UNIVERSITY OF MEDICINE AND PHARMACY "CAROL DAVILA" BUCHAREST

FACULTY OF MEDICINE

GENETIC MARKERS IN LOCALIZED PROSTATE CANCER

PHD THESIS SUMMARY

PhD supervisor:
Professor Viorel JINGA, PhD

Doctoral candidate
Doctor Dan-Gabriel ROȘOGA

Bucharest, 2022

CONTENTS

1.Introduction

Prostate cancer is the most common non-cutaneous type of cancer and the 3rd most common neoplasia present within the male population, with over 1,000,000 cases discovered annually and over 300,000 deaths.

Studies show that the current risk for a male in a Western society of developing microscopic prostate cancer throughout his lifetime is approximately 30%. The risk of developing the clinical illness is 10%, and the risk of dying from this condition is 3%.

The revolutionary development in terms of new screening methods has improved the discovery rate of this pathology in the last decades, allowing for an early diagnosis in localized stages of the disease.

A correct screening for these patients that facilitates the diagnosis and establishment of a therapeutic management procedure customised for each individual, mainly focused on improving their quality of life, constitutes a challenge for present generations of urologists and researchers. There is still a tendency to over-treat some patients, especially in the case of those with a low risk of developing the disease, which actually increases morbidity and does not entail any actual clinical benefits.

The risk of over-diagnosis and overtreatment of a possible cancer condition in men who have not presented symptoms of the disease must be taken into consideration and monitored carefully.

Two significant randomized clinical trials (PLC and ERSPC) have different findings regarding the effectiveness of prostate cancer screening in terms of reducing the rate (frequency) of mortality from the disease.

A recent meta-analysis of randomized clinical trials showed that PSA screening has no significant effect on prostate cancer mortality, when compared to overall mortality.

Throughout the years, at a global level, there has been concern in terms of the discovery and analysis of various genetic factors involved in prostate tumour pathology.

Multiple studies, particularly epidemiologic studies and genome-wide association studies (GWAS), have demonstrated a genetic component in what

concerns the etiology of prostate cancer, which represented the starting point for this study.

The ultimate target of GWAS studies is to use genetic risk factors, in order to predict who is at risk of developing a particular disease, and to identify the biological underpinnings of disease susceptibility, in order to develop new prevention and treatment strategies.

A significant number of oncogenes are currently described as being in connection with this pathology, but the goal is to provide these markers with clinical utility.

Their use in current practice would facilitate a better determination of the risk of disease, as well as the improvement of therapeutic strategies.

The objective of this doctoral thesis is to discover and analyse the genetic variations occurring in individuals with prostate cancer, diagnosed in Romania, and to assess their risk in relation to the development and prognosis of the disease.

2.Study objectives

Despite recent medical advances, prostate cancer still remains a significant medical issue for men. This study focuses on the assessment of the genetic component of this respective pathology and summarizes the opportunities to reduce prostate cancer morbidity and mortality in Romania.

Prostate cancer in Romania is one of the most common forms of cancer in men, with an annual incidence of 32.2 / 100,000 cases[1,2].

The analysis of the classic methods currently used in order to determine the diagnosis, as well as the population at risk, represented viable screening methods, as they are validated in current medical practice. An example relates to the introduction of blood sample-based PSA as a screening and diagnostic test for prostate cancer; this investigation raises the diagnosis rate of disease forms up to 50%, but artificially increases treatment rate for cases that, probably, would not have shown any clinical manifestations or would have led to the death of the patient. This aspect has led to increased costs and a series of false positive diagnoses[2,3].

Starting from this issue that has been reported by the scientific community, the main objective of this study was to identify the different genetic mutations present in prostate cancer patients diagnosed in Romania, in order to determine a set of biomarkers that possess clinical utility for diagnosis and prognosis of the development of this condition.

As a secondary objective, I tried to assess the risk of these markers, in relation to disease prognosis, by validating these estimates, using the internationally available databases.

2.1Studied population

The subjects included in this study were male patients, hospitalized between 2008 and 2016 in four clinics in Bucharest for various medical conditions. The study batch consisted of 1064 histopathologically PCa confirmed patients, out of which I subselected 166 cases with abnormal PSA levels and age of onset less than 60 years and 1104 control subjects, consisting of patients admitted for non-oncological urological and surgical conditions.

DNA was extracted from blood or buccal smear at deCODE Genetics (Reykjavik, Iceland) and genotyped using Illumina SNP arrays[4,5].

Plasma PSA levels were measured, in what concerns all subjects, upon hospital admission, but were not used as an exclusion criteria. All subjects signed an informed consent form before study enrolment and agreed to the use of personal, clinical data, and biological samples for genetic research purposes. The Bioethics Committee of the Romanian College of Physicians approved the study, and the protocols were approved by the National Ethics Committee of the Romanian Medical Association[6].

Trained medical staff conducted face-to-face interviews using standardized questionnaires, in order to collect personal data (ethnicity, marital status, education, height and weight), lifestyle data (occupation, smoking habits, coffee and tea, alcohol consumption) and medical history (personal and family history). All subjects were of self-reported European origin. No significant differences were observed in terms of other epidemiological characteristics: body mass index, smoking habits or alcohol consumption.

The inclusion criteria for patients in the control group were as follows: the absence of any type of cancer at the time of inclusion, the signing by the participants of an informed consent for inclusion in the research program and the filling in by the participants of the medical and personal data questionnaire.

The gathering of clinical data was performed with the help of a questionnaire used in the doctoral study that has an important value within the research methodology. The questionnaire includes the following chapters:

a) patient information

b) phenotypic data

c) medical history

d) risk-bearing behaviours

e) information related to lifestyle

Based on the predetermined protocol, all of the aforementioned information was collected by trained personnel, by means of using a standardized form within the study. The form encompasses 11 questions that inquire about medical aspects useful for the diagnosis of the subjects included in the study who demonstrate neoplastic pathology. The questionnaire includes information related to diagnosis, TNM, PSA and Gleason score value.

The main purpose of the study is represented by the elaboration of a set of genetic markers that are strongly associated with prostate cancer at the level of the entire human genome in the Romanian population. I would like to mention that this study uses data from the Romcan project, representing one of the multiple efforts of the Romanian urological scientific community, in an attempt to elucidate the etiology of prostate neoplastic processes.

The assessment of this pathology is carried out by means of a series of generally accepted clinical parameters, such as: cTNM staging, blood-collected PSA value and Gleason score - in order to classify patients diagnosed with prostate carcinoma into different risk groups in terms of biochemical recurrence, a classification that influences the choice of therapeutic management, as well [7].

Two similar studies were previously carried out; the first was conducted on a limited number of markers and was carried out on a number of 34 markers associated with this pathology in Romania. However, this result is somewhat unsatisfactory for medical practice, analysing a much too reduced number of SNPs, for replication purposes [8,9].

A second study was carried out, analysing the entire PROMARK cohort, a 'genome wide association study" (GWAS) that analysed, for the first time, the genetic correlation between the genetic profile and prostate cancer in the Romanian population [9] .

The study presented in this work is a direct continuation of the above-mentioned study, including a number of 24 million SNPs analysed with modern statistical methods, correlating a binary phenotype represented by the presence of prostate cancer with the aforementioned data set. The applied markers were qualitatively assessed, and their final structure was finalized following the imputation of the 1000 Genomes data set[10]. The data resulting from the imputation constitutes an overview of the entire human genome for each patient studied. This technology grants a unique analysis perspective in terms of the associations between the 24 million markers and the studied phenotype.

The applied methodology allows us to analyse all genes, loci or markers with precision, subsequently having, at the end of this study, a set of results that assesses the risk of each marker or gene based on the P-value obtained in

the relevant cohort. The data used in this study are part of the ROMCAN scientific project, which was carried out in Romania in partnership with the DeCODE Genetics Iceland institute, between 2014 and 2017[11].

In this doctoral thesis, I investigated two hypotheses, with the objective of validating the results obtained in relation to the Romanian cohort. The first stage was represented by the analysis of the Romanian population included in the study, as compared to previously published reference data, in order to catalogue and compare the two available data sets. The final goal of this study is represented by the identification of significant results obtained within the statistical tests, as well as their interpretation and replication within the study.

2.2Genotyping and SNP data analysis

A total of 716,503 SNPs were genotyped for each individual included in the study. Genotype data were filtered with the help of Plink! v1.07 [12,13]. Approximately 10% of the genotyped SNPs were removed using a Hardy-Weinberg equilibrium significance threshold of 5×10^{-6} and by means of excluding markers with a minor allele frequency of less than 1%.

Markers present in the '1000 Genomes" reference set - phase III from 2014 [13] were imputed to 2168 individuals, using the IMPUTE2 software [14] with a posterior probability of 0.9 acting as correction threshold for the quality of the analysed genotypes imputation process. The genotypes set was tested for the purpose of assessing population heterogeneity, by using main component analysis with the help of ADMIXTURE software [15], and the results were consistent with the model of a homogeneous population.

A total of 24,295,558 markers were generated by means of imputation for each individual in the study. Quality control for the imputation results was performed by means of removing markers with a minor allele frequency of less than 1%, with a correct identification rate of 0.95 and an estimated imputation concordance rate with the actual model of 0.8.

In total, 8,506,022 markers met the filter criteria. An association test between the 8.5 million imputed markers and a phenotype represented by a positive biopsy for prostate cancer was performed. The association test was calculated with the help of SNPTEST [16], with a single binary variable as response; all reported P-values are two-tailed.

2.3Selection of SNPs in order to replicate previous findings

A systematic specialised literature review of prostate cancer-associated variants from previous GWAS studies was concluded with the help of the NHGRI catalogue of these published genome-wide association studies [17], as a starting point. A query of this database was performed using "prostate cancer" as a keyword, and the inclusion criteria for selection were as follows: P-values $<5 \times 10^{-8}$ and a minor allele frequency of more than 5%.

For each study, the following variables were collected: country and ethnicity of participants, genotyping method, source of control elements, source of replication cohort, and multiple cases and controls in both the discovery and replication studies. A total of 63 articles were initially obtained from the GWAS catalogue, based on the keyword search. Twelve of the studies reported outcomes only tangentially related to prostate cancer, whilst 29 of the studies reported associations with prostate cancer risk. We identified 139 unique markers previously associated with PCa.

3.Results

The first two results were for less frequent variants (minor allele frequency estimated at 2% in the sample and 9% in the control sample) from chromosomes 22 and 12 SNP, RS9621053 and RS11059458. We believe the result of this SNP should be viewed with caution.

Another novel variant (RS42929) also reached genome-wide significance. The genome-wide results are graphically illustrated in **Figure 1**, and the main findings are presented in **Table 1**.

Table 1. Summary of main GWAS results related to early onset

RS	Chromosome	Position	Allele	P-value	OR
rs9621053	22	30635146	T	3.65E-10	3.419
rs11059458	12	128052616	T	7.12E-09	7.14
rs42929	22	30560432	T	2.12E-09	4521
rs1859169	23	107319661	G	6.41E-08	6628
rs7576486	2	183710710	A	5.14E-08	12.25
rs16886053	6	75053443	A	1.12E-07	13.62
rs12664835	6	75055012	T	2.12E-07	15.73
rs986633	8	86623223	A	5.12E-07	2,215
rs10490219	2	8260765	T	5.21E-07	1,621
rs2215220	6	28521097	T	1.051E-07	3,612
rs846277	7	42021903	T	4.14E-07	2,731
rs1366031	12	115611338	G	1.66E-06	16.66
rs7036724	9	86221371	C	2.65E-06	18.21
rs16888388	6	77015323	A	2.61E-06	13.67
rs2258265	8	3941805	T	2.55E-06	2,891
rs953920	10	116187631	C	4.12E-06	2,920

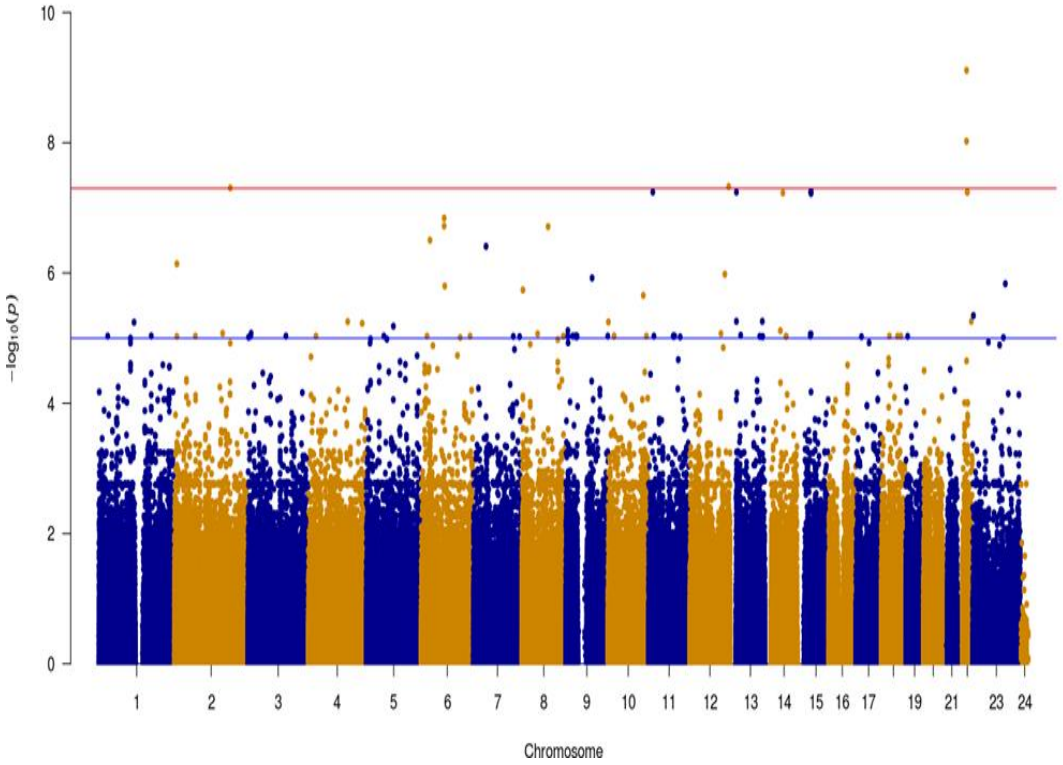

Figure1: GWAS results for early onset

A similar test was carried out for 898 cases diagnosed after age 60 with mixed status of additional phenotypic information and control patients from the same cohort. The genome-wide results are graphically illustrated in **Figure 2**, whilst the main findings are presented in **Table 2**.

In the second test, we observe lower P-values, as compared to the results observed in the case of the GWAS-type study that analysed cases with early onset, this being due to the larger size of the study sub-cohort. 14 SNPs from the 13 loci reached P-values lower than $5x10^{-8}$ in the Romanian cohort.

Table 2. Summary of main results of the GWAS-type study for late-onset prostate cancer patient cohort

RS	Chromosome	Position	Allele	P-value	OR
rs8110536	19	756985	3	4.14E-10	0,531
rs2827540	21	22474931	3	4.64E-10	0.4512
rs12090877	1	160406270	4	1.91E-09	0.12316
rs4828787	23	150996411	4	1.99E-09	2008
rs2598392	7	33581287	2	3.21E-09	0.5784
rs9866700	3	180563658	2	4.46E-09	0.6147
rs4810663	20	47711762	4	4.60E-09	0.2314
rs2135720	10	53995731	4	5.57E-09	0.6064
rs12067141	1	18033086	1	6.71E-09	0.6092
rs7211084	17	29049811	4	6.88E-09	0.6023
rs6602234	10	7275164	2	7.63E-09	0.5289
rs7563088	2	56737504	2	1.03E-08	0.6159
rs2974554	5	152677932	4	1.30E-08	0.5622
rs4711143	6	27205620	4	2.23E-08	0.4328

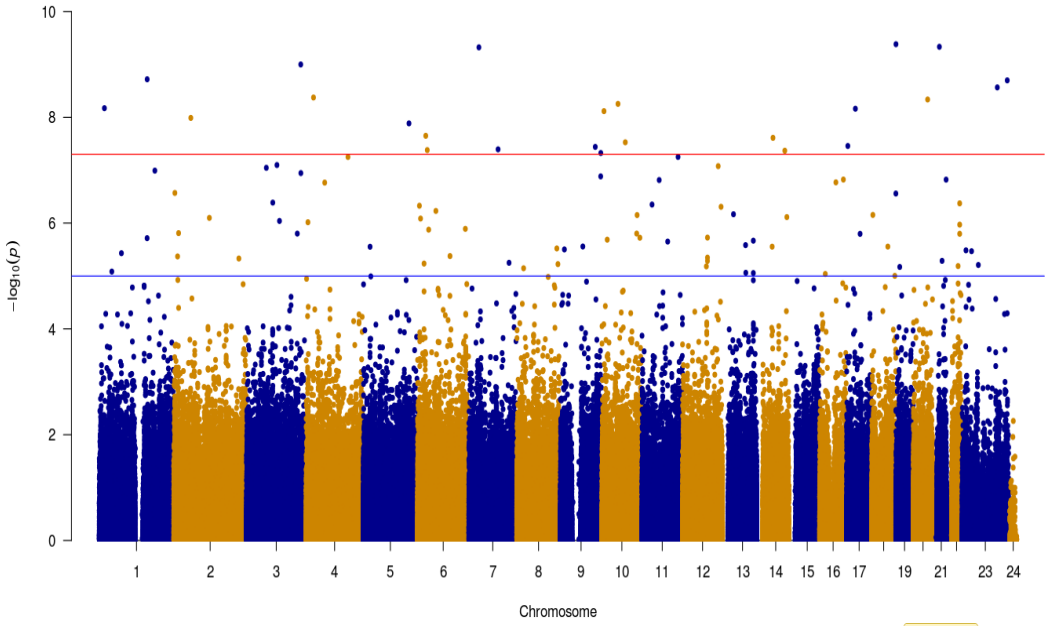

Figure 2: GWAS results for late onset

GWAS-type studies in European populations have provided insight into cancer-specific variation and indicated susceptibility areas that are of particular importance in certain populations. The discovery and characterization of cancer risk loci in various populations are essential for understanding the underlying biological mechanisms and for the future development of genetic models for risk prediction in populations of European ancestry.

Our prostate cancer study did not identify significant novel genome-wide variants. However, the findings demonstrate that the risk variants established in relation to prostate cancer significantly contribute to the risk of prostate cancer (PCa) amongst the Romanian population.

Early-onset prostate cancer has been shown to be significantly associated with increased family history of the disease, providing evidence of a stronger underlying genetic etiology for early-onset disease, as compared to late-onset disease [18].

To date, several multistage GWAS studies have been conducted in relation to prostate cancer, using a variety of case inclusion rules. In the case of GWAS based on PCa cases $\leq$ 60 years and cases with an existing family history of PCa, Eeles et al. [19] demonstrated the increased power to detect SNPs associated with prostate cancer that can be achieved by including cases with enriched genetic disease susceptibility.

4.Discussions

As a result of the genetic analysis, we identified a gene subset at the level of which consequences associated with the markers analysed in connection with prostate cancer occur.

The identified genes in question were TERT, PEX14, KCNN3, SIDT1, SLC22A3, PCAT2, PRNCR1, TET2, CASC1, VAV2 and MROH1.

A large volume of information on SNPs associated with prostate cancer risk is available for European populations. However, it is less known if these associations can be consistently replicated in Eastern European populations.

On a large scale, population-based studies regarding genetic epidemiology are currently underway or planned in several countries, and the results of these studies will define the landscape of genetic epidemiology in the near future. Also, next-generation sequencing technologies are established as the platforms of choice for routine screening of tumour samples in a clinical setting.

As whole-exome or whole-genome sequencing of patients becomes accessible to many hospitals and academic institutions, we believe that genetic screening can be a financially viable solution for medical practice. Both approaches show great potential in terms of integration of genetic epidemiological elements in medical practice [20].

GWAS-type studies have many limitations, such as their inability to fully explain the genetic risk related to common diseases. Despite these limitations, the variants obtained from these studies could be used to stratify the population as per the relevant risk level for certain diseases. Another important role of GWAS studies may be to identify disease subtypes that have different causes or responses to treatment[21].

Analysis of the main genes involved in PCa in the Romanian population

TERT gene

This gene is important in the pathogenesis of prostate cancer, in light of the fact that the RS2242652 and RS7725218 markers associated with the studied phenotype represent a risk factor for this pathology. There is evidence in the specialized literature that supports the involvement of TERT in the biological mechanisms that influence prostate cancer oncogenesis. In the following sections, the essential aspects that highlight the current knowledge level regarding the role of the TERT gene in the pathology of prostate cancer are described.

Information regarding the TERT gene in relation to prostate cancer

The analysis of mutations present in individual genes in tumour genomes is motivated by the hope of discovering important changes in terms of pathways that lead to a better understanding of the process of carcinogenesis. The availability of new and sensitive sequencing technologies has accelerated this search and has led to the identification of new areas, which are important for mutations involved in a variety of cancer cases [22].

Recently, mutations in the promoter of the telomerase reverse transcriptase (TERT) gene encoding the catalytic subunit of telomerase have been identified in familial melanoma with a high frequency [23]. Cell longevity is still a classic hallmark of tumours, and telomerase reactivation leading to telomere maintenance remains a fundamental process in carcinogenesis. Alterations in the coding region of the TERT gene represent rare events in what concerns various types of cancer. Therefore, the identification of mutations in the central promoter of the TERT gene that can lead to different particularities of tumour cells has generated great interest in the field of cancer research [24]

In prostate cancer, TERT expression has been associated with a poor prognosis and a higher risk of disease recurrence. The mechanisms underlying elevated TERT expression in prostate cancer have not yet been elucidated. To date, data on TERT promoter mutation analysis in PCa cases are scarce.

According to the Cbio Portal, 3436 mutant genetic variants have been identified, at the level of this gene. This increased variability rate represents an

additional factor that supports the hypothesis regarding the involvement of this gene in oncological cases.

Telomerase reverse transcriptase (TERT) expression is crucial for tumour survival and cancer cells escaping apoptosis. Several TERT locus variants have been found in association with cancer risk, but the underlying mechanisms and clinical impact remain unclear.

Human telomeres are a protection of chromosome ends, whose main function is to maintain telomeric DNA length and chromosomal stability. Most malignant cells, including prostate cancer, achieve unlimited replicative capacity, a hallmark of cancer, by activating telomerase to maintain telomeres. The key element for limiting the speed of telomerase activity was found to be the TERT gene, which encodes an essential catalytic subunit of telomerase that is aberrantly expressed in many cancer types. Understanding the mechanisms of aberrant TERT gene expression is a fundamental inquiry in what concerns human cancer, and TERT is also a potential clinical target for improving cancer diagnosis and prognosis.

PEX14 gene

This gene is known to be important in the etiology of prostate cancer, in light of the fact that the RS636291 marker associated with this phenotype studied in the previous analysis represents a risk factor for this pathology. This aspect is also supported by the fact that, in the specialized literature, there is evidence supporting the involvement of PEX14 in various biological processes that favourably influence prostate cancer.

Information regarding PEX14 in relation to prostate cancer

The PEX14 gene produces an integral membrane protein essential for protein docking on peroxisomes and is a bifunctional protein capable of acting as a transcriptional co-repressor and a polypeptide transport modulator.

Further studies have revealed that PEX14 is the only peroxin that exhibits a unique dual function in peroxisome formation and selective degradation. This gene encodes an essential component of the peroxisomal import machinery.

The protein is integrated into peroxisomal membranes with the C-terminus exposed to cytosol and interacts with the cytosolic receptor for proteins containing a PTS1 peroxisomal targeting signal. The protein also functions as a transcriptional co-repressor and interacts with histone deacetylase.

Genetic variants of this gene have previously been associated with breast cancers. SNPs such as rs616488 (PEX14) have been shown to be associated with breast cancer in particular.

This SNP is much more strongly associated with PEX14 expression in tumour tissues, as compared to normal tissues. Replication of these results in future studies must further analyse whether the association of PEX14 with breast cancer risk may be due, in part, to an involvement of this gene in neoplastic etiological processes.

According to the Cbio Portal, 146 mutant genetic variants have been identified, at the level of this gene.

This increased variability rate represents an additional factor that supports the hypothesis regarding the involvement of this gene in oncological cases.

SIDT1 gene

This gene also has a role in the etiology of prostate cancer, which is indicated by the association of the RS7611694 marker with the studied phenotype, a substantial also from a molecular point of view, as well. The specialized literature presents a series of information regarding the involvement of SIDT1 in prostate cancer oncogenesis.

General data about the SIDT1 gene

The protein encoded by this gene belongs to the SID-1 family of dsRNA transmembrane channels. These channels transport dsRNA into cells and are necessary for systemic RNA interference (RNAi). [25]

It has been shown that SIDT1 facilitates the influx of siRNA into the human body, as well. The increase in extracellular siRNA influx into human cells mediated by SIDT1 can cause highly specific post-transcriptional gene silencing [26].

In what concerns the prostate, the gene expression is 10.15 transcripts per million (tpm).

SIDT1 facilitates rapid, contact-dependent, bidirectional transfer of RNA between human cells, resulting in RNAi. SIDT1-mediated intercellular transport of microRNA-21(miR-21) increases resistance to the nucleoside analogue gemcitabine in human adenocarcinoma cells [27].

Information regarding SIDT1 in relation to prostate cancer

Since SIDT1 facilitates the transport of miR-21, its involvement in oncogenesis and, implicitly, in prostate cancer, is closely related to this function. The roles of miR-21 include controlling the expression of several mRNAs involved in microvascular proliferation and tumour invasion.

In what concerns prostate cancer, there is dysregulation of several miRNAs that may function as tumour suppressors or oncogenes.

MiR-21 is a relatively new member of the group of oncogenic microRNAs. It was initially found to be overexpressed in glioblastomas, and was later described as an antiapoptotic factor predicted to down-regulate genes associated with advanced apoptosis [28]. Subsequently, miR-21 overexpression was observed in a variety of human tumours, such as those originating from the breast, colon, liver, brain, pancreas, and prostate [29].

In prostate cancer patients, an overexpression of miR-21 correlates with poor biochemical recurrence-free survival and has predictive value in what concerns the risk of biochemical recurrence in patients treated via radical prostatectomy [30]. Also, its expression correlates with metastatic disease and increases in accordance with clinical parameters (Gleason score, lymph node metastases). Therefore, miR-21 is useful as a biomarker, in order to predict tumour progression [31].

Although an assessment of miR-21 expression in larger populations is required, the results existing in the current literature indicate the fact that miR-21 may be a promising candidate for a molecular prognostic biomarker and a possible therapeutic target for prostate cancer.

Gene therapy strategies involving miR-21 inhibition may prove useful in the treatment of prostate cancer[32].

SLC22A3 gene

This gene is important in the pathogenesis of prostate cancer, in light of the fact that the RS9364554 marker associated with the studied phenotype shows consequences at its level.

There is evidence in the specialized literature that supports the involvement of SLC22A3 in the biological mechanisms that influence prostate cancer oncogenesis. In the following sections, the essential aspects that highlight the current knowledge level regarding the role of the SLC22A3 gene in the pathology of prostate cancer are described.

Information regarding SLC22A3 in relation to prostate cancer

Throughout the progression of prostate cancer and in high-grade prostate cancer (according to the Gleason score), SLC22A3 has a low expression, as compared to normal tissues. Immunohistochemistry confirmed decreased SLC22A3 expression during tumour progression (from benign epithelium to prostate cancer, $P = 0.003$; from localized prostate cancer to metastatic cancer, $P = 0.009$), as well as in high-grade cancer compared to low-grade cancer, $P = 1.1 \times 10^{-5}$. [33].

In the study "Genetic and functional analyses implicate the NUDT11, HNF1B, and SLC22A3 genes in prostate cancer pathogenesis", RS9364554 is associated with low expression levels of SLC22A3. However, functional tests show that the suppression of this transcript leads to a reduced viability and proliferation of the analysed cell lines.

Further characterization will be required, in order to elucidate the functional consequences of changes in SLC22A3 levels, but the gene appears to be involved in the initiation of prostate cancer carcinogenesis [34].

PCAT2 and PRNCR1 genes

These genes are important in the pathogenesis of prostate cancer, in light of the fact that the RS1016343 marker associated with the studied phenotype shows consequences at its level.

There is evidence in the specialized literature that supports the involvement of PCAT2 and PRNCR1 in the biological mechanisms that influence prostate cancer oncogenesis. In the following sections, the essential

aspects that highlight the current knowledge level regarding the role of the two genes in the pathology of prostate cancer are described.

General data regarding the PCAT2 and PRNCR1 genes in relation to prostate cancer

PCAT2 (Prostate Cancer Associated Transcript 2) is an RNA gene and belongs to the class of lncRNAs (long noncoding RNAs).

Diseases associated with PCAT2 include prostate cancer and visual agnosia. (GeneCards).

PCAT2 expression is up-regulated in prostate cancer, this type of expression being specific for testicles, but not to normal prostate tissue.

PRNCR1 (Prostate Cancer Associated Non-Coding RNA 1) is an RNA gene and belongs to the class of non-coding RNAs. Diseases associated with PCAT2 include prostate cancer and malignant glioma. (GeneCards) Also, PRNCR1 is a potential oncogene involved in colorectal, gastric and breast cancer [35].

Early studies showed that PRNCR1 expression was up-regulated in certain prostate cancer and PIN tumour cells. Decrease of PRNCR1 expression on LNPCa and PC3 cell lines reduced cell viability and androgen receptor (AR) transactivation activity [36].

TET2 gene

This gene is important in the pathogenesis of prostate cancer, in light of the fact that the RS7679673 marker associated with the studied phenotype shows consequences at its level.

There is evidence in the specialized literature that supports the involvement of TET2 in the biological mechanisms that influence prostate cancer oncogenesis. In the following sections, the essential aspects that highlight the current knowledge level regarding the role of the TET2 gene in the pathology of prostate cancer are described.

Information regarding TET2 in relation to prostate cancer

DNA methylation represents an epigenetic pattern that describes the state of global gene regulation [37]. This process that regulates gene expression is deemed to regulate various biological mechanisms, including the oncogenesis of neoplastic tissues[37].

5-Hydroxymethylcytosine (5-hmC) is a recently identified genetic marker, acting as a potential indicator of diseases, such as cancer. The mechanisms favouring its presence as a triggering factor in tumours and its role on gene expression and the development of tumour cells are unclear [38].

The study "TET2 binds the androgen receptor and loss is associated with prostate cancer" (2016), by means of genetic mapping, revealed six SNPs located in intronic regions that are significantly associated with prostate cancer, supporting TET2 as the causal gene associated with risk SNP RS7679673 [39].

Somatic changes occurring in 6% of primary tumours and 20% of metastatic tumours, reduced mRNA expression in a subset of tumours, and experimental evidence that TET2 KD increases LNPCa proliferation and cell invasion indicate the fact that TET2 is a tumour suppressor in prostate cancer.

The relationship between TET2 and androgenic hormones constitutes an essential element in understanding the involvement of this gene in the oncogenesis process related to prostate cancer.

The study "TET2 repression by androgen hormone regulates global hydroxymethylation status and prostate cancer progression" [40] allowed the formulation of certain conclusions regarding TET2 - AR interaction:

TET2 expression is repressed by androgen hormones in tumour cells:
-TET2 is an important target gene of androgen-regulated miRNAs
-AR represses the activity of TET2-enhancers in HRPC.

AR-mediated induction of the miR-29 family, which targets TET2, is proportionally increased in hormone-refractory prostate cancer (HRPC), and its value predicts a poor prognosis for prostate cancer patients.

A reduction in 5-hmC activity activates key pathways related to prostate cancer, such as mTOR and AR. Thus, DNA modification causally links the TET2-mediated and AR-regulated epigenetic pathway to 5-hmC-dependent cancer progression.

In the same study, TET2 and 5-hmC values were assessed in clinical tumour samples by means of immunohistochemistry. Decreased expression of TET2 together with that of 5-hmC could be a prognostic factor predicting the survival of patients after surgery. The low level of TET2 expression is not associated with the Gleason score, but with advanced cancer stages and metastases.

It was confirmed that TET2 expression is significantly repressed in metastatic prostate cancer, as compared to localized tumours. Decreased TET2 expression is associated with disease progression and, in particular, with metastases.

TET2-dependent mechanisms are, therefore, responsible for tumour growth.

Increasing or decreasing TET2 expression has a significant effect on cell proliferation, demonstrating the growth-inhibitory effect of TET2 in prostate cancer.

Increased repression of TET2 mediated by the miR-29 family is involved in prostate cancer progression to HRPC.

In conclusion, miR-29 is a key epigenetic regulator that represses TET2 in cancer progression. Thus, the development of novel epigenetic approaches to inhibit miR29 or modify the TET2-mediated pathway may have significant implications for the treatment of advanced prostate cancer.

5. Conclusions

In this doctoral thesis, I have carried out an assessment of the impact of early diagnosis by way of genetic screening methods in the prognosis of PCa in Romanian patients. Our research did not discover new mutations at genome level, but the results show that the known risk variants for prostate cancer influence the probability of the emergence of this pathology among the Romanian population.

We have defined the particular genetic profile of the "early-onset" population correlated with genetic, metabolic and physiological factors, an aspect that can have a role in the post-therapeutic management of these patients. In the association test (GWAS) between early-onset PCa cases and PCa cases with a family history, we found statistically significant evidence supporting the association of 13 studied SNPs with PCa cases < 60 years old.

The increasing trend of risk alleles in the young population, as compared to subjects over 60 was reported only in cases with early-onset, a fact that denotes and emphasizes the importance of the impact of common genetic risk factors in men with PCa discovered under the age of 60.

The implementation of genetic testing in clinical practice, especially in what concerns young patients with aggressive tumours or those with a positive family history, represents a new challenge for the coming years and will identify males with pathogenic variants who may benefit from screening and early intervention and specific therapeutic options .

Another essential point was represented by the optimization of the treatment management of patients with prostate cancer depending on the identified genetic profile. Some of the types of genetic variants identified in this PhD thesis particularly predispose patients to aggressive prostate cancer and confer a more reserved prognosis, as compared to the prognosis of patients who do not carry these mutations.

Clinical applications of molecularly targeted treatments have much better results in what concerns these types of patients, as they allow the establishment of risk groups and, thus, optimize therapeutic management. On the basis of

genetic analysis, high-risk patients can be detected early, in order for them to benefit from a radical treatment.

The definition of a set of markers as a possible future alternative in the detection and monitoring of PCa patients, that will replace PSA, with higher sensitivity and specificity levels, but similar costs, is also a desire objective of this paper. As evidenced by the variety of studies focusing on different genetic mutations associated with PCa, this is a rapidly growing area of interest.

Although the genetic aspects of PCa were previously underestimated, we now have evidence supporting the impact of identifying a set of genetic markers useful in the assessment of PCa in Romania. This is also emphasized by the current recommendations related to genetic testing in various specialist guidelines.

The costs associated with this pathology are currently high, as many cases are discovered at a later stage and, thus, solving the complications requires time, as well as material and human resources. The early detection of localized PCa with the help of genetic markers, corroborated with current methods, will lead to the optimization of diagnosis, treatment schemes, as well as the reduction of the number of complications, costs and overtreatment of these patients.

At the same time, as a result of this study, we have identified a genetic profile that can be applied both in Eastern European populations and internationally, which is usable in the optimal management of PCa patients.

Another benefit is the capacity of this test to establish an early detectable genetic risk for first-degree relatives of diagnosed patients. Early-onset neoplasm is strongly associated with family history, suggesting the importance of genetic etiology in early-onset PCa, more than for late-onset disease.

Due to its non-invasive nature, the increased specificity and sensitivity levels demonstrated in this study, I deem it appropriate to integrate this test into current practice, as it is based on genetic markers specific to the studied Romanian population.

6.References

1. Iordache, Paul D et al. “Profile of common prostate cancer risk variants in an unscreened Romanian population.” Journal of cellular and molecular medicine vol. 22,3 (2018): 1574-1582. doi:10.1111/jcmm.13433
2. Kamal, Baher A. “The value of screening tests for detection of prostate cancer in 1000 saudi men.” Journal of family & community medicine vol. 11,3 (2004): 97-102
3. Thompson, Ian M, and Donna P Ankerst. “Prostate-specific antigen in the early detection of prostate cancer.” CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne vol. 176,13 (2007): 1853-8. doi:10.1503/cmaj.060955
4. Auton A, Abecasis GR, Altshuler DM, et al A global reference for human genetic variation. Nature. 2015; 526: 68–74.
5. Howie BN, Donnelly P, Marchini J, et al A flexible and accurate genotype Imputation Method for the Next Generation of Genome-Wide Association Studies. Schork NJ, ed. PLoS Genet. 2009; 5: e1000529
6. Jinga, Viorel et al. “Replication study of 34 common SNPs associated with prostate cancer in the Romanian population.” Journal of cellular and molecular medicine vol. 20,4 (2016): 594-600. doi:10.1111/jcmm.12729
7. Hoogland, A Marije et al. “Prognostic histopathological and molecular markers on prostate cancer needle-biopsies: a review.” BioMed research international vol. 2014 (2014): 341324. doi:10.1155/2014/341324
8. Iordache, Paul D et al. “Identification of Lynch syndrome risk variants in the Romanian population.” Journal of cellular and molecular medicine vol. 22,12 (2018): 6068-6076. doi:10.1111/jcmm.13881
9. Huang, Jie et al. “1000 Genomes-based imputation identifies novel and refined associations for the Wellcome Trust Case Control Consortium phase 1 Data.” European journal of human genetics : EJHG vol. 20,7 (2012): 801-5. doi:10.1038/ejhg.2012.3

10. Beyter, Doruk et al. “Long-read sequencing of 3,622 Icelanders provides insight into the role of structural variants in human diseases and other traits.” Nature genetics vol. 53,6 (2021): 779-786. doi:10.1038/s41588-021-00865-4
11. Bruinsma, Fiona J et al. “The utility of DNA extracted from saliva for genome-wide molecular research platforms.” BMC research notes vol. 11,1 8. 8 Jan. 2018, doi:10.1186/s13104-017-3110-y
12. Howie, Bryan N et al. “A flexible and accurate genotype imputation method for the next generation of genome-wide association studies.” PLoS genetics vol. 5,6 (2009): e1000529. doi:10.1371/journal.pgen.1000529
13. Alexander, David H et al. “Fast model-based estimation of ancestry in unrelated individuals.” Genome research vol. 19,9 (2009): 1655-64. doi:10.1101/gr.094052.109
14. Marchini, Jonathan, and Bryan Howie. “Genotype imputation for genome-wide association studies.” Nature reviews. Genetics vol. 11,7 (2010): 499-511. doi:10.1038/nrg2796
15. Welter, Danielle et al. “The NHGRI GWAS Catalog, a curated resource of SNP-trait associations.” Nucleic acids research vol. 42,Database issue (2014): D1001-6. doi:10.1093/nar/gkt1229
16. Pei, Yu-Fang et al. “Analyses and comparison of imputation-based association methods.” PloS one vol. 5,5 e10827. 26 May. 2010, doi:10.1371/journal.pone.0010827
17. Benafif, Sarah et al. “A Review of Prostate Cancer Genome-Wide Association Studies (GWAS).” Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology vol. 27,8 (2018): 845-857. doi:10.1158/1055-9965.EPI-16-1046
18. Lange, Ethan M et al. “Early onset prostate cancer has a significant genetic component.” The Prostate vol. 72,2 (2012): 147-56. doi:10.1002/pros.21414
19. Schumacher, Fredrick R et al. “Association analyses of more than 140,000 men identify 63 new prostate cancer susceptibility loci.” Nature genetics vol. 50,7 (2018): 928-936. doi:10.1038/s41588-018-0142-8
20. Katsanis, Sara Huston, and Nicholas Katsanis. “Molecular genetic testing and the future of clinical genomics.” Nature reviews. Genetics vol. 14,6 (2013): 415-26. doi:10.1038/nrg3493

21. Witte, John S. “Genome-wide association studies and beyond.” Annual review of public health vol. 31 (2010): 9-20 4 p following 20. doi:10.1146/annurev.publhealth.012809.103723
22. Mardis, Elaine R, and Richard K Wilson. “Cancer genome sequencing: a review.” Human molecular genetics vol. 18,R2 (2009): R163-8. doi:10.1093/hmg/ddp396
23. Griewank, Klaus G et al. “TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma.” Journal of the National Cancer Institute vol. 106,9 dju246. 13 Sep. 2014, doi:10.1093/jnci/dju246
24. Dratwa, Marta et al. “TERT-Regulation and Roles in Cancer Formation.” Frontiers in immunology vol. 11 589929. 19 Nov. 2020, doi:10.3389/fimmu.2020.589929
25. Feinberg, Evan H, and Craig P Hunter. “Transport of dsRNA into cells by the transmembrane protein SID-1.” Science (New York, N.Y.) vol. 301,5639 (2003): 1545-7. doi:10.1126/science.1087117
26. Kim, Daniel H et al. “Argonaute-1 directs siRNA-mediated transcriptional gene silencing in human cells.” Nature structural & molecular biology vol. 13,9 (2006): 793-7. doi:10.1038/nsmb1142
27. Elhassan, Mohamed O et al. “Homo sapiens systemic RNA interference-defective-1 transmembrane family member 1 (SIDT1) protein mediates contact-dependent small RNA transfer and microRNA-21-driven chemoresistance.” The Journal of biological chemistry vol. 287,8 (2012): 5267-77. doi:10.1074/jbc.M111.318865
28. Gabriely, Galina et al. “MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators.” Molecular and cellular biology vol. 28,17 (2008): 5369-80. doi:10.1128/MCB.00479-08
29. Bautista-Sánchez, Diana et al. “The Promising Role of miR-21 as a Cancer Biomarker and Its Importance in RNA-Based Therapeutics.” Molecular therapy. Nucleic acids vol. 20 (2020): 409-420. doi:10.1016/j.omtn.2020.03.003
30. Li, Tao et al. “miR-21 as an independent biochemical recurrence predictor and potential therapeutic target for prostate cancer.” The Journal of urology vol. 187,4 (2012): 1466-72. doi:10.1016/j.juro.2011.11.082

31. Fan, Bo et al. “Expression of miR-451a in Prostate Cancer and Its Effect on Prognosis.” Iranian journal of public health vol. 50,4 (2021): 772-779. doi:10.18502/ijph.v50i4.6002
32. Ibrahim, Noha H et al. “Diagnostic significance of miR-21, miR-141, miR-18a and miR-221 as novel biomarkers in prostate cancer among Egyptian patients.” Andrologia vol. 51,10 (2019): e13384. doi:10.1111/and.13384
33. Hoogland, A Marije et al. “Gene-expression analysis of gleason grade 3 tumor glands embedded in low- and high-risk prostate cancer.” Oncotarget vol. 7,25 (2016): 37846-37856. doi:10.18632/oncotarget.9344
34. Witte, John S. “Prostate cancer genomics: towards a new understanding.” Nature reviews. Genetics vol. 10,2 (2009): 77-82. doi:10.1038/nrg2507
35. Bardhan, Abhishek et al. “PRNCR1: a long non-coding RNA with a pivotal oncogenic role in cancer.” Human genetics vol. 141,1 (2022): 15-29. doi:10.1007/s00439-021-02396-8
36. Aird, John et al. “Carcinogenesis in prostate cancer: The role of long non-coding RNAs.” Non-coding RNA research vol. 3,1 29-38. 1 Feb. 2018, doi:10.1016/j.ncrna.2018.01.001
37. Moore, Lisa D et al. “DNA methylation and its basic function.” Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology vol. 38,1 (2013): 23-38. doi:10.1038/npp.2012.112
38. Laird, Alexander et al. “5-hydroxymethylcytosine profiling as an indicator of cellular state.” Epigenomics vol. 5,6 (2013): 655-69. doi:10.2217/epi.13.69
39. Nickerson, M L et al. “TET2 binds the androgen receptor and loss is associated with prostate cancer.” Oncogene vol. 36,15 (2017): 2172-2183. doi:10.1038/onc.2016.376
40. Takayama, Ken-ichi et al. “TET2 repression by androgen hormone regulates global hydroxymethylation status and prostate cancer progression.” Nature communications vol. 6 8219. 25 Sep. 2015, doi:10.1038/ncomms9219

Papers published

1. G.Roșoga, R. Petca, D. Diaconescu, R. Dănău, S. Raşcu, C. Sima, C. Toma, V. Jinga. Impact of Body Mass Index on PSA values in a Population-Based Study. *Romanian Journal of Urology*, vol 17,pag 39-44, 2018.

2. G.Roşoga , D. Diaconescu, R. Dănău, S. Rașcu, C. Sima, C. Toma, P. Iordache, V. Jinga. Follow-Up GWAS study for Novel Variants Associated with Early-Onset Prostate Cancer in Romania. *Romanian journal of Urology,* vol 18, pag 34-40, 2019.