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Glutathione-S-Transferase (GST) polymorphisms are associated with relapse after radical prostatectomy

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Abstract

Background—Organ confined prostate cancer (PCa) can be cured by radical retropubic prostatectomy (RRP); however, some tumors will still recur. Current tools fail to identify patients at risk of recurrence. Glutathione-S-Transferases (GSTs) are involved in the metabolism of carcinogens, hormones and drugs. Thus, genetic polymorphisms that modify the GSTs activities may modify the risk of PCa recurrence.

Methods—We retrospectively recruited Argentine PCa patients treated with RRP to study the association between GSTs polymorphisms and PCa biochemical relapse after RRP. We genotyped germline DNA in 105 patients for: *GSTP1* c.313 A>G (p.105 Ile>Val, rs1695) by PCR-RFLP; and *GSTT1* null and *GSTM1* null polymorphisms by multiplex-PCR. Kaplan-Meier curves and Cox proportional hazard models were used to evaluate these associations.

Results—Patients with *GSTP1* c.313 GG genotype showed shorter biochemical relapse-free survival (BRFS) (p=0.003) and higher risk for recurrence in unadjusted (Hazard Ratio (HR)=3.16, 95% Confidence Interval (95% CI)=1.41–7.06, p=0.005) and multivariate models (HR=3.01, 95% CI=1.13–8.02, p=0.028). We did not find significant associations for *GSTT1* and *GSTM1* genotypes. In addition, we found shorter BRFS (p=0.010) and increased risk for recurrence for patients having 2 or more risk alleles when we combined the genotypes of the three GSTs in multivariate models (HR=3.06, 95% CI=1.20–7.80, p=0.019).

Conclusions—Our results give support to the implementation of GSTs genotyping for personalized therapies as a novel alternative for PCa management for patients who undergo RRP.

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Conflict of interest
The authors declare that they have no competing interests.

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This is the first study that examined GST polymorphisms in PCa progression in Argentine men. Replication of our findings in larger cohort is warranted.

Keywords

prostate cancer; biochemical relapse; GST; glutathione-S-transferase; polymorphism

Introduction

Prostate cancer (PCa) is the second most common type of cancer diagnosed in men and the sixth leading cause of cancer-related deaths worldwide. However, the incidence and mortality rates are highly variable between countries and ethnicities.[1] In Argentina, PCa is the most frequent type of cancer and the second cause of death from cancer among Argentine men (http://www.msal.gov.ar/inc/equipos_analisis.php;).[2]

Early detection of PCa has resulted in more men being diagnosed with localized disease. Surgical resection of the entire gland, radical retropubic prostatectomy (RRP), is one therapeutic option with curative purposes for men with organ confined PCa. However, 30–40% of patients will have a biochemical relapse after RRP which may indicate clinical recurrence of aggressive disease. Current clinical indicators of PCa recurrence and mortality after RRP have limited sensitivity and specificity.[3–5] Thus, the ability to establish the risk of progression after surgery is of high importance for patient management and to avoid overtreatment.

The Glutathione-S-Transferases (GSTs) are phase II enzymes involved in detoxification of reactive oxygen species, environmental carcinogens, metabolism of steroid hormones, and metabolism of chemotherapeutic agents.[6] Different GST isoforms have diverse, but overlapping, substrate specificities and have been shown to be highly expressed in the prostate.[7]

Genetic polymorphisms that alter the activity of GSTs may affect the level of hormones and xenobiotics within the prostate which, in turn, may alter the risk of PCa development and progression.

Several studies have been conducted to determine the role of GSTs polymorphisms as risk factors for PCa development;[8,9] but only few sought to analyze the effect of these polymorphisms on PCa progression, with inconclusive results.[10–12] In addition, most studies that analyzed GST polymorphisms have been carried out in Caucasians, few in African-Americans and Asians, and only two were conducted in Hispanics to evaluate the effect of these polymorphisms on the risk PCa development.[13,14] Therefore, the role of GSTs in PCa in the Hispanic population is understudied.

This study is the first to analyze genetic polymorphisms in Hispanic PCa patients from Argentina and to evaluate their effect of these variations on PCa progression. We examined the *GSTT1* null, *GSTM1* null, and *GSTP1* c.313 A>G (p.105 Ile>Val) polymorphisms and their potential association with PCa biochemical relapse in a retrospective cohort of Argentine men who underwent RRP.

Materials and methods

Patients

We designed a hospital-based case-case study to determine the association between GSTs polymorphisms and PCa biochemical relapse after RRP. We retrospectively recruited 105

patients histologically diagnosed with PCa from August 2008 to November 2010 at the *Hospital de Clínicas José de San Martín*, Buenos Aires, Argentina. All patients underwent RRP as their primary therapeutic strategy (date of RRP from December 1998 to July 2010). Patient recruitment, follow-up and maintenance of updated medical records were performed by trained urologists and oncologists. All patients were Argentine citizens, and by definition Hispanics. Most of them had predominant Caucasian ancestry, although as reported for this population, some admixture of Amerindian and African ancestry is to be expected.[15]

The study protocol was approved by the Institutional Ethical Committee and followed the Ethical Principles enunciated by the Declaration of Helsinki. All patients who agreed to participate in the study signed a written informed consent.

Genotyping

Germline DNA was extracted from peripheral blood. We genotyped three polymorphisms in three GST genes: *GSTP1* c.313 A>G (NM_000852.3:c.313A>G; p.105 Ile>Val; rs1695), *GSTT1* null, and *GSTM1* null. The genotyping of *GSTP1* c.313 A>G was performed by PCR-RFLP assay. *GSTT1* null and *GSTM1* null genotypes were assessed by multiplex-PCR reaction. This method allowed us to discriminate the null genotype (homozygote deletion), determined by the absence of band in the electrophoresis, from the heterozygote and homozygote present genotypes. We called the null genotype when there was an absence of a band for either *GSTT1* or *GSTM1* with at least one band for one of the two genes being present (internal PCR control). Samples that did not amplify for both genes were repeated twice or three times to discard a PCR failure. These samples were called null for both *GSTT1* and *GSTM1* only when the following criteria were met: i) all replications were concordant, ii) other samples within the same PCR reaction using the same PCR mix amplified (reaction control), and iii) PCR reactions for double-null samples showed the specific amplicon for other genes (DNA quality control). Details of methods are available online as supplemental information SI1. Genotyping call rates were 98% for *GSTP1*, 99% for *GSTT1* and 98% for *GSTM1*. All PCR reactions were performed in a DNA Engine™ Thermocycler (Bio-rad, California, USA). PCR reactions and digested products were analyzed by 2% agarose (Genbiotech SRL, Buenos Aires, Argentina) gel electrophoresis in 1× TAE buffer (0.8 M Tris; 0.4 M sodium acetate; 0.04 M EDTA; pH 8.3) and dyed with ethidium bromide (Promega, Wisconsin, USA). Gels were photographed and analyzed with the G-Box system (Syngene, USA) and the Genesnap software (Syngene, USA). Samples that failed were repeated once or twice as needed. Genotyping outputs were read by 2 independent laboratory members, and 10–12% of blindly random selected samples were re-analyzed as quality control of the experiments. The results were considered for the final analyses when there was 100% agreement between the two independent readers, and when there was a 100% concordance between samples and blinded repeats.

Statistical analysis

Biochemical relapse was defined as a rise in serum PSA above 0.2 ng/ml after RRP. Univariate and multivariate analyses were conducted using Cox proportional hazard models to study the association between polymorphisms and PCa biochemical relapse and to estimate Hazard Ratios (HR) and 95% Confidence Intervals (95% CI). Multivariate models included margin involvement of the resected prostate, pathologic Gleason score, pathologic T stage, serum PSA level at diagnosis, family history of PCa in first-degree relatives, smoking status and age at diagnosis as covariates. Smoking status was stratified as follows: i) never smokers: patients that never smoke, ii) former smokers: patients that quit smoking at least 1 year prior PCa diagnosis, and iii) current smokers: patients that smoke at the time of PCa diagnosis or patients that quit smoking no more than 1 year prior PCa diagnosis. To study biochemical-relapse-free survival, time was calculated from date of RP to date of

biochemical relapse or last follow up. Kaplan-Meier plots were used to evaluate the association between clinical variables or genotypes and biochemical relapse, and the comparison between groups was done using the log-rank test.

All statistical analyses were carried out using Stata/SE 11.2 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

Results

The clinico-pathological characteristics of the studied patients are shown in Table 1. Twelve (11.4%) patients were diagnosed with PCa with normal serum PSA levels (≤4 ng/ml). Nearly half presented with a Gleason score <7, and 66% of patients with a combined Gleason score ≥7 showed a 7(3+4) score. Most resected prostates showed tumor-free margins (77.5%) and 50.5% of patients were diagnosed with pT2-stage tumors. One-third of patients developed a relapse during follow-up. The median follow-up time for patients without relapse was 84 months (8–156), and 36 months (3–132) for patients who recurred.

Association analyses between clinico-pathological variables and biochemical relapse

Kaplan-Meier curves were used to study biochemical recurrence-free survival (BRFS) across Gleason score categories, pathologic T stage, and margin involvement of the resected prostate, which are known risk factors for biochemical relapse. Gleason score was evaluated using the following categories: ≤6, 7 (3+4), 7 (4+3), and ≥8 (Figure 1A). Considering that the survival curve of patients with a Gleason score of 7 (3+4) is similar to the curve of patients with a score ≤6, we combined these two groups into a low-risk Gleason score. We also combined the Gleason scores 7 (4+3) and ≥8 into a high-risk Gleason score. Analysis of the dichotomized Gleason score showed a statistically significant difference in BRFS (Figure 1B) and was associated with a nearly 2.5-fold increased risk for biochemical relapse for patients with high-risk Gleason score (HR=2.45, 95% CI=1.18–5.09, p=0.016). Advanced tumors (pT3 stage) were also associated with higher risk for developing a biochemical relapse (HR=2.20, 95% CI=1.07–4.52, p=0.032) and shorter BRFS compared to patients with localized PCa (pT2) (Figure 1C). BRFS was also significantly different based on involvement of surgical margins (Figure 1D), and associated with more than 3-fold higher risk of biochemical relapse for positive surgical margins (HR=3.33, 95% CI=1.67–6.62, p=0.001).

A recent study showed that margin involvement predicts biochemical relapse only in intermediate risk disease (PSA=10–20 ng/ml, stage pT2 and/or Gleason score=7).[16] We found that high risk patients (PSA>20 ng/ml, stage pT3 or Gleason score ≥8; stratification according to D'Amico)[3] with tumor-positive margins had shorter BRFS (log-rank p=0.026, data not shown). However, we observed no association with the other two groups, which might be due to small numbers.

Overall, the studied patients followed the current clinical criteria used to evaluate PCa biochemical relapse risk.

Analyses of genotypes and risk of biochemical relapse

Figure 2 shows one agarose gel electrophoresis for the multiplex PCR reaction. The genotypes distributions are shown in Table 2, and were similar to those reported for Caucasians and US Hispanics.

We found that carriers of the null genotypes had slightly shorter BRFS than carriers of the non-null genotypes, albeit these differences were not statistically significant (Figures 3A and 3B for *GSTT1* and *GSTM1*, respectively). Non-statistically significant associations were

found between these polymorphisms and biochemical relapse risk in the unadjusted and multivariate Cox models (Table 3 and supplemental information SI2).

Figure 4 shows one agarose gel electrophoresis for the enzymatic digestion of the *GSTP1* amplicon. The genotype distribution and allelic frequencies are shown in Table 2, and were similar to those reported for the Caucasian and US Hispanic populations.

For the analysis of the *GSTP1* SNP, we first considered an additive model with the c.313 AA genotype (p.105 Ile/Ile, highest enzymatic activity) as reference, the heterozygote genotype as intermediate activity, and the c.313 GG genotype (p.105 Val/Val) as the lowest activity. We observed that carriers of the *GSTP1* c.313 GG genotype (p.105 Val/Val) had shorter BRFS when compared to patients with the *GSTP1* c.313 AA (p.105 Ile/Ile) and c. 313 AG (p.105 Ile/Val, intermediate enzymatic activity) genotypes (Figure 3C). Given that we did not observe meaningful differences between survival of patients with the *GSTP1* c. 313 AA and c.313 AG genotypes, we considered a recessive model in which a modification of the enzymatic activity and phenotype is only obtainable when two G alleles are present. We found that homozygote G patients had shorter BRFS when compared to patients with the AA or AG combined genotypes (Figure 3D) and was associated with a 3-fold higher risk for recurrence in the unadjusted model (Table 3). This association remained statistically significant when the model was further adjusted for margin status, Gleason score, pT stage, PSA level at diagnosis, family history of PCa, smoking status and age at diagnosis (Table 3 and supplemental information SI2). The estimates did not significantly change when further adjusting for *GSTT1* and *GSTM1* genotypes (supplemental information SI2).

Because GSTs are enzymes that typically participate in the same metabolism pathways with overlapping substrate specificity, we considered an additive combined score that captures information on the genotypes of the three GSTs. We stratified the genotypes as follows: 0-risk-allele genotype (*GSTT1* present, *GSTM1* present, and *GSTP1* c.313 AA+AG), 1-risk-allele genotype (*GSTT1* null, *GSTM1* present, and *GSTP1* c.313 AA+AG; or *GSTT1* present, *GSTM1* null, and *GSTP1* c.313 AA+AG; or *GSTT1* present, *GSTM1* present, and *GSTP1* c.313 GG), 2-risk-allele genotype (*GSTT1* null, *GSTM1* null, and *GSTP1* c.313 AA +AG; or *GSTT1* null, *GSTM1* present, and *GSTP1* c.313 GG; or *GSTT1* present, *GSTM1* null, and *GSTP1* c.313 GG), and 3-risk-allele genotype (*GSTT1* null, *GSTM1* null, and *GSTP1* c.313 GG). The genotype distribution is shown in Table 2. Only one patient presented the 3-risk-allele genotype; therefore, he was pooled with the 2-risk-allele group. We found that patients who carried 2 or more (2+) GST risk alleles had shorter BRFS compared to patients with 0 or 1 risk allele (Figure 3E). The unadjusted proportional hazard model showed that patients with 2+ risk alleles had a nearly 3-fold increased risk for biochemical relapse compared to patients with 0 risk alleles (Table 3). This association remained statistically significant after adjustment for other potential risk factors in multivariate models (Table 3 and supplemental information SI2).

Discussion

The clinical course of localized PCa is difficult to predict given that men with similar tumor features can experience strikingly diverse outcomes. Clinicians face the struggle of efficiently identifying high-risk patients given the limited accuracy of currently available staging tools for defining patients at risk of progression to lethal disease. Our study is the first study conducted among Argentine patients to examine the association between GSTs polymorphisms and the risk of PCa biochemical relapse. We found that *GSTP1* c.313 A>G polymorphism and the combined genotype of three GSTs are associated with the risk of recurrence and the time to recurrence in Argentine men with localized PCa.

Whereas many studies have investigated the role of GSTs polymorphisms as risk factors for PCa development,[8,9,13,14] fewer have analyzed the potential role of these polymorphisms on PCa recurrence, progression and PCa-specific death.[10–12] In particular, none of these studies were conducted among Hispanics. Agalliu *et al.* found an increased risk for PCa-specific mortality among Caucasians who have the *GSTM1* null genotype after adjustment for potential confounders (HR=3.76, 95% CI=1.59–8.91); however, the small number of PCa-specific deaths limited the power of the study.[11] No associations with recurrence and disease progression were found.[11] Another study failed to find a statistically significant increased risk for recurrence in Caucasian men for individual polymorphisms (*GSTT1* null, *GSTM1* null and *GSTP1* c.313 A>G) and for the combined genotypes.[10] However, an increased risk for biochemical relapse was found for the *GSTM1* null genotype among patients with high-grade (Gleason score ≥8) or high stage (stage ≥T3a) tumors.[10] In our patient cohort we had few men with Gleason score ≥8; therefore, we were unable to do analyses among high-grade patients. However, overall, our findings are in agreement with the published data given that we found a positive non-significant association between the *GSTM1* and *GSTT1* null genotypes and risk for recurrence and shorter BRFS. This lack of significance might be partly due to the modest number of patients enrolled in our study.

The *GSTP1* c.313 A>G (p.105 Ile>Val) single nucleotide polymorphism (SNP) alters the substrate specificity, activity and thermostability of the GSTpi enzyme.[17] Our results also showed that the *GSTP1* c.313 A>G (p.105 Ile>Val) SNP is associated with the risk of biochemical relapse, and patients carrying the *GSTP1* c.313 GG genotype were at higher risk of recurrence. Two other studies that investigated this polymorphism did not find statistically significant associations with PCa recurrence.[10,11] These discrepancies might be partially due to the fact that the latter studies included Caucasian men, whereas our patients are Hispanic. Our results showed that the genotype frequencies among these Argentine men are more similar to Caucasians from Spain and Italy than to US Hispanics and Hispanics from Chile, in agreement with the large proportion of Spanish and Italian ancestry among Argentines from Buenos Aires, from where patients in our study came from. However, Argentines, as all Hispanics, have an admixed genetic background that includes Europeans, Amerindians, and African ancestries.[15] Our study did not include genetic ancestry estimation using Ancestry Informative Markers (AIMs); therefore, we were unable to adjust for potential confounding by population admixture. Our study did not include genetic ancestry estimation using ancestry informative markers (AIMs); therefore, we were unable to adjust for potential confounding by population admixture.

We also found that patients with more than two risk alleles of the combined *GSTT1*, *GSTM1* and *GSTP1* polymorphisms were at higher risk for developing a biochemical relapse. These results agree with the results reported by Nock *et al.* who found that Caucasian men with more than two GST risk alleles and more aggressive tumors (Gleason score ≥8 or pT stage ≥3a) had an increased risk for recurrence.[10]

The primary strength of our study is the inclusion of patients who underwent RRP without neoadjuvant therapy, which avoided the potential confounding effect of other types of treatment. Key limitations are the modest number of patients included, the possible long-survivorship bias due to the retrospective study design, and that eight men were only followed for periods shorter than the median time to relapse (36 months) and might develop a biochemical relapse later on. Moreover, we did not evaluate *GSTP1* promoter methylation, which is a frequent epigenetic event in PCa and is suggested to be a predictor for PCa recurrence.[18] It was reported that serum *GSTP1* hypermethylation was detected in 15% of the patients who recurred.[18] Therefore, it might be challenging to clearly determine the role of the *GSTP1* SNP in biochemical relapse without considering methylation status.

GSTT1 null, *GSTM1* null and *GSTP1* c.313 A>G polymorphisms might be relevant predictors of biochemical relapse of PCa in the Argentine population. However, these results need to be validated with a larger patient cohort. Our results give support to the inclusion of molecular markers into clinical practice in an effort to better classify men with localized PCa according to their risk of progression, which may lead to tailored post-surgery therapies and, therefore, will avoid overtreatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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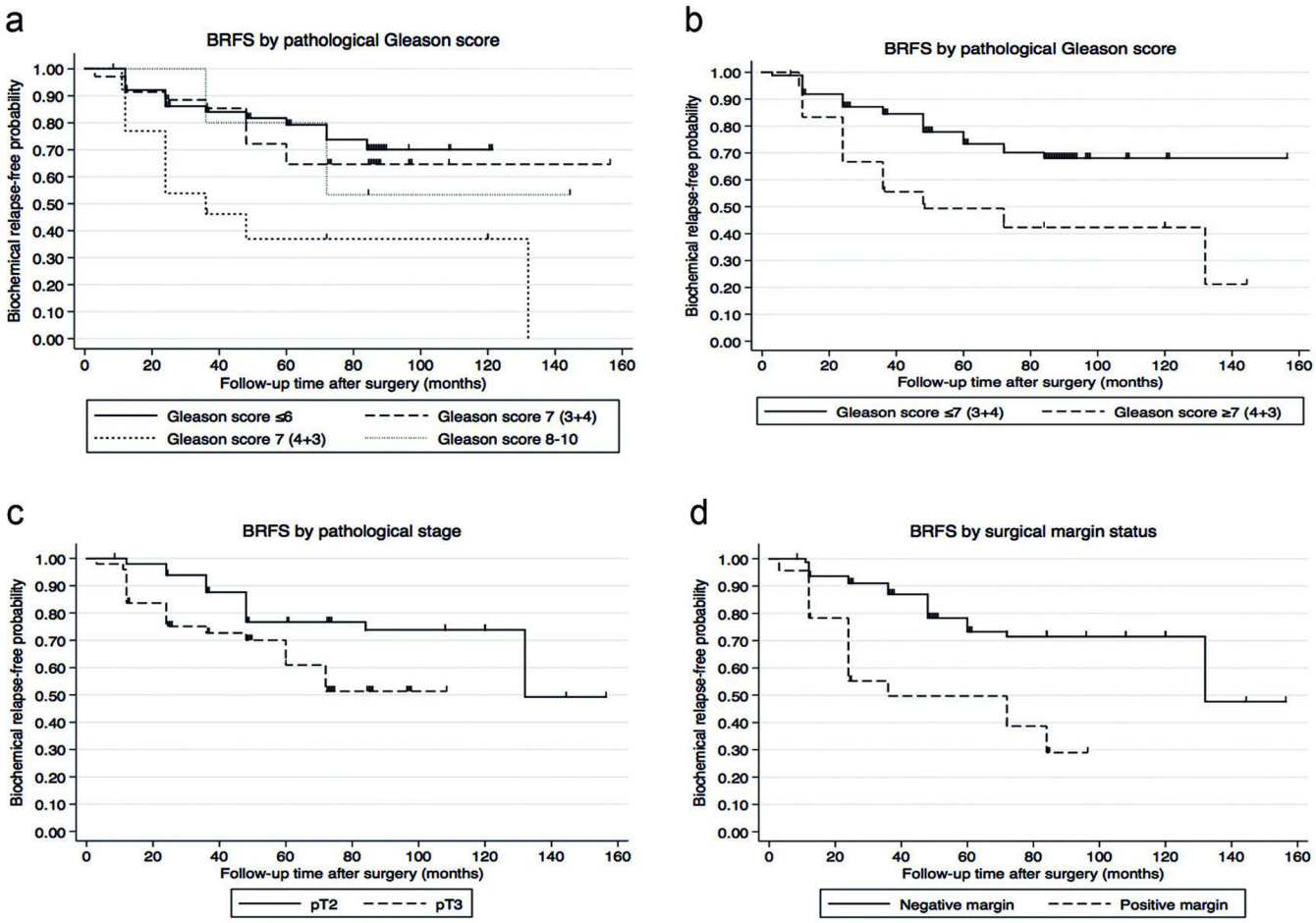

Figure 1. Biochemical-relapse free survival analysis by different clinico-pathological characteristics

To study biochemical-relapse free survival (BRFS), time was calculated from date of RRP to date of biochemical relapse or last follow up. The figure depicts the Kaplan-Meier survival analysis by: a) Gleason score (log-rank p=0.016), b) dichotomous Gleason score (log-rank p=0.011), c) pathological T stage (log-rank p=0.024), and d) margin status (log-rank p<0.001).

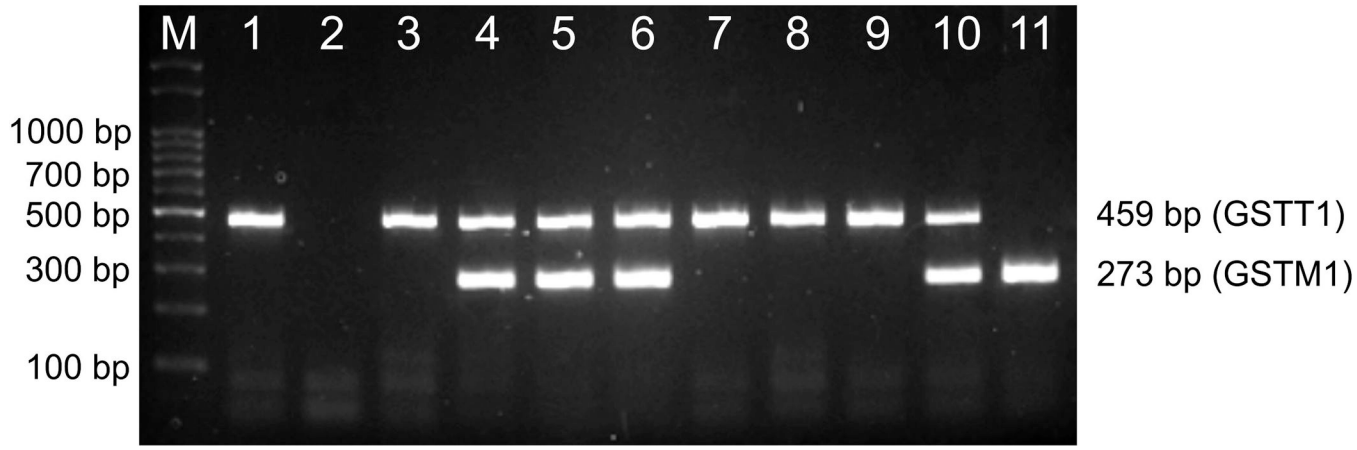

Figure 2. Agarose gel electrophoresis of the multiplex PCR reaction for *GSTT1* and *GSTM1* genotyping

The figure depicts an example of one 2% agarose gel electrophoresis dyed with ethidium bromide used to determine the *GSTT1* and *GSTM1* genotypes by multiplex PCR. Lanes 1, 3, 7, 8 and 9: *GSTT1* present/*GSTM1* null; lane 2: *GSTT1* null/*GSTM1* null; lanes 4, 5, 6 and 10: *GSTT1* present/*GSTM1* present; lane 11: *GSTT1* null/*GSTM1* present; M: 100 bp marker (Productos Bio-Lógicos, Buenos Aires, Argentina). Samples that did not amplify for both genes were repeated twice or three times to discard a PCR failure.

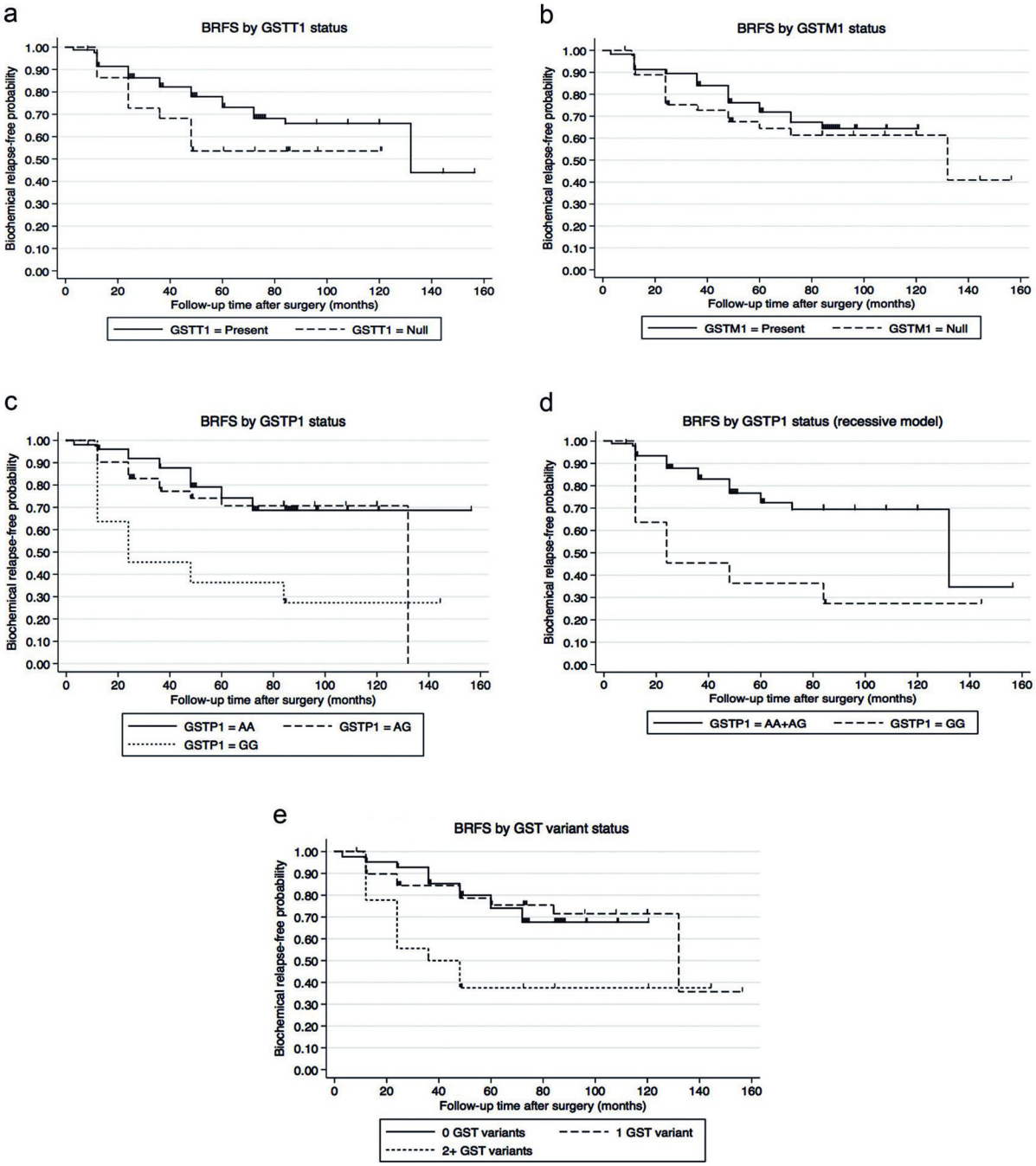

Figure 3. Biochemical-relapse free survival analysis by GST genotype

To study biochemical-relapse free survival (BRFS), time was calculated from date of RRP to date of biochemical relapse or last follow up. The figure depicts the Kaplan-Meier survival analysis by genotypes: a) *GSTT1* (log-rank p=0.1480), b) *GSTM1* (log-rank p=0.4901), c) *GSTP1* co-dominant model (log-rank p<0.010), d) *GSTP1* recessive model (log-rank p=0.003), and e) GST combined genotype using the *GSTP1* recessive model (log-rank p=0.010).

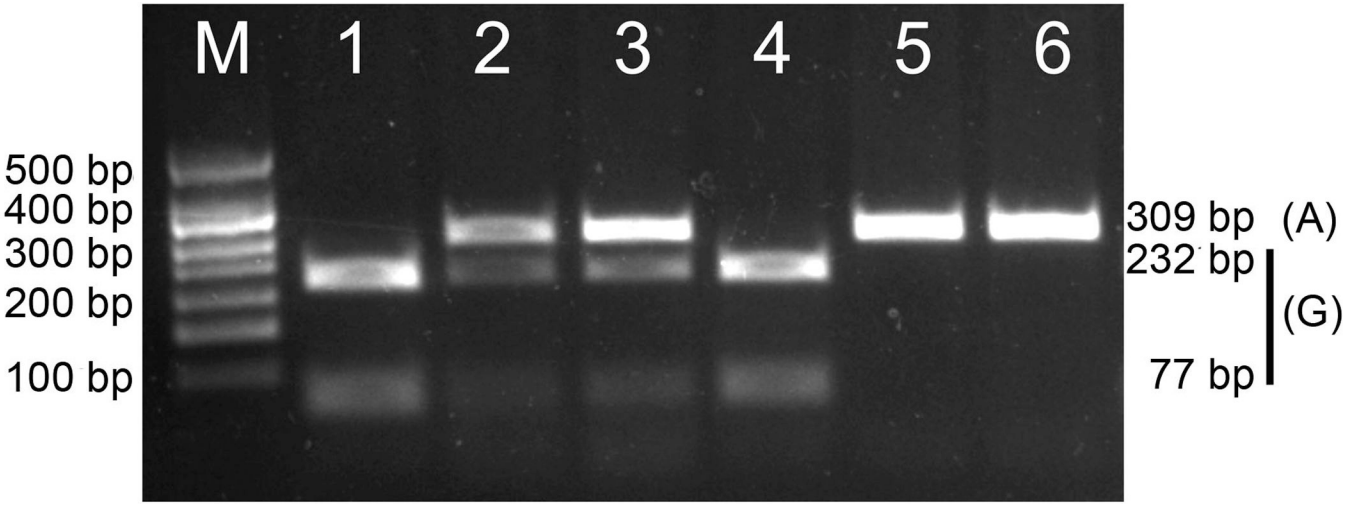

Figure 4. Agarose gel electrophoresis of GSTP1 amplicons digested with Alw26I restriction enzyme

The figure shows an example of one 2% agarose gel electrophoresis dyed with ethidium bromide used to determine the *GSTP1* genotype by PCR-RFLP. Lanes 1 and 4: c.313 GG; lanes 2 and 3: c.313 AG; lanes 5 and 6: c.313 AA; M: 50 bp marker (Genbiotech, Buenos Aires, Argentina).

Table 1

Clinico-pathological characteristics of the study group.

Variables	N	Percentage
Total cases	105	100.0
Age at diagnosis (years old) (median, range)	65	49 – 74
PSA at diagnosis (ng/ml) (median, range)	6.87	0.77 – 28.90
≤4	12	11.4
>4 – 10	63	60.0
>10	30	28.6
Smoking status		
Never smoker	29	27.6
Former smoker	56	53.3
Current smoker	20	19.1
Family History of PCa		
No	88	83.8
Yes	17	16.2
Pathological Gleason score		
5	6	5.7
6	46	43.8
7 (3+4)	35	33.3
7 (4+3)	13	12.4
8	5	4.8
Pathological T stage		
pT2	50	50.5
pT3a	47	47.5
pT3b	2	2.0
missing	6	
Risk group for biochemical relapse[a]		
Low	26	26.3
Intermediate	21	21.2
High	52	52.5
missing	6	
Weight of resected prostate (g) (median, range)	45	10 – 143
Missing data	14	
Tumor Volume (mm^3) (median, range)	1900	12 – 18000
Missing data	74	
Margin involvement of the resected prostate		

Variables	N	Percentage
No	79	77.5
Yes	23	22.5
Missing data	3	
Biochemical Relapse		
No	70	66.7
Yes	35	33.3

[a] risk groups were defined according to D'Amico as follows: low risk (PSA<10 ng/ml, pT2 stage and Gleason score ≤ 6), intermediate risk (PSA=10–20 ng/dL and pT2 stage and/or Gleason score 7), high risk (PSA>20 ng/dL or pT3 stage or Gleason score ≥ 8)[3].

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Table 2

Genotypic distribution and comparison with other populations.

Genotype	N	Percent	Reported percentage in US Hispanics[a]	Reported percentage in Hispanics from Chile[b]	Reported percentage in Caucasians[c]
Total cases	105	100.0	-	-	-
GSTT1 null					
Present	82	78.8	86	89–94	71–85
Null	22	21.2	14	6–11	15–29
Missing data	1				
GSTM1 null					
Present	57	55.3	57	64–77	52–55
Null	46	44.7	43	23–36	45–48
Missing data	2				
GSTP1 c.313 A>G (p.105 Ile>Val)					
AA (Ile/Ile)	51	49.5	52	NA	48–50
AG (Ile/Val)	41	39.8	35	NA	30–42
GG (Val/Val)	11	10.7	13	NA	10–22
A (Ile)	143	69.4	NA	NA	NA
G (Val)	63	30.6	NA	NA	NA
Missing data	2				
GSTT1 null + GSTM1 null + GSTP1 c.313 A>G [d]					
0 risk allele	42	42.0	NA	NA	NA
1 risk allele	40	40.0	NA	NA	NA
2 risk alleles	17	17.0	NA	NA	NA
3 risk alleles	1	1.0	NA	NA	NA
Missing data	5				

[a]Hispanic genotype frequency ranges were obtained from dbSNP (accessed in August 2012; http://www.ncbi.nlm.nih.gov/snp) for HISP1 population and a report by Block *et al.*.[19]

[b] Hispanic from Chile genotype frequency ranges reported by Acevedo *et al.*,[13] and Caceres *et al.*.[14]

[c] Genotype frequency ranges for Caucasians with Spanish and Italian ancestry were obtained from dbSNP (accessed in August 2012; http://www.ncbi.nlm.nih.gov/snp) for CAUC1 and AFD_EUR_PANEL populations, and reports by To-Figueras *et al.*,[20] Ladero *et al.*,[21] Raimondi *et al.*,[22] and Agudo *et al.*.[23]

[d] for GSTP1 c.313 A>G the recessive model was considered

Abbreviations: NA, not available

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Table 3

Unadjusted and multivariate Cox proportional hazard models for biochemical relapse by GST genotypes.

Genotypes	HR (95% CI)	HR_{adj}[a] (95% CI)
GSTT1		
Present	1.00 (reference)	1.00 (reference)
Null	1.69 (0.81–3.53)	2.05 (0.92–4.54)
p-value	0.164	0.078
GSTM1		
Present	1.00 (reference)	1.00 (reference)
Null	1.26 (0.64–2.47)	0.97 (0.47–2.01)
p-value	0.503	0.937
GSTP1 c.313 A>G		
AA+AG	1.00 (reference)	1.00 (reference)
GG	**3.16 (1.41–7.06)**	**3.01 (1.13–8.02)**
p-value	**0.005**	**0.028**
GSTP1 c.313 A>G + GSTT1 null + GSTM1 null[b]		
0 risk allele	1.00 (reference)	1.00 (reference)
1 risk allele	0.97 (0.43–2.21)	0.74 (0.30–1.84)
p-value (1 *vs* 0)	0.994	0.512
2+ risk alleles	**2.82 (1.23–6.49)**	**3.06 (1.20–7.80)**
p-value (2+ *vs* 0)	**0.015**	**0.019**

Statistical significant associations are bolded.

[a] adjusted for margin, Gleason score (low-risk *vs* high-risk), pathological T stage (pT2 *vs* pT3), PSA level at diagnosis (≤4 *vs* >4–10 *vs* >10), family history of PCa, smoking status (never *vs* former *vs* current) and age at diagnosis (continuous variable)

[b] for GSTP1 c.313 A>G the recessive model was considered

A Supplementary Table SI2 is available online showing the results of the other models tested.