HSPA1A rs1043618 and rs562047 polymorphism analysis and the assessment of their effect on tumor pathomorphological parameters and breast cancer patient prognosis

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Objective

Breast cancer became the most common cancer in women worldwide. There is a number of studies aiming to analyze different genetic variants and their effect on cancer phenotype and prognosis. Recently Heat shock proteins (HSPs) attracted scientific attention. HSPs participate in protein folding under stressors such as hypoxia, heat shock, and degradation process. HSPs also play a role across various types of cancers as they are implicated in cancer-related activities such as cell proliferation and metastasis. HSPs overexpression has been observed in various cancers such as ovarian, gastric, breast, colon, lung, and prostate cancers, however the data concerning germline HSP and carcinogenesis is limited. The aim of this study was to analyze the contribution of *HSPA1A* rs1043618 and rs562047 polymorphisms to tumor phenotype and breast cancer patient prognosis.

Methods

- This is a retrospective study, involving 100 breast cancer patients.
- The study research protocol was approved by Kaunas Regional Biomedical Research Ethical Committee (protocol number BE-2-10 and BE-2-10/2014).
- Patient blood samples, acquired by clinicians in a time-frame from 2014-2016, were utilized for the genomic DNA extraction. rs1043618 and rs562047 polymorphisms were analyzed with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. RFLP result are presented in Figure 1-2.
- Patient clinical information (the age at diagnosis, pT, pN, G, ER, PR, HER2, disease outcome parameters (PFS, MFS and OS)) was collected from clinical records. The statistical analysis was performed using IBM "SPSS".

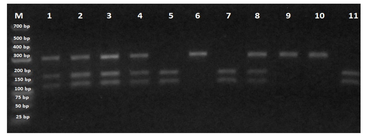

Fig. 1 Agarose gel electrophoresis for rs1043618 polymorphism analysis.
Lane M - DNA molecular marker GeneRuler Ultra Low Range DNA Ladder (Thermo Fisher Scientific Baltics, Lithuania); Lanes 6 ,9, and 10 -GG genotype; Lanes 1-4, 8 -GC genotype; Lane 5,7 and 11 CC genotype

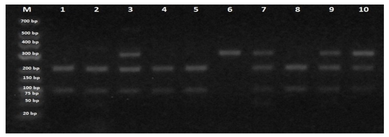

Fig.2. Agarose gel electrophoresis for rs562047 polymorphism analysis.
Lane M - DNA molecular marker GeneRuler Ultra Low Range DNA Ladder (Thermo Fisher Scientific Baltics, Lithuania); Lanes 1-2 ,4-5 and 8 CC genotype; Lanes 3,7, 9-10 -CG genotype; Lane 6 -GG genotype.

Results

- In our study the distribution of tumor pathomorphological parameters is presented in Table 1
- During a follow-up period, 26% of patients experienced distinct organ metastasis, 31% – local progress, 22% - deaths. The median follow-up of patients was 115 months.
- In our study, two polymorphisms in *HSPA1A rs1043618, rs562047* genes were analyzed. In *rs1043618* the distribution of genotypes was as follows: GG- 7%, CG- 54%, CC- 39%. In *rs562047* the distribution of genotypes was as follows: GG - 4%, CG - 25%, CC - 71%.
- The possible associations between *HSPA1A rs1043618* and *rs562047* polymorphisms and BC patient survival were assessed using Kaplan-Meier analysis (Log Rank test). No significant link between these SNP and PFS, MFS and OS were determined in both the genotype and allelic model.
- The association between the selected SNP's (genotype and allele model) and tumor pathomorphological characteristics (ER, PR, HER2 status, G, T, N, L, V) was investigated. There was no significant links determined between the analyzed rs1043618 and rs562047 polymorphisms (genotype and allelic model) and tumor pathomorphological characteristics (Table 2-3).

Characteristics	Subgroup and frequencies (%)
Age group	30-40 years – 35%, 41-50 years – 65%
Estrogen receptors (ERs)	ER negative - 43%, ER positive - 57%
Progesterone receptors (PRs)	PR negative - 52%, PR positive - 48%
Human epidermal growth factor receptor 2	HER2 negative - 78%, HER2 positive - 22%
Pathological lymph node involvement (N)	N0 - 54%, N1 - 46%
Tumor grade (G)	G1- 71%, G2 - 29%
Pathological tumor size (T)	T1 - 66%, T2 - 34%

Table 1 . The clinicopathological characteristics of the study group

SNP	Genotype or alleles	ER			PR			HER2		
		Odds	95% CI	p	Odds	95% CI	p	Odds	95% CI	p
HSPA1A rs1043618	CG versus CC	0.631	0.276-1.440	0.274	0.958	0.411-2.235	0.921	0.806	0.304-2.134	0.664
	GG versus CC	1.475	0.255-8.521	0.664	1.578	0.291-8.549	0.597	1.243	0.208-7.448	0.811
	The carriers of C allele versus the non-carriers	2.416	0.298-19.586	0.409	0.943	0.135-6.583	0.953	1.578	0.264-9.419	0.617
	The carriers of G allele versus the non-carriers	0.360	0.070-1.838	0.219	0.340	0.072-1.594	0.171	0.611	0.128-2.915	0.537
	CG versus GG	0.585	0.235-1.455	0.248	0.459	0.180-1.169	0.102	0.386	0.104-1.432	0.155
HSPA1A rs562047	GG versus CC	0.658	0.088-4.893	0.682	0.277	0.028-2.764	0.274	0.864	0.085-8.777	0.902
	The carriers of C allele versus the non-carriers	0.643	0.097-4.275	0.648	2.219	0.208-23.670	0.509	0.688	0.090-5.270	0.719
	The carriers of G allele versus the non-carriers	0.595	0.251-1.406	0.236	0.430	0.177-1.044	0.062	0.448	0.139-1.447	0.179

Table 2. Univariant logistic regression analysis. The odds ratio for the association between SNP's and tumor receptor status.

SNP	Genotype or alleles	Tumor grade			Tumor size			Lymph node involvement		
		Odds	95% CI	p	Odds	95% CI	p	Odds	95% CI	p
HSPA1A rs1043618	CG versus CC	0.660	0.268-1.624	0.366	0.903	0.382-2.139	0.817	1.078	0.479-2.424	0.857
	GG versus CC	1.552	0.303-7.936	0.598	2.610	0.511-13.319	0.249	0.935	0.186-4.699	0.935
	The carriers of C allele versus the non-carriers	0.301	0.036-2.548	0.961	0.271	0.057-1.894	0.214	0.322	0.038-2.733	0.299
	The carriers of G allele versus the non-carriers	0.696	0.157-3.085	0.633	0.411	0.098-1.729	0.225	1.614	0.373-6.986	0.522
	CG versus CC	1.054	0.383-2.900	0.919	1.029	0.401-2.645	0.952	0.985	0.396-2.449	0.975
HSPA1A rs562047	GG versus CC	7.566	0.744-76.898	0.087	0.000	0.00-	0.999	3.694	0.370-36.907	0.266
	The carriers of C allele versus the non-carriers	0.262	0.039-1.775	0.170	4.932	0.449-54.170	0.192	0.676	0.109-4.197	0.675
	The carriers of G allele versus the non-carriers	1.422	0.567-3.565	0.453	0.812	0.325-2.026	0.655	1.173	0.499-2.758	0.715

Table . 3. Univariant logistic regression analysis. The odds ratio for the association between SNP's and tumor grade, size and lymph node involvement.

Conclusions

The data indicate that rs1043618 and rs562047 polymorphisms in *HSPA1A* are not significantly related to tumor phenotypes and disease outcomes in this breast cancer patient group. For more precise analysis, studies, involving larger patient groups and implementing more advanced techniques in genetic testing, are suggested.

Key words: breast cancer, germline polymorphisms, *HSPA1A* .