ORIGINAL ARTICLE

Prognostic role of genetic biomarkers in clinical progression of prostate cancer

Maria Jesus Alvarez-Cubero1,2, Luis Javier Martinez-Gonzalez2, Maria Saiz1, Pedro Carmona-Saez2, Juan Carlos Alvarez1, Manrique Pascual-Geler3, Jose Antonio Lorente1,2,4 and Jose Manuel Cozar3,4

The aim of this study was to analyze the use of 12 single-nucleotide polymorphisms in genes ELAC2, RNASEL and MSR1 as biomarkers for prostate cancer (PCa) detection and progression, as well as perform a genetic classification of high-risk patients. A cohort of 451 men (235 patients and 216 controls) was studied. We calculated means of regression analysis using clinical values (stage, prostate-specific antigen, Gleason score and progression) in patients and controls at the basal stage and after a follow-up of 72 months. Significantly different allele frequencies between patients and controls were observed for rs1904577 and rs918 (MSR1 gene) and for rs17552022 and rs5030739 (ELAC2). We found evidence of increased risk for PCa in rs486907 and rs2127565 in variants AA and CC, respectively. In addition, rs627928 (TT–GT), rs486907 (AG) and rs3747531 (CG–CC) were associated with low tumor aggressiveness. Some had a weak linkage, such as rs1904577 and rs2127565, rs4792311 and rs17552022, and rs1904577 and rs918. Our study provides the proof-of-principle that some of the genetic variants (such as rs486907, rs627928 and rs2127565) in genes RNASEL, MSR1 and ELAC2 can be used as predictors of aggressiveness and progression of PCa. In the future, clinical use of these biomarkers, in combination with current ones, could potentially reduce the rate of unnecessary biopsies and specific treatments.

Experimental & Molecular Medicine (2015) 47, e176; doi:10.1038/emm.2015.43; published online 7 August 2015

INTRODUCTION

In recent times, several genetic germline polymorphisms have been associated with the risk of developing prostate cancer (PCa), as well as with its aggressiveness and the risk for biochemical recurrence. Results from some candidate genes and genome-wide association studies suggest that single-nucleotide polymorphisms (SNPs) in the angiogenesis pathway may be important in PCa progression and aggressiveness.

Nowadays, early detection of PCa remains a challenge for researchers and clinicians worldwide. The comprehensive understanding of PCa biology as well as a reliable, noninvasive biomarker for the detection of this cancer is urgently needed. The main objective is to avoid over- or undertreatment. At present, the parameters to adjust treatment are the D′amico guidelines (including total level of prostate-specific antigen (PSA)), Gleason score and clinical stage. These prediction tools have an accuracy of 70%–80%. The current prediction marker PSA is quite imprecise and subjective.

To evaluate the role of SNPs in PCa susceptibility and because of the heterogeneity of this pathology, the study has been focused on the most common variants of the European population in genes RNASEL, MSR1 and ELAC2.

The main aim of this study was to evaluate the effect of these variants (details in Table 1) in PCa by performing a genetic test among PCa patients and to change the current models for a more accurate prediction of PCa. Although the association between RNASEL, ELAC2 and MSR1 genes and PCa has been deeply studied, no conclusive results have been reported.

The RNASEL gene is an endoribonuclease with an important role as a tumor suppressor gene. The MSR1 gene encodes a macrophage scavenger receptor included in a signaling pathway related to apoptotic processes. Mutations in MSR1 may increase the risk of PCa by predisposing to chronic inflammation as a result of failure of viral RNA and bacterial degradation. The ELAC2 gene encodes for a hydrolase with a 3′ endoribonuclease processing activity (3′ tRNase) and interacts with the γ-tubulin complex. Although the exact
role of these genes in PCa carcinogenesis remains unknown, some of these processes and activities have been implicated in its development. There are previous reports about CYP24A1 genetic variants and PCa aggressiveness. In addition, abnormalities on the phosphoinositide 3-kinase/AKT pathway have been described in high-risk localized PCa. 

We strongly think that SNPs might be applied to personalized medicine in the context of cancer risk assessment and screening. The development of detailed SNP maps of the human genome coupled with high-throughput genotyping technologies may allow scientists to unravel complex genetic traits, such as multifactorial disease or drug response. This study will establish the first steps in predictive testing among PCa patients. It may be followed by whole-exome sequencing of the study cohort (Table 2). Nine SNPs out of the 21 had to be excluded because of lack of statistical differences among controls and patients, mainly because of ethnic differences in allele frequencies in these SNPs. 

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**MATERIALS AND METHODS**

**Study cohort**
The cohort of patients was from the Urology section of 'Virgen de las Nieves' Hospital (Granada, Spain). The men enrolled as patients (n = 235) had a Gleason score of 5–8 and the mean age at diagnosis was 67.4 years (range 47–86). Clinical diagnosis of primary prostate adenocarcinoma was histopathologically confirmed after abnormal serum PSA findings or lower urinary tract symptoms (Supplementary Table S1). Subjects were included as high-risk patients if they met the following indications of the European Association of Urology Guidelines: local stage with values ≥ T2c; Gleason score > 7; or PSA > 20 ng ml$^{-1}$. Healthy unrelated Caucasian men (n = 216) from the same geographical area and age group with no history of PCa were enrolled as controls. All controls were men with other urological health problems (renal lithiasis or andrological problems) with PSA < 4 ng ml$^{-1}$ and a normal rectal examination. Cancer progression parameters were also measured by the clinician analyzing increases of PSA (biochemical progression of PSA), appearance of pain, or prostatic obstruction during a period of 18, 36 and 72 months (clinical progression of PCa). If PSA increases were not observed, it was reported as good progression. Informed consent was required for all participants in the trial, and the study was approved by local institutional review boards and Ethics Committees. This study took place between 2007 and 2013.

**Genotyping**
A total of 21 SNPs were initially selected on the basis of previous reports of their association with PCa in the European population (Table 1). Nine SNPs out of the 21 had to be excluded because of lack of statistical differences among controls and patients, mainly because of ethnic differences in allele frequencies in these SNPs (Supplementary Table S3). Thus, the study focused on the analysis of 12 SNPs in the cohort (Table 2).

**Statistics**
To test the association of each SNP with binary variables such as PCa risk, Gleason grade (<7 or ≥7), disease stage (high-stage (C–D) or low-stage (A–B)) and progression, unconditional logistic regression was used. For continuous variables (PSA), linear regression analysis was performed. All analyses were adjusted by age and the Bonferroni correction was used to adjust P-values for multiple testing. P < 0.05 was considered statistically significant. Furthermore, Kaplan–Meier analysis was performed. Analyses were carried out using the SNP stats software package and R statistical environment.

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Table 1 SNP information in RNASEL, ELAC2 and MSR1 genes

| RNASEL gene—chromosome 1q22 | MSR1 gene—chromosome Bp22 | ELAC2 gene—chromosome 17p11 |
|-----------------------------|---------------------------|-----------------------------|
| M1I (3 G > A)              | P275A (823 C > G)         | T520T (1560A > G)           |
| rs74315365                 | rs3747531                 | rs11545302                  |
| G59F (175 G > A)           | E262X (784 G > T)         | 3' UTR (*366A > G)          |
| No rs#                     | rs627928                  | rs12718376                  |
| I97L (289A > G)            | E265X (793 G > T)         | 3' UTR (*516G > A)          |
| rs56250729                 | rs74315364                | rs918                       |
| S113S (339 T > C)          | S406F (1217C > T)         | Intron region               |
| rs3606971                  |                           | (1223–3957C > T)           |
| Del 471 AAAG               | R462Q (1384 G > A)        | S217L (650C > T)            |
| No rs#                     | rs486907                  | rs4792311                   |

Abbreviations: SNP, single-nucleotide polymorphism; UTR, untranslated region.
Table 2 Statistical analysis in patients and controls in RNASEL, ELAC2 and MSR1 genes

| Genotype | Control n(%) | Tumor n(%) | OR (95% CI) | P-value | Adj. P-value | HWE (P-value) |
|----------|--------------|------------|-------------|---------|-------------|---------------|
| **Chromosome 1q22-RNASEL gene**<sup>a</sup> | | | | |
| rs486907-R462Q | | | | |
| G/G | 61 (28.2) | 80 (33.8) | 1.00 | 0.38 | 1 | 0.29 |
| A/G | 114 (52.8) | 120 (50.6) | 0.80 (0.53–1.22) | 0.39 | 1 | 0.29 |
| A/A | 41 (19) | 37 (15.6) | 0.69 (0.39–1.20) | 0.5 | 1 | 0.38 |
| rs627928-D541E | | | | |
| G/G | 69 (31.9) | 78 (32.9) | 1.00 | 0.95 | 1 | 0.12 |
| A/G | 113 (52.3) | 124 (52.3) | 0.97 (0.64–1.47) | 0.85 | 1 | 0.12 |
| T/T | 34 (15.7) | 35 (15.6) | 0.91 (0.51–1.61) | 0.85 | 1 | 0.12 |
| rs56250729-I97L | | | | |
| T/T | 200 (99) | 212 (99.1) | 1.00 | 0.42 | 1 | 0.012 |
| G/T | 2 (1) | 1 (0.5) | 0.47 (0.04–5.24) | 0.5 | 1 | 0.012 |
| G/G | 0 (0) | 1 (0.5) | NA (0.00–NA) | 0.5 | 1 | 0.012 |
| **Chromosome 17p11-ELAC2 gene** | | | | |
| rs11545302-T520T | | | | |
| A/A | 120 (54.8) | 106 (45.3) | 1.00 | 0.079 | 0.948 | 0.21 |
| A/G | 88 (40.2) | 108 (46.1) | 1.39 (0.95–2.04) | 0.05 | 1 | 0.21 |
| G/G | 11 (5) | 20 (8.6) | 2.06 (0.94–4.49) | 0.05 | 1 | 0.21 |
| rs17552022-T631T | | | | |
| T/T | 166 (79) | 157 (66.8) | 1.00 | 0.01 | 0.12 | 0.1 |
| C/T | 40 (19.1) | 67 (28.5) | 1.77 (1.13–2.77) | 0.05 | 1 | 0.12 |
| C/C | 4 (1.9) | 11 (4.7) | 2.91 (0.91–9.32) | 0.05 | 1 | 0.12 |
| rs5030739-A541T | | | | |
| G/G | 121 (57.9) | 171 (72.8) | 1.00 | 0.0019 | 0.0228 | 0.08 |
| A/G | 81 (38.8) | 62 (26.4) | 0.54 (0.36–0.81) | 0.05 | 1 | 0.35 |
| A/A | 7 (3.4) | 2 (0.8) | 0.20 (0.04–0.99) | 0.05 | 1 | 0.35 |
| rs4792311-S217L | | | | |
| G/G | 114 (53.5) | 111 (47.8) | 1.00 | 0.34 | 1 | 0.57 |
| A/G | 83 (39) | 96 (41.4) | 1.19 (0.80–1.76) | 0.5 | 1 | 0.57 |
| A/A | 16 (7.5) | 25 (10.8) | 1.60 (0.81–3.17) | 0.5 | 1 | 0.57 |
| **Chromosome 8p22-MSR1 gene** | | | | |
| rs12718376 | | | | |
| C/C | 123 (57.2) | 143 (60.9) | 1.00 | 0.72 | 1 | 0.19 |
| C/T | 76 (35.4) | 77 (32.8) | 0.87 (0.59–1.30) | 0.72 | 1 | 0.19 |
| T/T | 16 (7.4) | 15 (6.4) | 0.81 (0.38–1.70) | 0.72 | 1 | 0.19 |
| rs918 | | | | |
| G/G | 170 (81) | 189 (80.4) | 1.00 | 0.0084 | 0.1008 | 0.0032 |
| A/G | 39 (18.6) | 35 (14.9) | 0.81 (0.49–1.33) | 0.81 | 1 | 0.19 |
| A/A | 1 (0.5) | 11 (4.7) | 9.89 (1.27–77.38) | 0.81 | 1 | 0.19 |
| rs1904577 | | | | |
| A/A | 133 (64.2) | 165 (71.1) | 1.00 | 0.037 | 0.444 | <0.0001 |
| A/G | 47 (22.7) | 53 (22.8) | 0.91 (0.58–1.43) | 0.81 | 1 | 0.19 |
| G/G | 27 (13) | 14 (6) | 0.42 (0.21–0.83) | 0.81 | 1 | 0.19 |
| rs2127565 | | | | |
| G/G | 141 (67.8) | 156 (69) | 1.00 | 0.96 | 1 | <0.0001 |
| C/G | 52 (25) | 54 (23.9) | 0.94 (0.60–1.46) | 0.96 | 1 | <0.0001 |
| C/C | 15 (7.2) | 16 (7.1) | 0.96 (0.46–2.02) | 0.96 | 1 | <0.0001 |
| rs3747531-P275A | | | | |
| G/G | 186 (87.3) | 194 (86.2) | 1.00 | 0.33 | 1 | <0.0001 |
| C/G | 22 (10.3) | 20 (8.9) | 0.87 (0.46–1.65) | 0.33 | 1 | <0.0001 |
| C/C | 5 (2.4) | 11 (4.9) | 2.11 (0.72–6.19) | 0.33 | 1 | <0.0001 |

Abbreviations: Adj. P-value, P-value Bonferroni correction; CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratios.

rs56250729 has an allele distribution in the European population that corresponds to TT around 97% and GT 2.90%, (population data obtained from HapMap).

*Only rs486907, rs627928 and rs56250729 were analyzed in the RNASEL gene, because the other SNPs (E262X, 471delAAAG, G265X and M1I) presented only one genotype among all the patients (details in Supplementary Table S3).

NOTE: As can be seen, the number of informative samples varied for each variant, ranging from 214 to 237 among patients and from 202 to 216 among controls because of some problems carried in the genotyping assay. Some samples could not be correctly genotyped by TaqMan SNP Genotyping and they were eliminated from the study in order to avoid the increase of repetitive analysis. Unconfirmed results were not included in the analysis, although it means a reduction in the number of samples in some variants.
Haplotype association was determined by IBM SPSS Statistics v.20 (IBM Corp., Armonk, NY, USA). Only haplotype variants with a frequency $\geq 2.5\%$ in either the PCa or the control group were subjected to further statistical analysis (Table 4). Association analysis was performed with the use of logistic regression with each single haplotype combination to validate the single affection of each haplotype.

Linkage disequilibrium and Hardy–Weinberg equilibrium analyses were performed among all SNPs using Arlequin v3.5 software (Supplementary Table S7).

RESULTS
Genes and relation to PCa
Two hundred and thirty-seven PCa cases and 216 controls were genotyped (patients’ characteristics, Supplementary Table S1). Several samples were excluded for some SNPs because of failure in genotyping.

All controls and patients were in Hardy–Weinberg equilibrium, except rs627928, rs918, rs1904577, rs2127565 and rs3747531 (Table 2). Linkage disequilibrium analysis revealed linkage between rs4792311/rs11545302/rs17552022 (ELAC2) and rs1904577/rs918/rs2127565 (MSR1) (Supplementary Table S7).

We first assessed whether any of the 12 SNPs were associated with PCa (Table 2). Using logistic regression analysis, we found that 4 of the 12 SNPs were nominally associated with PCa ($P<0.05$). Furthermore, a statistically significant increase in the overall PCa risk for the rs11545302 (ELAC2) GG polymorphic variation (0.079; odds ratio (OR) = 2.06, 95% confidence interval: 0.94–4.49) was found as far as in rs17552022 and rs918 (CC and AA variants, respectively) when compared with the other variants in the SNPs. However, a reduced risk for PCa in carriers of the A allele in rs5030739 and in carriers of the G allele in rs1904577 was reported (Table 2).

Analysis of clinical parameters
The discriminative accuracy of the statistical models in the polymorphic variants among patients and their clinical parameters, as well as the prognosis progression, is shown in Table 3. An association between rs627928, rs486907 and rs2127565 and PSA ($P=0.041, 0.043$ and $\leq 0.0001$, respectively) was noted, but only rs2127565 maintained significance after Bonferroni correction, with an increased risk in GG patients (difference = 295.49 (181.70–409.29)). No significant association was observed between the studied SNPs and Gleason score. When studying the stage of disease, two main groups were distinguished, depending on cancer aggressiveness: those with high-stage disease (stage C–D) and those with low-stage disease (A–B). The differences in SNP associations between low-stage and high-stage disease were statistically significant only in case analysis ($P<0.0001$ for rs486907 and $P<0.0001$ for rs627928). Genotype AA in rs486907 was associated with increased risk for high-stage disease (OR: 14.84 (5.43–40.57)). However, the TT genotype in rs627928 was associated with low-risk disease (OR: 0.08 (0.02–0.28)). The minor allele TT in the MSR1 gene (rs12718376) was associated with an increased risk for high-stage disease (stages C–D) (OR: 2.30 (0.77–6.90)).

Although not many significant statistical values were obtained (Tables 2 and 3), evidence of increased risk of some SNP associations to more aggressive parameters, as well as poor progression of the pathology, was found (Table 3 and Supplementary Table S7).

Analysis of cancer progression
As this is an analysis performed in a follow-up cohort of 72 months, we also have data on progression. The AA genotype of rs486907 has been associated with worst progression (OR = 3.83 (1.64–8.94); $P=0.0012$) in patients. However, T carriers in rs627928 ($P=0.016$) are linked to a better progression of the cancer (Table 3).

Haplotype relationship with PCa risk
Table 4 demonstrates that some haplotypes such as TT-GG-GG-GG and AA-TT-GA-GG for MSR1 and ELAC2 genes, respectively, were associated with a significant increase in PCa risk (OR: 1.112 and OR: 1.214, respectively). However, other haplotypes seemed to protect against PCa development, such as CT-GG-AA-GG-GG (MSR1), GA-CT-GG-GA (ELAC2) and GG-GT-TT (RNASEL) (OR: 0.876, 0.489 and 0.815, respectively). More details in Supplementary Material (Supplementary Tables S4).

DISCUSSION
The introduction of the PSA test into clinical practice significantly improved the early diagnosis and management of PCa. However, there is a lot of controversial data because of its low specificity in levels around 2 and 10 ng ml$^{-1}$ (Obert et al.$^3$ and Roddam et al.$^{20}$). Therefore, it would be desirable to have prognostic markers for personalized treatment and a precise diagnosis in the ranges where PSA is not accurate. In the present study, new complementary genetic biomarkers in the screening of PCa are presented.

The panel of 12 SNP markers in RNASEL, MSR1 and ELAC2 genes as biomarkers facilitates a better prediction of aggressiveness and would lead to clinically superior outcomes compared with current biomarkers used in clinical practice. In the future, this panel could be used as a decision aid in men. The identification of those SNPs that enable a better classification into low-, medium- and high-risk disease groups will offer accurate treatment options and earlier effective detection. This approach could prevent patients from suffering adverse effects of treatment or unnecessary biopsies, and thus improve the quality of life. In addition, it could assist patients with potentially aggressive cancers to give them the opportunity for flexible timed follow-ups of this pathology.

The main goal of this project is not only to make a unique genetic predictive tool for clinical and detection purposes but, in combination with current biomarkers and clinical support, to provide a more effective treatment and detection of PCa, as well as offer less invasive biomarker systems. Furthermore, the evaluation of the effect of these SNPs in the development of
| Genotype | OR (95% CI) | P-value | Adj. P-value | Difference (95% CI) | P-value | Adj. P-value | OR (95% CI) | P-value | Adj. P-value | OR (95% CI) | P-value | Adj. P-value | OR (95% CI) | P-value | Adj. P-value |
|----------|-------------|---------|--------------|---------------------|---------|--------------|-------------|---------|--------------|-------------|---------|--------------|-------------|---------|--------------|
| G/G      | 1.00        | <0.0001 | 0.0012       | 0.00                | 0.043   | 0.516        | 1.00        | 0.063   | 0.756        | 1.00        | 0.0012 | 0.0144       |             |         |              |
| A/G      | 0.99 (0.50–1.97) |         |              | 20.67 (–44.87–86.21) | 0.78 (0.42–1.46) | 0.94 (0.49–1.81) |             |         |              |
| A/A      | 14.84 (5.43–40.57) |         |              | 114.55 (23.56–205.54) | 1.96 (0.87–4.45) | 3.83 (1.64–8.94) |             |         |              |
| G/T      | 0.26 (0.14–0.48) |         |              | –79.61 (–145.13–14.09) | 0.49 (0.27–0.90) | 0.44 (0.24–0.81) |             |         |              |
| T/T      | 0.08 (0.02–0.28) |         |              | –85.85 (–176.54–4.84) | 0.64 (0.27–1.49) | 0.38 (0.15–0.97) |             |         |              |
| T/T      | 1.00        | 0.46    | 1            | 0.00                | 0.97    | 1            | 1.00        | 0.23    | 1            | 1.00        | 0.47    | 1            |             |         |              |
| G/T      | 0.00 (0.00–NA) |         |              | –20.09 (–324.40–284.22) | 0.00 (0.00–NA) | 0.00 (0.00–NA) |             |         |              |
| G/G      | 0.00 (0.00–NA) |         |              | –33.98 (–338.42–270.45) | NA (0.00–NA) | 0.00 (0.00–NA) |             |         |              |
| A/A      | 1.00        | 0.82    | 1            | 0.00                | 0.87    | 1            | 1.00        | 0.63    | 1            | 1.00        | 0.33    | 1            |             |         |              |
| A/G      | 1.10 (0.61–1.97) |         |              | 0.95 (–62.19–64.08) | 1.06    | (0.59–1.89) | 1.56        | (0.86–2.82) |             |             |         |              |
| G/G      | 1.38 (0.49–3.90) |         |              | –28.98 (–144.78–86.82) | 1.66    | (0.60–4.59) | 1.38        | (0.47–4.06) |             |             |         |              |
| T/T      | 1.00        | 0.42    | 1            | 0.00                | 0.92    | 1            | 1.00        | 0.43    | 1            | 1.00        | 0.85    | 1            |             |         |              |
| C/T      | 0.84 (0.44–1.59) |         |              | 12.74 (–54.79–80.27) | 0.70    | (0.37–1.33) | 1.16        | (0.62–2.20) |             |             |         |              |
| CC       | 2.13 (0.59–7.72) |         |              | –11.85 (–171.28–147.58) | 1.46    | (0.38–5.70) | 1.31        | (0.35–4.89) |             |             |         |              |
| A/A      | 2.02 (0.12–32.92) |         |              | –27.86 (–339.33–283.61) | NA (0.00–NA) | 1.85 (0.11–30.41) |             |         |              |             |         |              |             |         |              |
| A/G      | 0.88 (0.46–1.68) |         |              | 16.19 (–52.07–84.44) | 0.87    | (0.46–1.64) | 0.67        | (0.34–1.33) |             |             |         |              |             |         |              |
| A/A      | 2.02 (0.12–32.92) |         |              | –27.86 (–339.33–283.61) | NA (0.00–NA) | 1.85 (0.11–30.41) |             |         |              |             |         |              |             |         |              |
## Table 3 (Continued)

| Genotype            | Stage | PSA | OR (95% CI) | P-value | Adj. P-value | Difference (95% CI) | P-value | Adj. P-value | OR (95% CI) | P-value | Adj. P-value | OR (95% CI) | P-value | Adj. P-value |
|---------------------|-------|-----|-------------|---------|--------------|---------------------|---------|--------------|-------------|---------|--------------|-------------|---------|--------------|
| **S217L-rs4792311 ELAC2** |       |     |             |         |              |                     |         |              |             |         |              |             |         |              |
| G/G                 |       |     | 1.00        |         |              | 0.00               | 0.29    |              | 1.00        |         |              | 1.00        |         |              |
| A/G                 |       |     | 0.94 (0.52–1.70) | 0.48 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38) |         |              |             |         |              |             |         |              |
| A/A                 |       |     | 1.06 (0.41–2.75) | 3.22 | (−106.66–100.22) | 1.21 (0.48–3.07)  | 1.02 (0.38–2.73) |         |              |             |         |              |             |         |              |
| **3’UTR-rs12718376 MSR1** |       |     |             |         |              |                     |         |              |             |         |              |             |         |              |
| C/C                 |       |     | 1.00        |         |              | 0.00               | 0.67    |              | 1.00        |         |              | 1.00        |         |              |
| C/T                 |       |     | 0.96 (0.52–1.76) | 48.62 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38)  |         |              |             |         |              |             |         |              |
| T/T                 |       |     | 1.01 (0.41–2.75) | 3.22 | (−106.66–100.22) | 1.21 (0.48–3.07)  | 1.02 (0.38–2.73)  |         |              |             |         |              |             |         |              |
| **3’UTR-rs918 MSR1** |       |     |             |         |              |                     |         |              |             |         |              |             |         |              |
| G/G                 |       |     | 1.00        |         |              | 0.00               | 0.67    |              | 1.00        |         |              | 1.00        |         |              |
| A/G                 |       |     | 1.51 (0.70–3.23) | 0.72 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38)  |         |              |             |         |              |             |         |              |
| A/A                 |       |     | 0.47 (0.10–2.25) | 3.22 | (−106.66–100.22) | 1.21 (0.48–3.07)  | 1.02 (0.38–2.73)  |         |              |             |         |              |             |         |              |
| **Intron-rs1904577 MSR1** |       |     |             |         |              |                     |         |              |             |         |              |             |         |              |
| A/A                 |       |     | 1.00        |         |              | 0.00               | 0.67    |              | 1.00        |         |              | 1.00        |         |              |
| A/G                 |       |     | 0.67 (0.33–1.34) | 28.78 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38)  |         |              |             |         |              |             |         |              |
| G/G                 |       |     | 0.72 (0.22–2.41) | 28.78 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38)  |         |              |             |         |              |             |         |              |
| **Intron-rs2127565 MSR1** |       |     |             |         |              |                     |         |              |             |         |              |             |         |              |
| G/G                 |       |     | 1.00        |         |              | 0.00               | 0.67    |              | 1.00        |         |              | 1.00        |         |              |
| C/G                 |       |     | 0.56 (0.27–1.17) | 29.69 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38)  |         |              |             |         |              |             |         |              |
| C/C                 |       |     | 1.12 (0.38–3.27) | 29.69 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38)  |         |              |             |         |              |             |         |              |
| **P275A-rs3747531 MSR1** |       |     |             |         |              |                     |         |              |             |         |              |             |         |              |
| G/G                 |       |     | 1.00        |         |              | 0.00               | 0.67    |              | 1.00        |         |              | 1.00        |         |              |
| C/G                 |       |     | 1.18 (0.45–3.13) | 28.78 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38)  |         |              |             |         |              |             |         |              |
| C/C                 |       |     | 0.94 (0.23–3.77) | 28.78 | (−15.75–112.98) | 0.96 (0.53–1.72)  | 1.31 (0.72–2.38)  |         |              |             |         |              |             |         |              |

Abbreviations: Adj. P-value, Bonferroni corrected P-value; CI, 95% confidence interval; NA, not available; OR, odds ratio; PSA, prostate-specific antigen; SNP, single-nucleotide polymorphism.
PCa will enable to avoid the actual clinical cases of many undiagnosed PCa, because of a lack of signs or symptoms that indicate the need for a prostate biopsy, or the cases of false-negative biopsy results of the random nature of needle placements.

Our decision analysis showed that the use of the SNP biomarker model could reduce the number of unnecessary operations through a previous clinical and genetic stratification of these patients. It is established that a number of SNPs can be used to classify men and PCa risk, similar to the strategy employed in breast cancer patients and BRCA1 mutations.

However, this study is based on a rather small cohort of men and all of them were selected from the same geographical area of Spain, which could cause some statistical bias and limitations in results. Nevertheless, this is one of the first studies using these genetic markers on the Spanish population. The next step will be an external validation study on a widespread cohort.

The results reported for the genetic biomarker model as a predictive and prognostic factor in our population are consistent with previous studies evaluating the use of genetic biomarkers as detectors of progression and aggressiveness in cancer, such as adenomatous polyposis coli gene in colorectal cancer or BRCA1/2 in breast cancer. In PCa, previous analyses confirm the possibility of using genetic biomarkers such as SNPs in RNASEL, ELAC2 and RNASEL genes, in combination with family history, to assess an individual patient’s risk and the

### Table 4 The haplotype association with prostate cancer in a south Spanish population at MSR1, ELAC2 and RNASEL genes

| MSR1 Haplotype | Frequencya | Case, control frequencies | χ² | P-value | OR (95% CI) |
|----------------|------------|--------------------------|----|---------|-------------|
| CC-GG-AA-GG-GG | 0.2688     | 0.2739, 0.2449           | 4.767 | 0.312   |             |
| CT-GG-AA-GG-GG | 0.1219     | 0.1348, 0.0612           | 5197 | 0.023/0.032d | 0.876 (0.776–0.989) |
| CC-GG-AG-GC-GG | 0.0538     | 0.0478, 0.0816           | 0.621 | 0.431   |             |
| CC-GA-AA-GG-GG | 0.0430     | 0.0261, 0.1224           | 0.330 | 0.566   |             |
| TT-GG-AA-GG-GG | 0.0430     | 0.0261, 0.1224           | 4.265 | 0.049   | 1.112 (1.000–1.236) |
| CT-GA-AA-GG-GG | 0.0287     | 0.0304, 0.0204           | 0.662 | 0.416   |             |
| CC-GG-GA-CC-GG | 0.0215     | 0.0217, 0.0204           | 0.215 | 0.646   |             |
| CC-GG-GG-CC-GG | 0.0215     | 0.0130, 0.0612           | 0.633 | 0.426   |             |
| CC-GG-GG-GC-GG | 0.0143     | 0.0087, 0.0408           | 3.386 | 0.066   |             |
| CC-GG-GG-GG-GG | 0.0108     | 0.0087, 0.0204           | 0.119 | 0.730   |             |
| CC-GA-GA-GC-GG | 0.0108     | 0.0087, 0.0204           | 0.119 | 0.732   |             |
| CT-GG-GG-CC-GG | 0.0108     | 0.0043, 0.0408           | 0.688 | 0.407   |             |

| ELAC2 Haplotype | Frequencya | Case, control frequencies | χ² | P-value | OR (95% CI) |
|----------------|------------|--------------------------|----|---------|-------------|
| AA-TT-GG-GG    | 0.3043     | 0.3191, 0.2500           | 3.541 | 0.060   |             |
| AA-TT-GA-GG    | 0.1538     | 0.0979, 0.3594           | 18.417 | 0.0001/0.0001d | 1.214 (1.107–1.332) |
| GA-CT-GG-GA    | 0.1271     | 0.1489, 0.0469           | 6.241 | 0.012/0.020d | 0.815 (0.686–0.969) |
| GA-TT-GG-GA    | 0.1237     | 0.1362, 0.0781           | 2.847 | 0.092   |             |
| GA-TT-GA-GA    | 0.0669     | 0.0553, 0.1094           | 1.202 | 0.273   |             |
| GA-CT-GA-GA    | 0.0502     | 0.0426, 0.0781           | 0.621 | 0.431   |             |
| GG-CT-GA-AA    | 0.0234     | 0.0255, 0.0156           | 0.422 | 0.516   |             |
| GA-TT-GG-GG    | 0.0100     | 0.0085, 0.0156           | 0.119 | 0.730   |             |

| RNASEL Haplotype | Frequencya | Case, control frequencies | χ² | P-value | OR (95% CI) |
|-----------------|------------|--------------------------|----|---------|-------------|
| GA-GT-TT        | 0.3667     | 0.3505, 0.4286           | 0.021 | 0.885   |             |
| GG-GT-TT        | 0.1370     | 0.1589, 0.0536           | 6.241 | 0.012/0.020 | 0.815 (0.686–0.969) |
| GA-GG-TT        | 0.1444     | 0.1355, 0.1786           | 0.018 | 0.892   |             |
| AA-GG-TT        | 0.1296     | 0.1262, 0.1429           | 0.077 | 0.782   |             |
| GG-TT-TT        | 0.1333     | 0.1215, 0.1786           | 0.198 | 0.656   |             |
| GG-GG-TT        | 0.0519     | 0.0607, 0.0179           | 2.441 | 0.118   |             |

Abbreviations: CI, confidence interval; OR, odds ratio.
aFrequency calculated with the whole data of the analyzed population.
bFrequency of cases and controls individually.
cThe haplotypes were generated from SNPs rs12718376, rs918, rs1904577, rs2127565 and rs3747531, in that order.
dP-value logistic regression was only calculated when χ² P-value was significant.
eThe haplotypes were generated from SNPs rs11545302, rs17552022, rs5030739 and rs4792311, in that order.
fThe haplotypes were generated from SNPs rs486907, rs627928 and rs56250729, in that order.
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Supplementary Information accompanies the paper on Experimental & Molecular Medicine website (http://www.nature.com/emm)

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