GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Frequencies of -308G/A (*TNFA*) and -509C/T (*TGFB1*) polymorphisms in sickle cell anemia patients from Brazil

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Genet. Mol. Res. 12 (4): 6762-6766 (2013)
Received January 11, 2013
Accepted August 5, 2013
Published December 16, 2013
DOI http://dx.doi.org/10.4238/2013.December.16.1

ABSTRACT. Sickle cell anemia is an affection that causes chronic inflammation, with consequences for vaso-occlusion, oxidative stress and cytokine production. Genetic polymorphisms in markers involved in this process can modulate the inflammatory response, including polymorphisms -308G/A of TNFA (tumor necrosis factor alpha) and -509C/T of TGFB1 (transforming growth factor beta 1), reported to increase TNF-α and TGF-β1 production, respectively. Changes in the cytokine balance are important risk factors for clinical events; consequently, we examined the frequencies of these polymorphisms in 240 Brazilian sickle cell anemia patients from southeast Brazil. PCR-RFLP was used to detect these polymorphisms. The -509C/T (TGFB1) polymorphism was more frequent than -308G/A (TNFA), with allelic frequency of 0.3 for the mutant allele T (TGFB) agaist 0.1 for the mutant

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allele A (TNFA). These allelic frequencies are similar to those known from populations with ethnicity similar to the Brazilian population. Inheritance of these polymorphisms does not seem to be associated with that of the Hb S mutation; however, this information could be useful in analyses of specific clinical characteristics of sickle cell anemia.

Key words: Allelic frequency; Genetic polymorphisms; PCR-RFLP; Sickle cell disease; SNPs; Hemoglobin S

INTRODUCTION

Sickle cell anemia (SCA) is a hemolytic anemia caused by a mutation in the sixth codon of the beta globin chain (GAG → GTG), resulting in homozygous hemoglobin (Hb) S (Honig and Adams III, 1986; Frenette and Atweh, 2007). The inflammatory process that occurs in SCA patients may play an important role in vaso-occlusion, oxidative stress stimulation and cytokine production, contributing to the disease pathogenesis (Chiang and Frenette, 2005).

Evidence shows the involvement of genetic polymorphisms in the inflammatory response in SCA patients, among them, some association studies suggest that *TNFA* (tumor necrosis factor alpha) and *TGFB1* (transforming growth factor beta 1) genes interfere in the SCA clinical profile (Nolan et al., 2006; Cajado et al., 2010). TNF-α and TGF-β cytokine production may be genetically influenced by polymorphisms that affect gene regulation. The -308G/A (*TNFA*) (rs1800629) and -509C/T (*TGFB1)* (rs1800469) polymorphisms are reported to increase the production of TNF-α (Rodriguez-Rodriguez et al., 2011) and TGF-β1 (Bhayal et al., 2011), respectively.

Changes in cytokine balance in SCA patients are important risk factors for clinical events, and thus, the objective of this study was to determine the frequencies of the -308G/A (*TNFA*) and -509C/T (*TGFB1*) polymorphisms in Brazilian SCA patients, and to compare our results with the frequencies reported in some recent studies in the literature.

MATERIAL AND METHODS

We analyzed 240 peripheral blood samples of SCA patients from southeast Brazil. All samples were submitted to a classical Hb diagnostic, including Hb electrophoresis at alkaline and acid pH (Marengo-Rowe, 1965; Vella, 1968) and high-performance liquid chromatography for Hb fraction quantification (VARIANT, Bio-Rad) (Bonini-Domingos, 2006). For molecular analysis, genomic DNA was extracted by the phenol-chloroform method (Sambrook et al., 1989). Amplification of the segment that encodes Hb S was performed using specific primers, and fragments were cleaved with the restriction endonuclease Fast*Dde*I (Fermentas, USA) (Belini et al., 2010). -308G/A (*TNFA*) and -509C/T (*TGFB1*) were determined by amplification of the specific genomic segment, with subsequent treatment with specific restriction enzymes for mutations in the *TNFA* (Fast*Nco*I; Fermentas) and *TGFB1* (Fast*Bsu*36I; Fermentas) genes (Wilson et al., 1992; Silverman et al., 2004).

Statistical analyses were performed for comparing the frequencies obtained against those of other studies and databases, using the Statistica 10.0 software with the chi-square test. The level of significance was set at $P < 0.05$.

RESULTS

The genotypic and allelic frequencies for the polymorphisms evaluated in SCA patients are shown in Table 1. The -509C/T (*TGFB1*) polymorphism was more frequent than -308G/A (*TNFA*), with the frequency of the mutant allele being 0.3 versus 0.1. Table 2 presents the literature frequencies for the -308G/A (*TNFA*) and -509C/T (*TGFB1*) polymorphisms obtained by other authors and in databases, as well as a comparison with our data, showing that our data were in accordance with other studies with similar populations.

Table 1. Genotypic and allelic frequency of the -308G/A (*TNFA*) and -509C/T (*TGFB1*) polymorphisms in sickle cell anemia patients.

Polymorphism	Genotype	Genotypic frequency [N (%)]	Allelic frequency
TNFA	GG	195 (81.25)	G = 0.90
(-308G/A)	GA	43 (17.92)	A = 0.10
	AA	2 (0.83)	
TGFB1	CC	109 (45.42)	C = 0.70
(-509C/T)	CT	116 (48.33)	T = 0.30
	TT	15 (6.25)	

Table 2. Allelic frequency found to -308G/A (*TNFA*) and -509C/T (*TGFB1*) polymorphisms in some recent studies and database, comparing with the present study.

	Population group	N	Allelic frequency		P	References
			G	A		
-308G/A (*TNFA*)	General population (Brazil)	200	0.86	0.14	0.1956	(Cajado et al., 2011)
	SCA (Brazil)	210	0.88	0.12	0.5175	(Cajado et al., 2011)
	SCA (Brazil)	49	0.92	0.08	0.6920	(Vicari et al., 2011)
	General population (India)	216	0.94	0.06	0.1200	(Ghosh et al., 2010)
	HapMap_CEU European	113	0.83	0.17	0.0678	**
	HapMap_YRI African	113	0.91	0.09	0.6712	**
	*SCA (Brazil)	240	0.90	0.10	-	-
-509C/T (*TGFB1*)	General population (Korea)	352	0.52	0.48	<0.0001	(Kim et al., 2010)
	General population (Hungary)	30	0.58	0.42	0.1382	(Rovo et al., 2010)
	Severe asthma population (Brazil)	38	0.60	0.40	0.2419	(de Faria et al., 2008)
	HapMap_CEU European	113	0.71	0.29	0.8507	-
	HapMap_YRI African	113	0.79	0.21	0.0843	-
	*SCA (Brazil)	240	0.70	0.30	-	-

SCA = sickle cell anemia; CEU = Utah residents with ancestry from Northern and Western European; YRI = Yoruba in Ibadan, Nigeria. Statistical test: Chi-square test ($\alpha < 0.05$). *Presente study; **available in dbSNP, NCBI [http://www.ncbi.nlm.nih.gov/projects/SNP].

DISCUSSION

The frequencies found for the -308G/A (*TNFA*) and -509C/T (*TGFB1*) polymorphisms are similar to those provided by databases for Caucasian and African populations from NCBI databases (http://www.ncbi.nlm.nih.gov/projects/SNP), reflecting the contribution of these groups to the ethnic composition of the Brazilian population. For the -308G/A (*TNFA*) polymorphism, the frequencies obtained were similar to those results of other SCA studies in Brazil (Cajado et al., 2010; Vicari et al., 2011). Our results also did not differ from those found for the Brazilian population without hemoglobinopathies (Cajado et al., 2011), suggesting that the inheritance of the polymorphisms studied is independent of the β^S gene inheritance. In re-

lation to the Indian population, the frequencies found in our population did not differ (Ghosh et al., 2010), probably due to the ethnic heterogeneity of both.

For the -509C/T (*TGFB1*) polymorphism, our data were similar to findings from a Brazilian study in severe asthma patients (de Faria et al., 2008). Regarding that study, there was no association between presence of polymorphism and risk of developing asthma, which is a chronic inflammatory disease. The similarity with the frequencies obtained in our study is probably due to resemblance of the two populations, but not to the chronic inflammatory process. Regarding a study in Hungary with healthy individuals (Rovo et al., 2010), a population with different ethnic characteristics from the Brazilian one, the absence of a difference can be explained by the small sample number (N = 30), which probably was not representative of the Hungarian population. Differences relative to Korean individuals (Kim et al., 2010), in turn, demonstrate the low influence of this ethnicity in the formation of the Brazilian population.

In summary, the frequencies for the polymorphisms evaluated were not associated with inheritance of Hb S and were similar between populations with the same ethnic characteristics. Furthermore, the polymorphisms do not appear to be associated with other inflammatory diseases.

CONCLUSION

Allelic frequencies found in this study for the -308G/A (*TNFA*) and -509C/T (*TGFB1*) polymorphisms are in agreement with the literature for population groups with similar ethnicity as compared to the Brazilian population. The inheritance of the two polymorphisms does not seem to be associated with the Hb S mutation, but knowledge about them can be useful in future studies involving specific clinical characteristics of SCA, since they are involved in the control of inflammation.

ACKNOWLEDGMENTS

Reasearch supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Ministério da Saúde (#MS3072/2007), and Fundação Pró-Hemorio RJ (FUNDARJ). English text revised by Carolina Grünig Humberto da Silva.

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