P = 0.046, OR, 0.20; 95% CI, 0.04-1.02). Genetic variants 764A>G, 714G>T and 649G>A were not detected in our study group. There is a body of evidence regarding an inverse correlation of the NOS3 894G>T minor allele with high serum PSA (>20 ng/ml) (dominant model, P = 0.013, OR, 0.37; 95% CI, 0.17-0.82). Our results suggest that NOS3 gene polymorphisms are genetic susceptibility factors for the progression of PCa and patient outcome (Branković et al. 2013a).

To elucidate the involvement of these variants in prostate carcinogenesis, we conducted a meta-analysis of previously published case-control and relevant case-only studies. Eleven studies comprising 3806 cases and 4466 controls in total were included in the meta-analysis, which yielded evidence of the association of rs2070744 and intron 4a/b variant with PCa risk under a recessive and dominant model, respectively. Furthermore, PCa patients carrying the 4a/b allele were found to have an increased risk of cancer progression to a less differentiated form, characterized by a high Gleason score. These results support the involvement of NOS3 variants in the molecular pathogenesis of PCa (Nikolić et al. 2015a).

Genome-wide association analyses have identified more than 100 PCa risk-associated SNPs. The molecular mechanisms are unclear for most of these SNPs (Chen et al. 2015). The results of initial GWASs showed that most of the PCa-associated genetic variants are located in the so-called "gene-deserts" and are suspected of affecting gene expression levels and genome/chromatin organization (Van den Broeck et al. 2014).

One of the first GWASs on PCa identified 17q12 as the region carrying PCa susceptibility variants. Together with 8q24, this region remained the most significant region associated with PCa risk and it was replicated in multiple populations and different ethnic groups (Gudmundsson et al. 2007; Zheng et al. 2008).

In the population-based case-control study, we tested the association between PCa risk and the SNPs rs1447295, rs4242382, rs6983267, rs7017300 and rs7837688 at 8q24, as well as rs7501939 and rs3760511 at 17q12, in the Serbian population. Additionally, we evaluated the possible link between these SNPs and the standard prognostic parameters of PCa progression. Furthermore, we conducted a meta-analysis for rs7501939 and rs3760511 at the 17q12 region (Branković et al. 2013b; Nikolić et al. 2014a).

There was a significant positive association between allele A of the SNP rs4242382 and PCa risk. We found evidence of association between PCa risk and rs7017300 when comparing genotype distributions in PCa and BPH patients. The association between allele T rs7837688 and PCa risk was determined in PCa vs. BPH comparison. The odds ratios for carriers of rs6983267 TT genotype under a recessive model of association with PCa was found to be 0.36. For rs1447295, deviation from the Hardy-Weinberg equilibrium was observed in BPH patients and controls. We found no association between parameters of PCa progression and five 8q24 SNPs. Locus 8q24 harbors genetic variants associated with PCa risk in the Serbian population (Branković et al. 2013b). In recent years, by utilizing clinical data and a case-only design, both GWASs and validation studies have provided evidence of an association of several loci within 8q24 with PCa aggressiveness or survival (Nikolić et al. 2016). In a follow-up study of the Cancer Genetic Markers of Susceptibility (CGEMS) study, 8q24 rs4242382 and rs6983267 were proved to be associated with the risk for metastatic prostate cancer (Chen et al. 2013).

At the same time, we examinedthe possible association between the genotypes and alleles of two 17q12 polymorphisms (rs3760511 and rs7501939) and PCa risk and progression in the Serbian population. The association between allele T of rs7501939 and PCa risk was determined in PCa patients vs. controls. We found no association between the parameters of PCa progression and the analyzed SNPs. Meta-analysis showed a strong association between these variants and PCa risk. Our study shows an association between SNPs at locus 17q12 and the risk of prostatic diseases in the Serbian population. Results of the meta-analysis also suggested the association of these SNPs with PCa risk (Nikolić et al. 2014a).

Initial studies that aimed to analyze the association between genetic variants potentially influencing the expression and/or function of non-coding RNA molecules with PCa risk in the Serbian population did not provide evidence of the supposed effect of selected variants (Brajušković et al. 2013; Nikolić et al. 2013). The two chosen genetic variants for these case-control studies were rs374458 located in the most studied PCa-related region, 8q24 (Meyer et al. 2011), as well as rs3787016, which was identified through a meta-analysis of two previous genome-wide association studies targeting potential PCa-associated loci among putative long noncoding RNA (lcnRNA) genes (Jin et al. 2011).

The next approach in identifying PCa-associated genetic variants was based on microRNA-related mechanisms of RNA interference. Several genetic variants located in the genes encoding microRNAs that were previously studied in a few populations of Asian origin (Xu et al. 2010; George et al. 2011; Parlayan et al. 2014) were selected for analysis in the Serbian population, which is also the first population of European descent in which the association of the mentioned genetic variants with PCa risk and progression was tested.

The potential mechanisms of the effect of the selected variants on microRNA functions are based on their location and the presumed influence of mature microRNA biogenesis and/or the interaction of mature microRNAs with target mRNAs, including the target specificity changes depending on the allelic variant (Xu et al. 2010; George et al. 2011).

The results obtained in our initial study involving microRNA-related genetic variants have provided evidence of an association between rs2910164 located in a gene encoding miR-146a with the Gleason score, as well as with the presence of distant metastases and PCa aggressiveness (Nikolić et al. 2014b). These results significantly differ from those previously obtained in populations of Han Chinese (Xu et al. 2010) and North Indians (George et al. 2011), possibly reflecting the differences in ethnic background. For the association of this variant with PCa risk, statistical significance was not reached in our study, while previously mentioned studies provided contrasting results.

The obtained evidence of association between rs2910164 and the parameters of PCa progression encouraged our further analyses involving microRNA genetic variants. We next reported the lack of association between rs11614913 located in the gene encoding miR-196a2 and the risk of PCa onset and progression. On the other hand, we showed the association of rs3746444 located in hsa-miR-499 gene with the risk of PCa progression, which was not assessed in previous studies (Nikolić et al. 2015b).

Besides these genetic variants thatwere previously analyzed in other populations, rs895819 located in the gene encoding miR-27a was chosen to be tested for a possible association with PCa for the first time (Nikolić 2015b). The main reasons for choosing this genetic variant were its association with other malignant tumors and its potential effect on the biogenesis and/or function of miR-27a involved in the androgen signaling pathway (Bai et al. 2014; Fletcher et al. 2012). Our results showed an association of rs895819 with PCa risk when genotype distributions among PCa and BPH patients were compared. Furthermore, this genetic variant was shown to be associated with the clinical stage of localized PCa. Also, a minor allele C was found to confer increased risk of developing bone metastases (Nikolić et al. 2015b).

Since the potential effect on microRNA-related regulatory mechanisms is not restricted to genetic variants located in microRNA-encoding genes, the next phase in our research included the variants in genes encoding the proteins of the RNA-induced silencing complex (RISC). The only genetic variant among the selected ones that was previously analyzed was rs7813, located in *GEMIN4* (Liu et al. 2012). Our results showing the lack of association between this genetic variant and PCa risk contrasted the those previously obtained in the Han Chinese population. Nonetheless, the results showing the association of rs7813 with the clinical stage of PCa are in accordance with previous ones (Liu et al. 2012; Nikolić et al. 2017).

Taking these results together, most of the studied microRNA-related variants show the association with the parameters of PCa progression. Nevertheless, although promising, these results need to be validated in other European and non-European populations in order to clarify the effect of the analyzed genetic variants on PCa risk and progression.

In an attempt to identify the potential reason for discordances between our results and those previously obtained, we have conducted a meta-analysis involving rs2910164 located in the miR-146a gene (Nikolić et al. 2015c). Since the number of the included studies on PCa risk was relatively small (Xu et al. 2010; Nikolić et al. 2014b; Parlayan et al. 2014), we have also included studies on other types of malignant tumors, aiming to identify the potential effect of ethnic differences, study sizes, participant recruitment strategies and several other differences in study design on the results of association tests. Also, for the subgroup meta-analysis based on tumor type, the potential tissue-specific effect of rs2910164 on cancer risk was expected.

Although the association between rs2910164 and the overall cancer risk was not shown, our results have provided evidence of the association of this genetic variant with the risk of developing bladder, cervical, liver, gastric, lung and nasopharyngeal cancer, as well as oral squamous cell carcinoma. The association of rs2910164 with PCa risk was not found to be statistically significant. However, this subgroup meta-analysis on PCa risk included only the results of three studies, including ours. Stratified meta-analyses have also failed to show the significant effect of ethnic background and study size on the results of association studies involving rs2910164 (Nikolić et al. 2015c).

The main aim of genetic association studies of PCa is the identification of potential PCa genetic markers that could

improve our understanding of the molecular basis of PCa and serve for the evaluation of the risk for PCa development and/or PCa progression. Therefore, future research requires the integration of knowledge on genetic associations, cellular pathways, and statistical epistasis for the better understanding of neoplastic transformation of prostate tissue. We strongly believe that such investigations could greatly improve clinical protocols in monitoring and treating PCa (Nikolić et al. 2016).

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