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OMICs Role in Hereditarian Prostate Cancer †

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Abstract: Prostate cancer (PC) is one of the most prevalent tumours in the world, however, the hereditary (Hereditary PC; HPC) form is a rare pathology, without exceeding 6%. Despite its very low incidence, a family history of PC in a first-degree relative multiplies the risk to suffer from PC approximately twofold. Therefore, the search for genetic variables associated with detection, monitoring and treatment is unavoidable. With this study, we make a deep screening of exome by Next-Generation Sequencing (NGS) analysis in search of new biomarkers. We performed this analysis in a family of a high incidence of PC. Our data revealed that variants in some genes, such as HIBCH and DPP4, are present in all HPC patients. Moreover, high-risk patient has unique additional variants such as FANK1, TUBA3FP and ALDH3B2. These results provide a new set of promising biomarkers in HCP.

Keywords: hereditary prostate cancer; OMICs technologies; next-generation sequencing; biomarker

1. Introduction

Although all men are at increased risk for prostate cancer (PC) developing with increasing age, a family history of PC in a first-degree relative multiplies that risk approximately twofold. Most cases of PC occur sporadically in people with no family history of the condition. However, approximately 5% to 10% of PC cases are believed to be primarily caused by a genetic predisposition to the condition. Around 43% of men with a diagnosis of PC before age 55 have "hereditary" PC (HPC). HPC refers to a specific subtype of "familial" PC. The criterion for HPC is a family with three generations affected, three first-degree relatives affected, or two relatives affected before age 55 [2].

Knowing more about molecular pathways or regulation in HPC, is nearly to improve the role of initial steps of PC. Thanks to NGS (Next generation Sequencing technology) big advances in the knowledge of this tumour have been obtained [3]. Nevertheless, by the moment there is scarce data about large family cohorts to find out more details of HPC malignancy. It is crucial to obtain more information about the molecular basis of this disease in order to break down the alterations in the metabolic pathways of these families and how these alterations could influence in the appearance and evolution of this tumour in sporadic cases.

The aforementioned NGS has turn in to a significant tool for finding new altered genes, allowing the diagnosis of many diseases and with a special important role in those less known diseases, such as HPC. Moreover, it helps the knowledge of control mechanisms of genetic expression with a deep detail and accuracy, which is an important role for the comprehension of genotype-phenotype relationship among patients [4]. The implementation of "omics" data in clinical practice is generating a new framework in health and the causes of disease; including new knowledge and approaching precision medicine. Thanks to all the methodologies developed to generate libraries, it is reasonable to expect extraordinary progress in the knowledge of the genome, its expression and regulation related to the molecular mechanisms associated to health.

With this study we will make a deep analysis in data of exome analysed by NGS technology to understand the role of new reported variants as aggressiveness variants of this tumour.

2. Materials and Methods

2.1. Samples

We have performed an analysis in a family of a high incidence of PC (family pedigree details in Figure 1). Blood samples were collected in EDTA and Tempus™ tubes and processed 24 h before collection by a separation from plasma by a centrifugation of 120× *g* during 20 min. Samples were following stored at −80 °C until they will be processed. In this family mean tumour detection age was around 50 years, with PSA values between 7 to 12 ng/mL, Gleason score of 8 and TNM around 2 or 3 values. Samples were collected in 2017, so data about follow-up and quality of life of patients is available. All study participants provided a written informed consent before being enrolled, and the study was previously approved by the Research Ethics Committee of Granada Centre (CEI-Granada internal code 0166-N-19) following Helsinki ethical declaration.

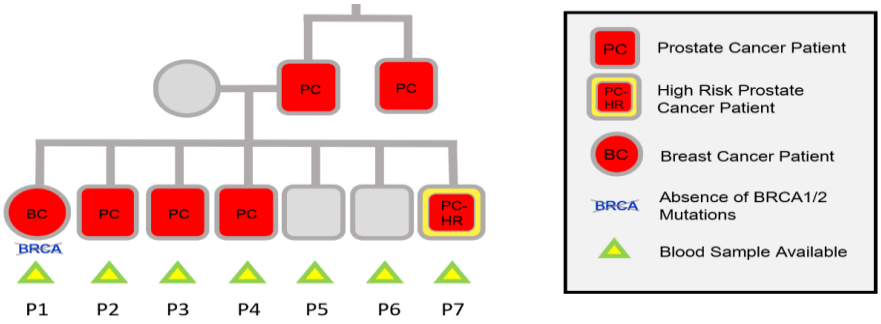

Figure 1. Family pedigree representation.

2.2. Next-Generation Whole-Exome Sequencing (WES) for Candidate Gene Identification

DNA was extracted using kit "DNA Blood Mini" (Qiagen, Hilden, Germany) by an automatized extraction using Qiacube (Qiagen, Hilden, Germany). For performing the library, 0.5 µg of DNA from all de samples were collected after quantification by Qubit v4 (Thermo Fisher Scientific, MA, USA). DNA from all samples was fragmented using Covaris E200 (Covaris, MA, USA) following the recommendations "Hybridization-Based Target Enrichment" by Nimblegen Roche for exome capture, using reagents "HyperCapWorkflow v2.0" (Roche Sequencing Solutions, CA, USA) and "SeqCap® EZ MedExome" (Nimblegen Roche, Basel, Switzerland). Libraries were next sequenced by NextSeq 500 (Illumina, CA, USA) using the kit NextSeq 500/550 High Output Kit v2.5 (300 Cycles) for producing paired sequences 150 × 2.

2.3. Bioinformatic Analysis

To find significant genetic variations within the family of high-risk PC, some treatments were carried out on the sequencing data obtained. The most significant genetic variations, according to the parameters provided by the *SIFT* [5] and *PolyPhen* [6] predictors, were selected. Among the selected

variants, those that met two specific criteria were chosen. First criteria included those variants that are expressed only in PC patients and not in healthy patients, in others words, variants expressed in patients P2, P3, P4 and P7, and not in P1, P5 and P6 (code assigned to each patient shown in Figure 1). On the other hand, the variants found only in patients with high-risk PC (most aggressive form of the disease), P7, were included in the second pattern.

3. Results

To investigate the aetiology and help in understanding this disease, exome sequencing was performed in the seven siblings belonging to the study family. Sequencing analyses reveal (i) the presence of variations in five genes for only the patients with PC (P2, P3, P4 and P7); *HIBCH*, *DPP4*, *MOK PPP4R3A* and *STK31*. All of them, except *STK31*, are also found in the patient with breast cancer. (ii) The patient with aggressive PC (P7) shows a total of ten genes with unique variations, not present in the other patients; *ACE*, *ALDH3B2*, *CD63*, *EFCAB13*, *FAM86HP*, *FANK1*, *MYO15B*, *SCL25A5*, *TUBA3FP* and *ZNF142*. All genes and their associated single nucleotide polymorphisms (SNPs) are shown in Table 1.

Table 1. List of variations obtained after bioinformatics analysis in PC patients.

Variations in all PC Patients	
Gene	**SNP**
DPP4	rs116302758
HIBCH	rs291466
MOK	rs56377169
PPP4R3A	.
STK31	.
Variations in High Risk PC Patient	
Gene	**SNP**
ACE	.
ALDH3B2	rs7947754
CD63	.
EFCAB13	rs2271803
FAM86HP	rs16834628
FANK1	.
MYO15B	rs73998360
SLC25A5	.
TUBA3FP	rs2075276
ZNF142	.

4. Discussion

In the current study, we have shown how genetic variation correlates to PC depending on being in healthy or affected patients (deepening in different aggressiveness stages). Whole-exome sequencing was performed to identify variants, within the coding region of the genome, associated with HPC aggressiveness. The aggressiveness of the disease was monitored by Gleason score, PSA levels and quality of life of patients. A death of the high-risk PC patient (P7) has been recently reported.

Our data support that several genes such as *HIBCH*, *DPP4*, *MOK* and *PPP4R3A* are just present in all HPC patients. Although scarce data, there are previous publications relating *HIBCH* with clinical implication in PC [7]; or accelerating PC progression following androgen deprivation therapy in *DPP4* [8]. These data support our results as promise biomarkers in aggressiveness mainly due to a bad response of the therapy, which is also confirmed in other tumours such as colorectal cancer in *HIBCH* [9]. Furthermore, this is the first time that *PPP4R3A* and *MOK* have been related to PC. *PPP4R3A* has some related pathways it is included breast cancer and glucagon signalling [10].

Carcinogenesis event could be explained by its role during DNA replication and DNA double-strand break repair. Besides, *MOK* belongs to the *MAP* kinase superfamily and among its related pathways are IL-2 [10]. Moreover, high-risk patient has unique additional variants such as *ACE*, *CD63* and *EFCAB13*, that have already been associated with PC. These results provide a new set of promising biomarkers that can help in the stratification and diagnosis in HCP.

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Conflicts of Interest: The authors declare no conflict of interest.

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