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Study of some candidate genes for treatment in prostate cancer †

Veronica Arenas-Rodriguez [1,*], Patricia Maria Porras-Quesada [2], Victor Sanchez-Conde [3], Ignacio Puche-Sanz [3], Fernando Vazquez-Alonso [3], Sergio Cuenca-Lopez [1], Blanca Cano-Gutierrez [3], Sara Martin-Esteban [4], Maria Jesus Alvarez-Cubero [1,2] and Luis Javier Martinez-Gonzalez [1,*]

[1] GENYO (Pfizer-University of Granada-Andalusian Regional Government Centre for Genomics and Oncological Research), Av. de la Ilustracion, 114, 18016 Granada, Spain; scuenca@ugr.es (S.C.-L.); mjesusac@ugr.es (M.J.A.-C.)
[2] Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, University of Granada, Av. de la Investigacion, 11, 18016 Granada, Spain; patriciaporras@correo.ugr.es
[3] Urology Department, University Hospital Virgen de las Nieves, Av. de las Fuerzas Armadas, 2, 18014 Granada, Spain; vaitor1991@gmail.com (V.S.-C.); nacho.puchesanz@gmail.com (I.P.-S.); fvazquezalonso@gmail.com (F.V.-A.); blancacanoguti@gmail.com (B.C.-G.)
[4] Andalusian Health Service, Andalusian Regional Government, Spain; sary_126@hotmail.com
* Correspondence: veronica.arenas@genyo.es (V.A.-R.); luisjavier.martinez@genyo.es (L.J.M.-G.)
† Presented at the 1st International Electronic Conference on Genes: Theoretical and Applied Genomics, 2–30 November 2020; Available online: https://iecge.sciforum.net/.

Received: date; Accepted: date; Published: date

Abstract: The androgenic signalling pathway is essential for carcinogenesis and tumour development in prostate cancer (PC). The importance of that pathway makes it the main target of treatments against PC, among which androgen deprivation therapy (ADT) stands out. The heterogeneity of the response against the same treatment shows the importance of the search for molecular biomarkers which enable the prediction of the response to the therapy in each case. This work focuses on the characterization of the response to treatment in several patients of PC through the analysis of different genetic variants [rs10877012 (*CYP27B1*); rs3768490 (*GSTM5*); rs1004446 (*IGF2*)]. The statistical analysis revealed a certain tendency to resistance in A/G genotype carriers in rs1004446 (*IGF2*). Furthermore, a significant statistical relation between aggressive phenotypes was confirmed in single nucleotide polymorphism (SNP) rs10877012 (*CYP27B1*, $p = 0.013$).

Keywords: prostate cancer; androgen receptor; androgen deprivation therapy; castration-resistant prostate cancer

1. Introduction

Prostate cancer (PC) is the most frequent tumour in men in Spain and the second worldwide [1]. The activation of the androgenic signalling pathway in PC is essential for carcinogenesis and tumour development [2]. The relevance of that pathway makes it the main target of treatments against PC, among which androgen deprivation therapy (ADT) is the most commonly used. This therapy is efficient in the early stages of tumour development however, in most instances, the tumour evolves into castration-resistant prostate cancer (CRPC). In such cases, the tumour becomes more aggressive and a metastatic process usually occurs [3].

Currently, the main strategy for the stratification of patients with PC is based on Gleason score and prostate-specific antigen levels (PSA). PSA is the most widely used biomarker in PC, but it is also correlated with other disorders. This fact together with the heterogeneous response against the same

treatment makes it necessary to search for new biomarkers that make it possible to predict the most effective treatment [4].

This work focuses on the characterization of the response to treatment in several patients of PC through the analysis of different genetic variants. Thereby, candidate genetic positions implicated in the development of resistance against ADT were selected. The search of single nucleotide polymorphisms (SNPs) was carried out to establish a possible connection between the genotype of each patient and the response to treatment.

2. Materials and Methods

2.1. Patients

A total of 59 subjects with PSA levels above 4 ng/mL and positive biopsy were included in this study. Peripheral blood and buccal swabs samples were obtained and clinical data such as age, Gleason score or response to hormone therapy were obtained from medical records. All subjects of the study provided written informed consent to be enrolled. The study was previously approved by the Research Ethics Committee of Granada Center (CEI-Granada) following Helsinki ethical declaration.

2.2. SNPs Selection

Three genes involved in resistance to hormone therapy were selected after a thorough bibliography review in *GeneCards* and *Pubmed* databases. Besides, possible SNPs of the candidate genes were identified using *SNPedia* and their allelic frequencies in the Caucasian population were obtained from *Ensembl*. Those SNPs with allele frequencies higher than 20% in the minor allele frequency (MAF) were selected. Finally, three genetic variant were chosen for this study: rs10877012 (*CYP27B1*), rs3768490 (*GSTM5*) and rs1004446 (*IGF2).*

2.3. DNA Extraction and Genotyping

Standard organic DNA extraction was performed from blood and buccal swab samples. Purity and concentration of DNA extracts were determined with a NanoDrop 2000c (*Thermo Scientific, USA*). Then, DNA genotyping was performed using TaqMan SNP Genotyping in a QuantStudio 6 Flex (*Applied Biosystems, USA*).

2.4. Statistical Analysis

Software package SPSS v.19.0 was used for statistical analyses. The association between different genotypes and the response of each patient to hormonal treatment was analysed by chi-square and statistical signification was considered with p values < 0.05.

3. Results and Discussion

A cohort of 59 PC samples was divided into sensitive or resistant to treatment according to clinical data. Association results between genotype and response to treatment are shown in Table 1.

Table 1. Association between genotype and response to hormonal treatment.

SNP	Sensitive to Treatment	Resistant to Treatment	*p*
rs10877012 (*CYP27B1*)			0.656
G/G	8 (44.45%)	21 (52.50%)	
T/T	2 (11.10%)	2 (5.00%)	
G/T	8 (44.45%)	17 (42.50%)	
rs3768490 (*GSTM5*)			0.629
G/G	5 (29.41%)	13 (34.21%)	
T/T	4 (23.53%)	5 (13.16%)	

G/T	8 (47.06%)	20 (52.63%)	
rs1004446 (*IGF2*)			0.391
G/G	11 (61.11%)	17 (42.50%)	
A/A	1 (5.56%)	5 (12.50%)	
A/G	6 (33.33%)	18 (45.00%)	

Our results show a trend to resistance in A/G genotype in rs1004446 (*IGF2*). However, owing to the lack of statistical significance we cannot confirm a direct association between the genotype of these SNPs and the response to treatment. We consider that these inconclusive results are due to the low number of samples analysed. Thus, bigger sample size will be necessary to reinforce these preliminary findings.

On the other hand, we have found a statistically significant (p = 0.013) association between the genotype of the SNP rs10877012 (*CYP27B1*) and the aggressiveness of phenotypes measured with the Gleason score. These results are shown in Table 2.

Table 2. Association between genotype and Gleason score.

SNP	Gleason Score ≤ 7	Gleason Score > 7	p
rs10877012 (*CYP27B1*)			0.013
G/G	6 (25.00%)	22 (59.46%)	
T/T	1 (4.17%)	3 (8.10%)	
G/T	17 (70.83%)	12 (32.44%)	
rs3768490 (*GSTM5*)			0.908
G/G	11 (44.00%)	15 (42.86%)	
T/T	2 (8.00%)	4 (11.43%)	
G/T	12 (48.00%)	16 (45.71%)	
rs1004446 (*IGF2*)			0.784
G/G	13 (52.00%)	16 (43.24%)	
A/A	2 (8.00%)	4 (10.81%)	
A/G	10 (40.00%)	17 (45.95%)	

As represented in Figure 1, we observe that 78.57% of the patients with G/G genotype for rs10877012 had a Gleason score above 7, which is associated with greater aggressiveness of PC. In the case of the heterozygous genotype, it was found that 56.67% of those presented a Gleason score lower or equal to 7. In the case of the T/T genotype, no relevant conclusion can be reached since only 4 of all the samples that participated in the study presented that genotype. These results would help to predict the future severity of the PC when the tumour is still in the early stages.

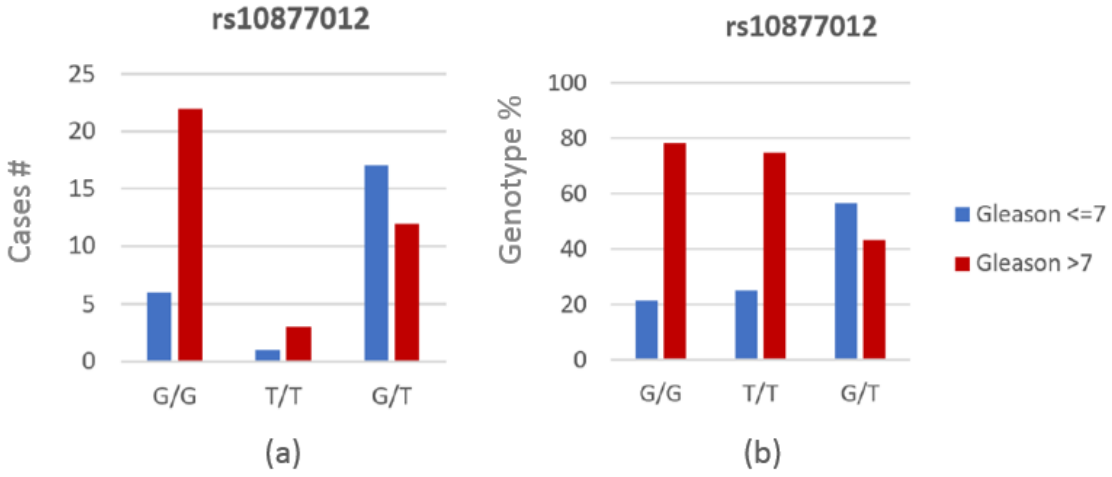

Figure 1. Representation of genotype association and Gleason score: (**a**) Expressed in number of cases; (**b**) Expressed as a percentage.

4. Conclusions

This study reveals that PC patients with A/G genotype in rs1004446 (*IGF2*) show a certain tendency to resistance to hormonal treatment, although a relationship with statistical significance was not confirmed. More importantly, a significant statistical relation between aggressive phenotypes was confirmed in rs10877012 (*CYP27B1*). Further confirmation of these results in a larger cohort will open up a wide range of possibilities for the clinical implementation of these SNPs.

Funding: This research is part of the project "Application of genomics in the treatment of prostate cancer, a new step to precision medicine" which was funded by Consejeria de Salud, Junta de Andalucia, Proyectos Estrategicos de I+D+i, (Spanish Government Institution). PE-0448-2019, 01/01/2020-31/12/2024.

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

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