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Original Article

Lack of Association between Glutathione S-Transferase -M1 and -T1 Gene Polymorphisms with Clinicopathological Parameters in Prostate Cancer

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Abstract

Glutathione S-transferase (GST) enzymes play a role in detoxification of several carcinogens. Inherited absence of both alleles are common in human in two subtypes of GST gene superfamily, -M1 and -T1, by varying frequencies among different populations. The absence of both alleles result in loss of enzymatic activity and are related to increased risk for development of various cancer types. In this study, we investigated the association of GSTM1 and GSTT1 genetic profiles with clinicopathological parameters in prostate cancer. GSTM1 and GSTT1 genotypes were assessed by multiplex PCR and high resolution melting curve analysis method in 162 patients that underwent radical prostatectomy for localised prostate cancer. Their association with prognostic parameters were analysed. Frequencies of GSTM1 null and GSTT1 null genotypes were 82/162 (50.6%) and 33/162 (20.4%), respectively. No any significant differences were observed between different genotype groups and clinicopathological parameters including Gleason score, pathological stage, tumor volume, surgical margin status, extraprostatic extension, seminal vesicle invasion, perineural invasion or patient age. Although the roles of GSTM1 and GSTT1 deletions on prostate cancer risk was indicated in Turkish population, our results revealed that null or wild type genotypes of GSTM1 and GSTT1 genes are not associated with prognostic parameters in prostate cancer.

Introduction

Glutathione S-transferase (GST) gene superfamily are cancer-susceptibility genes and they encode GST enzymes, which play a key role in detoxification of several carcinogens such as electrophilic xenobiotics or aliphatic aromatic heterocyclic radicals present in tobacco (1). The gene superfamily consisted of eight classes of genes known as α (GSTA), μ (GSTM), θ (GSTT), π (GSTP), σ (GSTS), κ (GSTK), o (GSTO), and τ (GSTZ) (2). Genetic polymorphisms or deletions of GST genes may result in decreased enzymatic activity, that lead to a loss in the cancer prevention mechanisms. Therefore, many members of the GST genes have been implicated to act as tumor supressor genes. Molecular alterations affecting the carcinogen metabolisms are usually seen as single nucleotide polymorphisms, whereas, complete deletion of a gene is rare. However, two members of the gene superfamily, GSTT1 and GSTM1 genes, have been attracted the researchers, because homozygous deletions of two alleles (null genotype) are relatively common in these genes.

GSTT1 null or GSTM1 null genotypes are potential risk markers for several cancers such as lung, bladder, and head & neck cancers (3). The results of studies in prostate cancer with regard to these genes are confusing. While some publications indicate that null genotypes are predisposing risk factors for prostate cancer (4-6), others do not provide a such association (7-8).

Although the association of GST gene deletions and prostate cancer risk has been studied by many, their prognostic values or association with histopathologic

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parameters are not well documented. Prostate cancer is a complex, heterogeneous disease by its molecular, histological and clinical aspects. Specific molecular alterations may reflect distinct biologic and histological features, which may have clinical implications. In the present study we investigated the utility of deletions in these genes as possible prognostic markers by their correlation with histopathologic parameters in radical prostatectomy specimens.

In the majority of the earlier studies, conventional PCR and subsequent gel electrophoresis was used for demonstration of GSTM1 and GSTT1 null genotypes. Since those methods are time consuming and shorter amplicons are more suitable for DNA derived from formallin fixed paraffin embedded (FFPE) tissues, we used a multiplex PCR and high resolution melting curve (HRMC) analysis method to screen null and wild type genotypes.

Material and Methods

Patients

The study was performed in accordance with Declaration of Helsinki and approved by institutional ethics comittee. We selected 162 eligible prostate cancer patients that underwent radical prostatectomy between years 2001 and 2011. Clinical data for patient demographics were obtained from hospital records. Mean patient age was 60.4 years old (range 49 - 77 years). Hematoxylin eosin stained sections of the corresponding prostatectomy specimens were reviewed for evaluation of tumor characteristics. Gleason score (GS), tumor volume (TV), pathological tumor stage (pT), extraprostatic extension (EPE), perineural invasion (PNI), seminal vesicle invasion (SVI), and presence of positive surgical margin (PSM) were recorded by a single pathologist during the reevaluation. Modified ISUP 2005 Gleason scoring system was used for reevaluating histological tumor grades. In the study group, mean TV was 3.32 cm³ and median GS was 7. Seventy nine patients (48.8%) had a GS of ≤ 6, sixty nine patients (42.6%) had score 7, and a subset of 14 patients (8.6%) had score ≥ 8 acinary adenocarcinomas. The majority of cases (n=136, 84.0%) had localised (pT < 3), and a subset of 26 patients (16.0%) had advanced stage (pT ≥ 3) disease. EPE was seen in 13 (8.0%), PSM was observed in 27 (16.7%), SVI was seen in 6 (3.7%), and lymph node metastasis was detected in 2 (1.2%) patients.

DNA extraction and genotype analysis

Genomic DNA was isolated from FFPE nontumoral prostatic tissues, by using QIAamp DNA FFPE tissue kit (Qiagen) according to the manufacturer's instructions. PCR amplifications were carried out on RotorGene Q 5-Plex (Qiagen) in a final volume of 50 μL containing 100 ng of genomic DNA as described before (9) by using the same primer sets. After amplification steps, generated HRMC and melting curves were visually compared to identify different melting patterns of GSTM1 and GSTT1 amplicons.

With this approach both amplicons served as an internal control in each tube for PCR efficiency. Whenever an amplification was not observed (ie. null - null genotype), seperate PCR amplifications were performed for both genes using glyceraldehyde 3-phosphate dehydrogenase gene as an internal control to evaluate the DNA integrity.

Statistical analysis

Results are presented as mean ± standart deviation for continious variables, and defined as percentages for cathegorical variables. We used 't test' to compare the distributions of continious variables and chi squared tests to analyse the differences of incontinious variables between null and wild type groups for both genes. Fischer's exact test was also used where appropriate. Histologic tumor grades were divided into three subgroups prior to statistical analysis as GS ≤ 6, GS = 7, and GS ≥ 8. Pathological tumor stages were also stratified as pT ≤ 2b, pT = 2c, and pT ≥ 3 to analyse whether different genotypes are associated with more aggressive behaviour. Statistical analyses were performed using 'SPSS for Windows software version 11.0'. All p values were two-tailed, and p < 0.05 was considered as significant.

Results

The results of histopathologic evaluation and genotype analysis of GSTM1 and GSTT1 genes are summarised in Table 1. The frequencies of GSTM1 null genotypes were 50.6% (82/162), and GSTT1 null genotypes were 20.4% (33/162) in the patient cohort. Only two patients were found to have loss of two alleles in both genes. As shown in Figure 1, melting curves revealed two peaks in the presence of GSTM1 and GSTT1 amplicons. Since visual discrimination of different genetic profiles were easier by HRMC analysis, we used normalised and subtracted df/dt graphs of HRMC analysis to identify GSTM1

Table 1. Comparison of selected clinicopathological variables in groups of different GSTM1 and GSTT1 genotypes.

	GSTM1-	GSTM1+	p	GSTT1-	GSTT1+	p	All
n (%)	82 (50.6)	80 (49.4)		33 (20.4)	129 (79.6)		162 (100)
Age [a]	62.4 ± 5.4	62.3 ± 6.2	0.98	62.5 ± 5.9	62.4 ± 5.7	0.92	62.4 ± 5.8
TV [a]	3.4 ± 4.6	3.3 ± 4.6	0.85	3.2 ± 3.7	3.4 ± 4.8	0.91	3.3 ± 4.6
PSM [b]	13 (15.9)	14 (17.5)	0.78	6 (18.2)	21 (16.3)	0.79	27 (16.7)
SVI [b]	3 (3.7)	3 (3.8)	1.00	1 (3.0)	5 (3.9)	1.00	6 (3.7)
PNI [b]	62 (75.6)	52 (65.0)	0.11	22 (66.7)	92 (71.3)	0.45	114 (70.4)
EPE [b]	8 (9.8)	5 (6.3)	0.41	2 (6.1)	11 (8.5)	1.00	13 (8.0)
GS [b]							
≤6	41 (50.0)	38 (47.5)	0.78	15 (45.5)	64 (49.6)	0.91	79 (48.8)
7	33 (40.2)	36 (45.0)	0.78	15 (45.5)	54 (41.9)	0.91	69 (42.6)
≥8	8 (9.8)	6 (7.5)	0.78	3 (9.1)	11 (8.5)	0.91	14 (8.6)
Pathological Stage b							
T2a-2b	34 (41.4)	35 (43.8)	0.94	12 (36.4)	57 (44.2)	0.41	69 (42.6)
T2c	35 (42.7)	32 (40.0)	0.94	17 (51.5)	50 (38.8)	0.41	67 (41.4)
≥ T3	13 (15.9)	13 (16.2)	0.94	4 (12.1)	22 (17.0)	0.41	26 (16.0)

[a] Data are given as mean ± standard deviation.

[b] Data are given as number of patients (% of patients).

Table 2. Distributions of prognostic parameters in groups with respect to combined GST genotypes.

Prognostic parameters	GST genotypes				
	M1+/T1+ n: 49 (30.2%)	M1+/T1- n: 31 (19.1%)	M1-/T1+ n: 80 (49.4%)	M1-/T1- n:2 (1.2%)	p
Age [a]	62.2 ± 6.29	62.6 ± 6.00	62.5 ± 5.43	60.0 ± 2.83	0.94
TV [a]	3.26 ± 5.09	3.26 ± 3.84	3.40 ± 4.61	3.00 ± 4.57	0.98
PSM, n (%)	8 (16.3)	6 (19.4)	13 (16.3)	0	0.98
SVI, n (%)	2 (4.1)	1 (3.2)	3 (3.8)	0	0.92
PNI, n (%)	37 (75.5)	24 (77.4)	52 (65.0)	1 (50.0)	0.29
EPE, n (%)	3 (6.1)	2 (6.4)	8 (10.0)	0	0.69
GS, n (%)					
≤6	24 (49.0)	14 (45.2)	40 (50.0)	1 (50.0)	0.92
7	22 (44.9)	14 (45.2)	32 (40.0)	1 (50.0)	0.92
≥8	3 (6.1)	3 (9.7)	8 (10.0)		0.92
Pathological stage, n(%)					
T2a-2b	24 (49.0)	11 (35.5)	33 (41.3)	1 (50.0)	0.57
T2c	16 (32.6)	16 (51.6)	34 (42.5)	1 (50.0)	0.57
≥T3	9 (18.4)	4 (12.9)	13 (16.3)		0.57

[a] Data are given as mean ± standard deviation.

and GSTT1 genotypes in our study. An example of HRMC analysis result used to identify GSTM1-/GSTT1+, GSTM1+/GSTT1-, and GSTM1+/GSTT1+ genotypes are demonstrated in Figure 2.

Mean patient ages were similar for all groups. EPE rate was slightly higher in GSTM1 null genotypes (9.8% vs 6.3%) but did not reach the significancy level (p=0.41). A slight but nonsignificant difference was also observed in PNI rates between GSTM1 wild type and null genotype groups (p=0.11). With respect to PSM, SVI, or TV, the differences between null and wild type genotypes for both gene groups were far from statistical significi-
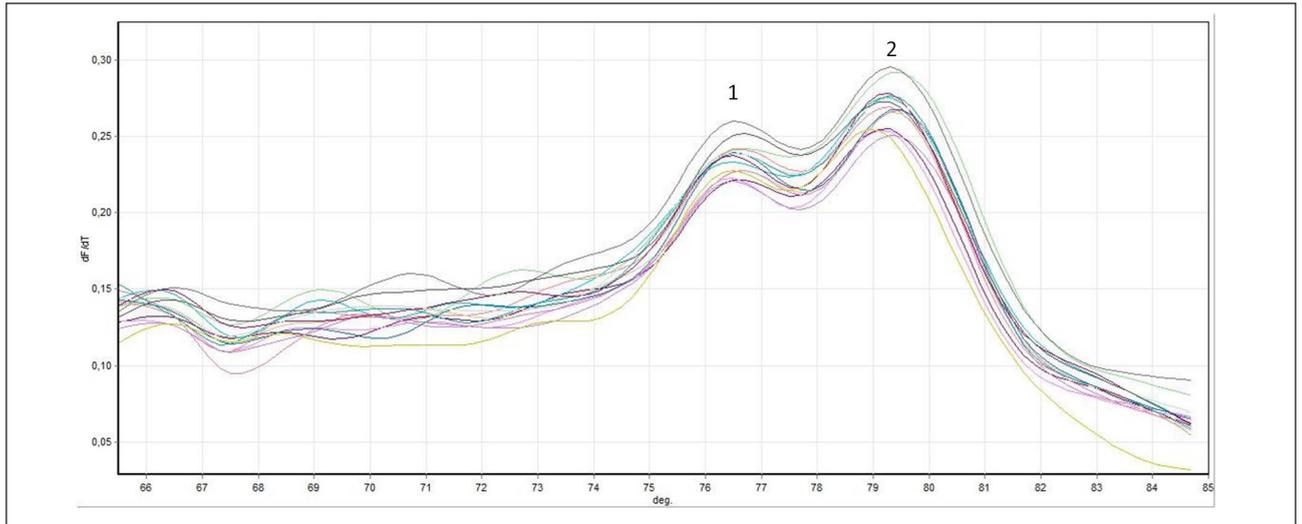
Figure 1. Melting curves generated after multiplex PCR. Different melting points enable the identification of GSTM1 (peak 1) and GSTT1 (peak 2) amplicons.
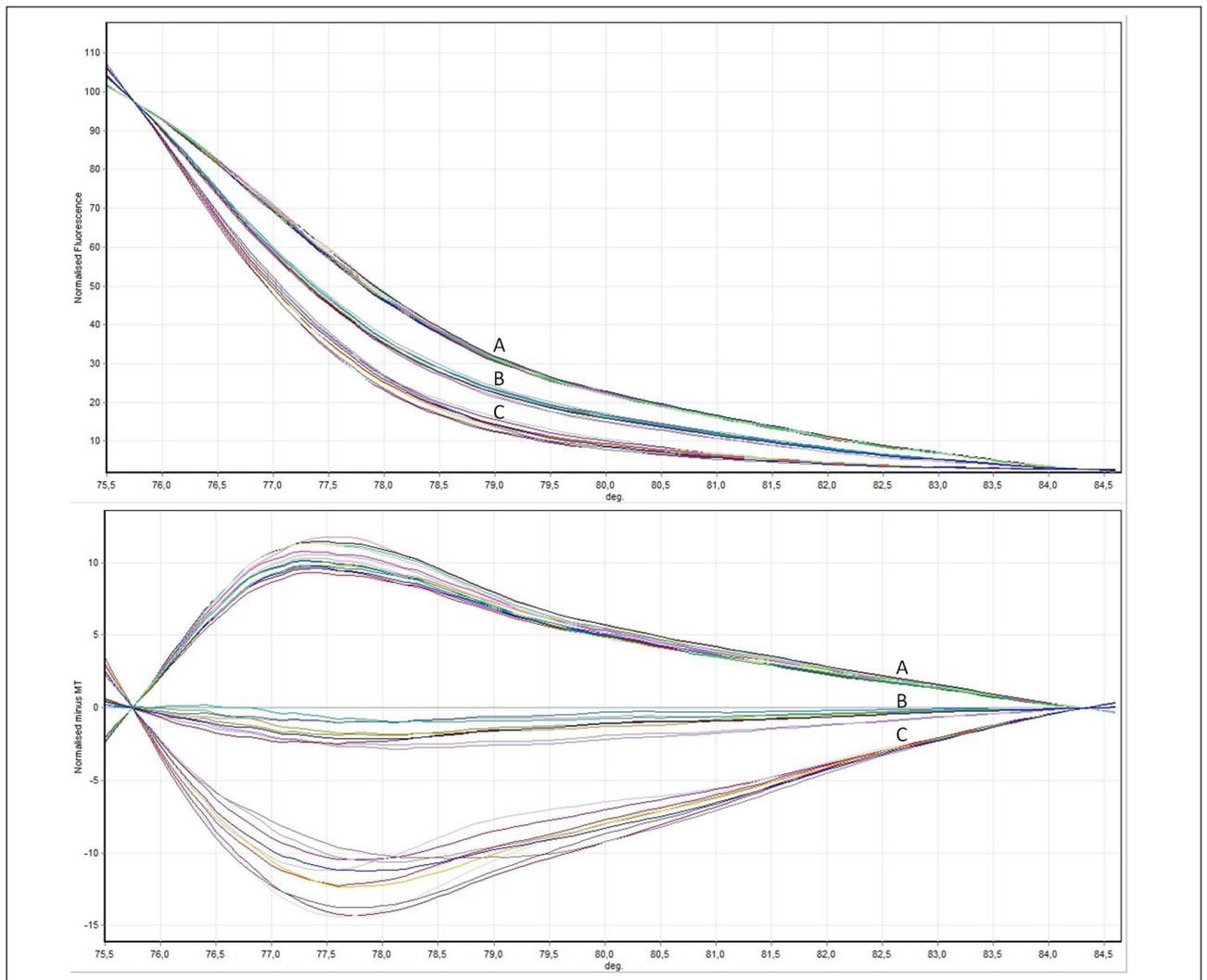

Figure 2. High resolution melting curves generated after multiplex PCR for screening GSTM1 and GSTT1 genotypes. Up; normalised graph, down; subtracted graph (Normalised minus GSTM1+/GSTT1+ genotype). 'A' corresponds to presence of only GSTT1 amplicons (GSTM1 null), 'B' to GSTM1+/GSTT1+ genotype, and 'C' to presence of only GSTM1 amplicons (GSTT1 null).

cance (all p>0.5). Stratification of patients according to GS and pathological tumor stage did not show any statistically significant differences across any of the subgroups.

Statistical analysis for combined genotypes of GSTM1 and GSTT1 genes also did not reveal any significant differences for selected prognostic parameters between different groups (Table 2).

Discussion

The incidence of prostate cancer varies in a wide range between different ethnic / social groups worldwide, however, little is known about the causes of racial differences (10). Genetic factors are implied to be one probable reason for this difference. Polymorphisms in metabolic pathways is one of the major subjects that researchers have investigated in an effort to clarify polygenic models of cancer development. Unfortunately, the effects and interactions of low penetrence genes in cancer susceptibility is largely unknown due to the requirement of thousands of patients to identify their roles. GST genes belong to such low penetrence genes and they play a role in carcinogenesis of various tumor types. Previous studies revealed that about 50% of the Caucasians lack the GSTM1 and 20% lack the GSTT1 genes due to the inherited loss of both alleles (2). Both genes encode GST enzymes which are involved in the metabolic detoxification of several carcinogenes. For prostate cancer, their involvement in metabolism and intracellular transportation of steroid hormones provide additional support for these genes to may have a role in prostate carcinogenesis (11).

Hence hypothecising that specific molecular alterations may be related to distinct behavioral or histological features, we conducted the present study to investigate whether two common deletions in GST gene superfamily associate with clinicopathological parameters in prostate cancer. In the current literature, although only a few investigated the relationship between null genotypes of GSTM1 and GSTT1 genes and clinicopathologic parameters in prostate cancer, results of those studies revealed some significant associations. Medeiros et al., reported that GSTT1 null genotype was associated with advanced stage disease in prostate cancer patients (12). Mittal RD et al., found that ages between 50-60 years exhibited significant variation between the control and the cancer patients for GSTT1 and GSTM1 null genotypes (13). Chen et al., also observed that the prostate can-

cer patients with a GSTM1 null genotype were younger than those with the GSTM1 wild type genotypes, and reported the association of GSTM1 null genotype and early age of onset (14). A more recent study also demonstrated that GSTM1 null and GSTT1 null genotype was observed in a higher frequency in patients with Gleason score >7 (15). In our patient cohort, we evaluated the relationship between null genotypes and tumor characteristics by histopathologic analysis of corresponding radical prostatectomy specimens. As best of our knowledge the present study is the first to investigate such association between GSTM1 and GSTT1 null genotypes and tumor characteristics in radical prostatectomy specimens in Turkish population. However, we were unable to demonstrate any significant relationship between genetic profiles of both genes and selected clinicopathological variables.

On the other hand, we have to take into consideration that the wild type genotypes are consisted of both heterozygote (+/-) and homozygote (+/+) patients in our study. Discrimination of (+/-) and (+/+) genotypes may reveal significant differences which would be masked in the present study design. Such discrimination was reported to highlight the association between GSTM1 and GSTT1 genotypes in colorectal adenomas (16). Therefore, effects of having one or two alleles should be identified with further projects to clarify their association with prognostic parameters in prostate cancer.

In conclusion, in contrast to previous reports, our results revealed that no genotypes of GSTM1 and GSTT1 genes were associated with either high Gleason score, high tumor volume, advanced stage, involvement of extraprostatic tissues, surgical margin positivity, nor patient age in prostate cancer, and suggest that these genotypes may have no impact on patient prognostication. Furthermore, the lack of association found in this study also supports the view that GSTM1 null or GSTT1 null genotypes are not major determinants for prostate cancer development.

Conflict of Interest

No conflict of interest was declared by the authors.

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