Analysis of the polymorphic variant rs5498 of ICAM-1 in melanoma patients from southwestern Brazil

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Abstract

Melanoma is a highly lethal skin cancer whose worldwide frequency increases every year. Metastatic melanoma is aggressive and resistant to conventional therapy. Cell adhesion molecules play a central role in cell-cell interactions, especially in metastasis and immunity. Among cell adhesion molecules, which are potential risk factors for melanoma, ICAM-1 was previously correlated with melanoma progression. This study aimed to verify the presence and frequency of the polymorphic variant rs5498 (-1548 A/G, K469E) of the *ICAM1* gene in a sample of melanoma patients from southeastern Brazil. A total of 42 melanoma patients and 16 controls were genotyped and the frequencies of each variant were calculated. Our results indicate a frequency of 0.655 for the K allele in cases and of 0.719 for the control group. In our population, no correlation was identified between the rs5498 SNP and melanoma risk, although an increase in the sample size as well as the introduction of other variants of this and other adhesion molecules are needed in order to better establish the real role of such molecules in melanoma development and clinics.

Introduction

Melanoma accounts for 2-4% of all skin cancers worldwide, although it responds by 75% of the deaths by skin cancer (1-4). It is estimated that melanoma frequency, which has increased every year, will continues to grow (1, 5-7). Although almost always curable in its early stages, metastases to other regions of the body are very likely to occur if this condition is not diagnosed early (2).

Melanoma is a multifactorial disease (7-9). Risk factors include pigmentation phenotypes, number of melanocytic nevi and family history (9, 10). The main environmental risk factor is ultraviolet (UV) radiation exposure (8, 11). Research on genetic risk factors are being conducted to identify genes correlated to development and prognostic of melanoma, and the identified findings include genes related to cell proliferation and signaling, and some smoking behavior-related alleles (7-10, 12-14).

With tumor proliferation and neoangiogenesis, tumor cells interact with their microenvironment, including the nearby epithelial cells and immune system cells (15, 16). These interactions could allow tumor cell migration to different tissues, resulting in a process of metastasis. Metastatic melanoma is an aggressive disease resistant to conventional therapy (16).

Cell adhesion molecules play a central role in cell-cell interactions, especially in metastasis and immunity. These molecules belong to different groups, such as the integrin, cadherin and immunoglobulin superfamilies. In what concerns melanoma, molecules such as Intercellular Adhesion Molecule-1 (ICAM-1), Carcinoembryonic Antigen-Related Cell Adhesion Molecule 1 (CEACAM1), Activated Leucocyte Cell Adhesion Molecule (ALCAM), Vascular Cell Adhesion Molecule-1 (VCAM-1) and Melanoma Cell Adhesion Molecule (MCAM) have been reported in studies of both

susceptibility and development (3, 14, 17-21). The above mentioned molecules belong to the immunoglobulin superfamily, which is essential for the formation and maintenance of tissue integrity and known by its involvement in different steps of the metastatic process (21).

Amongst the previously cited molecules, ICAM-1 was correlated to melanoma progression (20, 22, 23). ICAM-1 is encoded by the *ICAM1* gene, and it is a cell surface glycoprotein which is typically expressed on endothelial and immune system cells (24). Increase on *ICAM1* expression was correlated with higher risk of metastasis (25). Increased expression of this gene is generally correlated with increased immune responses while the reduction of expression can potentially interfere with the adherence and migration patterns of immune cells (24, 26). The ICAM-1 glycoprotein is an adhesion molecule involved in the control of immune cell trafficking across vessel walls, being an essential mediator of the inflammatory pathway (24, 27). The rs5498 variant is a single nucleotide polymorphism (SNP) in exon 6 with the replacement of the nucleotide A for G, which results in an exchange of a lysine (AAG, K allele) for a glutamic acid (GAG, E allele) (24, 26, 28).

The rs5498 SNP affects the fifth immunoglobulin-like domain of the ICAM-1 glycoprotein (29, 30). This domain is essential for dimerization, presentation at the cell surface, and solubilization of the glycoprotein (29, 31). The amino acid residue change can compromise normal immune function and inflammation, so the rs5498 SNP could be a factor involved in many diseases with inflammatory and/or proliferative components.

The polymorphic variant rs5498 has been evaluated in European populations for melanoma and no correlation has been established in the studies (20, 22). Tang et al. stated that the polymorphic variant rs5498 probably contributes to decreased

susceptibility to cancer, especially in Caucasians, in melanoma and colorectal cancer subgroup (23).

Since no data was found regarding analyzes of the above-mentioned variant in Brazilian populations with melanoma, the aim of the present study was to verify the presence and frequency of the polymorphic variant rs5498 (-1548 A/G, K469E) of the *ICAM1* gene in a sample of melanoma patients from southeastern Brazil.

Material and methods

Case and control DNA samples were provided in a context of a collaboration established with the laboratory of Dr. Alessandra Pontillo from the Instituto de Ciências Biológicas, Universidade de São Paulo.

Genotyping was performed by PCR-RFLP using specific primers previously described (32). PCR samples were prepared to a final volume of 26ul containing the following reagents: 2ul of DNA (0,2 - 0,5ug), 2,5ul of 10X PCR buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl], 1ul of 50mM MgCl2, 1ul of 3mM dNTP mix, 1ul of 10pmol primer mix and 1U of Taq DNA polymerase (Invitrogen Corporation, California, USA). RFLP cleavage was performed with Bsh 1236I restriction enzyme (Thermo Scientific, BstU I isoesquisomer) in overnight incubation.

Genotypic and allelic frequencies were calculated for case and control groups by counting, and Hardy-Weinberg (H-W) equilibrium expectations were evaluated. Chi-square test was performed for populational differences evaluation and $p < 0.05$ was accepted as statistically significant.

Results

A total of 42 cases and 16 controls have been genotyped so far (Table 1). From the 42 cases, 18 were genotyped as KK (genotypic frequency: 0.429), 12 as KE (genotypic frequency: 0.452) and 4 were genotyped as EE (genotypic frequency: 0.119). The frequency of allele K in cases was 0.655 while the frequency of the allele E was 0.345. The cases group was in H-W equilibrium.

Amongst the 16 controls, 10 were KK (genotypic frequency: 0.625), 3 KE (genotypic frequency: 0.188) and 3 were EE (genotypic frequency: 0.188). The frequency of allele K in controls was 0.719 while the frequency of the allele E was 0.281. The control group was in H-W equilibrium.

Discussion

The *ICAM1* gene encodes a cell surface glycoprotein typically expressed on endothelial and immune system cells. The ICAM-1 molecule is an essential mediator of the inflammatory pathway (24, 27). The rs5498 variant is a SNP in exon 6 with the replacement of the nucleotide A for G, which results in an exchange of a lysine (K) for a glutamic acid (E) (24, 26, 28).

Due to technical difficulties, only a small part of the total sample available (198 patients and 142 controls) has been genotyped so far. Even though the preliminary results point to an absence of correlation between the rs5498 polymorphism and melanoma risk (Table 1), what is in accordance with previous studies in European populations (20, 23), it is obvious that our analyzed sample is ratter small and an increase in sample size can change the frequency values in cases and controls.

The rs5498 SNP could be a factor involved in many diseases with inflammatory and/or proliferative components due to the compromise of normal immune function and inflammation. In fact, in the past years, several studies have evaluated the potential association of this polymorphism and diseases, such as migraine, periodontal disease, type 2 diabetes, asthma, coronary disease, endometriosis, various types of tumors and obesity, among others (20, 23, 26, 28-30, 33-43). This SNP has also been correlated with susceptibility to infectious diseases as exemplified by malaria (44, 45). In most studies, the E allele seems to be the disease-related allele, and interestingly, it is also the less frequent allele in all studies.

For example, a Malaysian study with type 2 diabetes (T2D) and diabetic nephropathy (DN) analyzed DNA polymorphisms and methylation status of the *ICAM1* gene with TaqMan allelic discrimination and pyrosequencing (29). This study successfully correlated the rs5498 SNP with susceptibility to T2D and DN, with a high heterozygous index associated, which differs from other Asian populations (29). A Chinese meta-analysis associated the E variant with retinopathy in T2D in a dominant model (EE + KE vs KK) (30). A study from Slovenia reported that the rs5498 SNP (specifically the EE genotype) affects the progression of carotid atherosclerosis in patients with T2D (42). The previously referred study included 595 patients with T2DM and 200 subjects without T2DM in the control group, and genotyping was performed with RT-PCR.

Nevertheless, although adhesion molecules are important factor for inflammation development, not all pro-inflammatory diseases are directly affected by polymorphic variants on such genes, as revealed by studies approaching endometriosis. For instance, both a Brazilian study involving 200 women divided in 100 control and 100 cases (26), and an Italian study, with 363 women divided in 188 controls and 175 cases (28), were not able to identify a correlation between this variant and the disease. The Brazilian

study used the PCR-RFLP technique for genotyping while the Italian study used an allele-specific PCR (AS-PCR) technique.

Also, a research on variants of ICAM-1 and β3 integrin in patients with brain tumors from Turkey, covering 92 patients with primary brain tumors and 92 age-matched healthy control subjects, did not find statistically significant differences for the rs5498 SNP (33). Genotyping in this study was performed by PCR-RFLP. A Korean study with 181 control and 144 obese subjects genotyped by direct sequencing did not find correlation between the variant ant obesity (34), and a Taiwanese study using real time PCR to genotype 339 cases of coronary artery disease and 186 controls also did not find correlation for rs5498, but found correlation with another ICAM-1 variant, rs281432 (35). In this same direction, a Russian study performed with AS-PCR with 33 controls, 49 patients with colorectal cancer and 30 patients with breast cancer did not find correlation with the rs5498 SNP (36).

Investigating migraine in a Chinese Han population through PCR-RFLP, He et al. found significant higher frequencies of the EE genotype and E allele of rs5496 SNP between cases and controls (37). In this Chinese Han population study, the frequencies of the EE genotype and E allele were higher in the cases, compared with controls. In an evaluation of a Chinese population approaching periodontal disease, 584 patients and 182 healthy individuals were evaluated, and statistically significant associations were identified highlighting a potential involvement of the rs5498 gene polymorphism in this condition (38). In Germany, this same variant was correlated with pediatric bronchial asthma and elevated soluble levels of ICAM-1 (slICAM-1) (39). In this previously cited study, 352 children with asthma and 270 controls were genotyped by PCR-RFLP.

The rs5498 SNP was also correlated with epithelial ovarian cancer (EOC) in a Chinese study (40), and oral cancer and urothelial cell carcinoma (UCC) in Taiwanese studies

(41, 43). The Chinese study genotyped 408 patients with EOC and 520 controls using the MassARRAY system, with the E allele being associated with increased tumor grade and EOC risk (40). The oral cancer Taiwanese study genotyped 595 cases and 561 controls using real time PCR. The KE and EE genotypes conferred significantly higher risk to disease development (41). The UCC Taiwanese study genotyped 279 cases and 279 controls using real time PCR, as in the other Taiwanese study, KE and EE genotypes were statistically significantly correlated with UCC (43).

In order to evaluate potential differences on allelic and genotypic frequencies in distinct human populations classified according to the ethnic background, we pooled and compared the control groups used in different populational studies involving rs5498 SNP in the Table 2. A significant difference ($p < 0.01$, for all populations comparison) between populations could be observed. The frequencies of the alleles do differ in ethnic groups living in different geographical regions (Fig. 1), as it was previously reported in the literature (36, 46).

Nevertheless, even taking into consideration only populations living in a specific geographic region, a relatively heterogeneous situation can be reported. For example, comparing Asiatic populations, China, Korea and Taiwan ($p < 0.00001$), China and Korea ($p = 0.023$), China and Taiwan ($p < 0.00001$) and comparing Korea and Taiwan ($p < 0.00001$). This heterogeneity amongst Asiatic populations was already reported (30). In contrast, no statistically significant difference between the European populations from Germany and Italy ($p = 0.201$) were detected. Comparing these European populations with the Brazilian population (since Brazil has a strong genetic Germany and Italy ancestry), a significant difference is observed ($p = 0.04$), which could be related to admixture in our population and deserves more studies.

A comparison of control data from this study with the endometriosis study of rs5498 SNP in a Brazilian population (26) and previous data from our group (Personal communication) shows no significant differences ($p = 0.824$). Even though the referred study with endometriosis was carried out only with women, no data in the literature supports differences on rs5498 allelic frequencies according to sex. Brazil is a highly diverse country with mainly European, African and Amerindian ancestrally, with an European ancestry predominating in all regions (47). Brazil also shows interethnic admixture what difficult ethnic groups separation (48). With the actual data, no correlation between rs5498 SNP and melanoma development was observed.

Perspectives

We expect to expand sample size with better quality sample provided by the collaborating group. With a higher sample size for both cases and controls we will be able to verify whether indeed there is no correlation between melanoma and the rs5498 SNP in our population directly in our results to confirm our current conclusions. An analysis of our data subgrouping patients according to the ethnic origin will also be performed.

Conflicts of interests

There are no conflicts of interest.

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Table 1. Genotypic and allelic frequencies of genotyped cases and controls			
Cases		Controls	
Genotype	Frequency n (freq.)	Genotype	Frequency n (freq.)
KK	18 (0.429)	KK	10 (0.625)
KE	19 (0.452)	KE	3 (0.188)
EE	5 (0.119)	EE	3 (0.188)
$\chi^2 = 3.463$ / p = 0.177			
Allele	Frequency n (freq.)	Allele	Frequency n (freq.)
K	55 (0.655)	K	23 (0.719)
E	29 (0.345)	E	9 (0.281)
$\chi^2 = 0.431$ / p = 0.512			

Table 2. Comparison of ICAM-1 rs5498 SNP allelic frequencies in different populations (control groups in selected studies).

	K n (freq.)	E n (freq.)	Ref.
Brazil	134 (0.67)	66 (0.43)	(26)
Brazil’	271 (0.69)	123 (0.31)	Wieck, A. (Personal communication,)
China	702 (0.682)	328 (0.318)	(40)
Germany	308 (0.572)	230 (0.428)	(39)
India	233 (0.607)	151 (0.393)	(44)
Italy	69 (0.63)	39 (0,37)	(28)
Korea	223 (0.616)	139 (0.314)	(34)
Russia	36 (0.545)	30 (0.455)	(36)
Slovenia	223 (0.558)	177 (0.442)	(42)
Taiwan	882 (0.786)	240 (0.214)	(41)
Turkey	108 (0.587)	76 (0.413)	(33)
Brazil (control)”	23 (0.719)	9 (0.281)	This Study
Melanoma	55 (0.655)	29 (0.345)	This study.
$p < 0.01$ (all comparisons)			

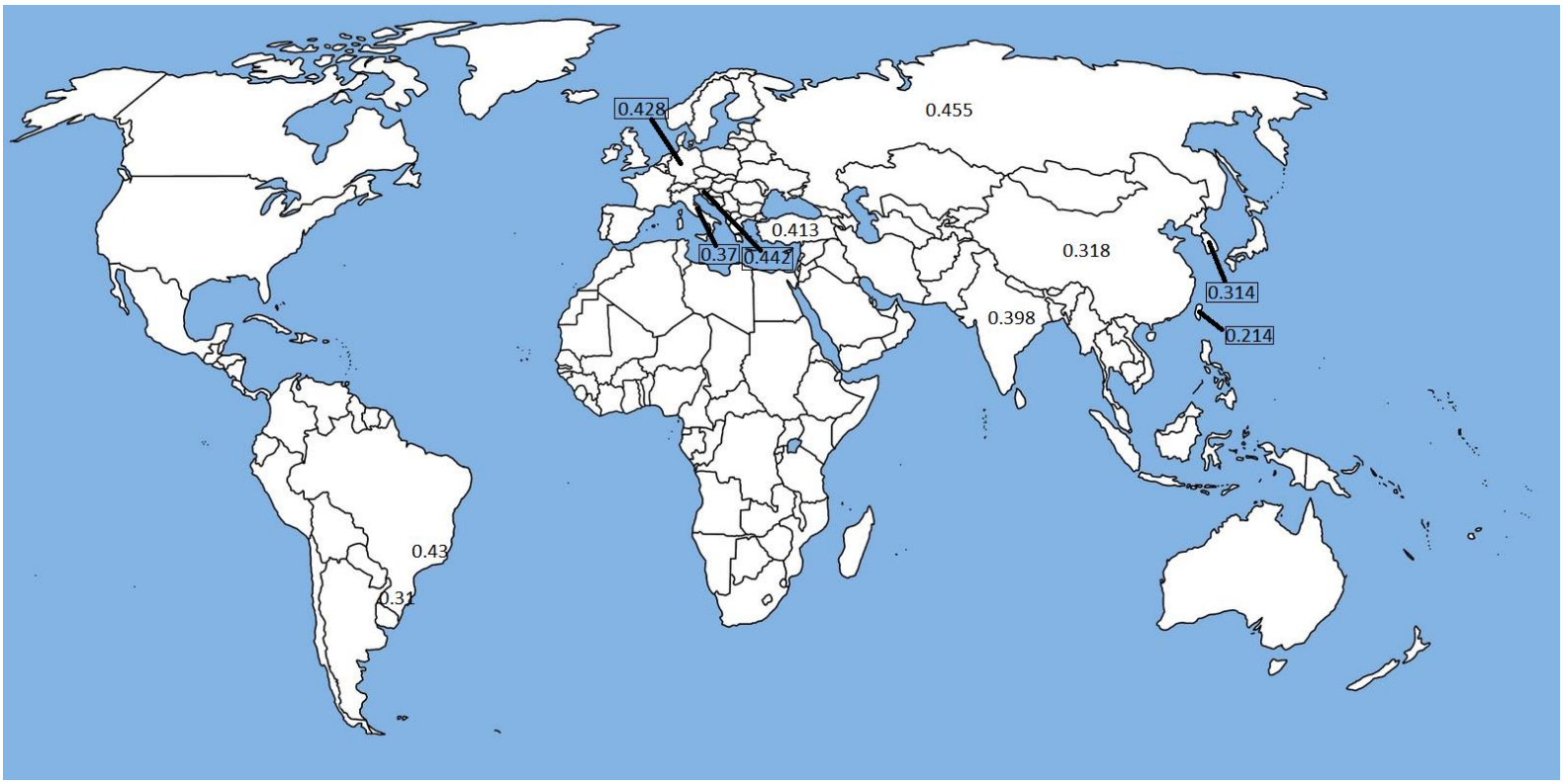

Figure 1. World distribution of selected frequencies of K allele.