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Os polimorfismos do gene *MBL2* não estão relacionados com a ocorrência de doença cerebrovascular na anemia falciforme

MBL2 gene polymorphisms are not related to the occurrence of cerebrovascular disease in sickle cell anemia

Los polimorfismos del gen *MBL2* no están relacionados con la aparición de enfermedad cerebrovascular en la anemia falciforme

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Resumo

Objetivo: Este estudo tem como objetivo verificar se os polimorfismos do gene *MBL2* estão relacionados com a ocorrência de doença cerebrovascular (DC) em pacientes com anemia falciforme (AF). **Métodos**: No total, 259 pacientes com AF não relacionados foram incluídos. Os pacientes foram divididos em três grupos: grupo controle, grupo acidente vascular cerebral (AVC) e faixa de risco. Amostras de sangue periférico foram coletadas e foi realizada extração de DNA. Todos os pacientes foram genotipados para o éxon 1, região promotora -221 e região promotora -550 do gene *MBL2*, juntamente com os haplótipos do gene da β-globina. **Resultados:** Em relação à genotipagem do *MBL2*, não houve diferença na frequência das variantes alélicas e genotípicas do éxon 1 e nas regiões promotoras -221 e -550 do gene *MBL2* entre os grupos estudados. **Conclusão:** Apesar do pequeno número de pacientes e da falta de associação entre polimorfismos do *MBL2* e DC, nosso estudo representa um esforço para entender o impacto dos polimorfismos do *MBL2* no curso clínico de pacientes com AF.

Palavras-chave: Anemia falciforme; Doença cerebrovascular; Gene *MBL2*; Polimorfismos.

Abstract

Objective: This study has as objective to verify whether *MBL2* gene polymorphisms are related to the occurrence of cerebrovascular disease (CD) in sickle cell anemia (SCA) patients. **Methods:** Overall, 259 unrelated SCA patients were enrolled. The patients were divided into three groups: control group, stroke group ad range of risk group. Peripheral blood samples were collected and DNA extraction was performed. All patients were genotyped for

exon 1, promoter region -221 and promoter region -550 of *MBL2* gene, along with β-globin gene haplotypes. **Results:** Concerning the genotyping of the *MBL2*, there was no difference in the frequency of allelic and genotypic variants of the exon 1 and the promoter regions -221 and -550 of the *MBL2* gene among the studied groups. **Conclusion:** Despite the small number of patients, and the lack of association between *MBL2* polymorphisms and CD, our study represents an effort to understand the impact of *MBL2* polymorphisms in the clinical outcome of patients with SCA.

Keywords: Sickle cell anemia; Cerebrovascular disease; *MBL2* gene; Polymorphism.

Resumen

Objetivo: Este estudio tiene como objetivo verificar si los polimorfismos del gen *MBL2* están relacionados con la aparición de enfermedad cerebrovascular (EC) en pacientes con anemia falciforme (AF). **Métodos:** en total, se incluyeron 259 pacientes con AF no relacionada. Los pacientes se dividieron en tres grupos: grupo de control, grupo de accidente cerebrovascular y rango de riesgo. Se recogieron muestras de sangre periférica y se realizó extracción de DNA. Todos los pacientes fueron genotipados para el exón 1, la región promotora -221 y la región promotora -550 del gen *MBL2*, junto con los haplotipos del gen de la β-globina. **Resultados:** con respecto al genotipo *MBL2*, no hubo diferencia en la frecuencia de variantes alélicas y genotípicas del exón 1 y en las regiones promotoras -221 y -550 del gen *MBL2* entre los grupos estudiados. **Conclusión:** a pesar del pequeño número de pacientes y la falta de asociación entre los polimorfismos *MBL2* y EC, nuestro estudio representa un esfuerzo por comprender el impacto de los polimorfismos *MBL2* en el curso clínico de los pacientes com AF.

Palabras clave: Anemia falciforme; Enfermedad cerebrovascular; Gen *MBL2*; Polimorfismos.

1. Introduction

Sickle cell anemia (SCA) is a monogenic disease caused by a mutation in the the β globin gene (*HBB)*, characterized by chronic hemolytic anemia and vasooclusive episodes, which are responsible by the main clinical events present in the patients with this disorder (Kato *et al*, 2018). There is a wide variability in the clinical course of patients with SCA, and it is influenced by genetic features, including factors that affect HbF level and co-inheritance

of α-thalassaemia, and by environmental, social, and economic factors (Ngo *et al*, 2013; Kato *et al*, 2018).

Among the clinical events present in SCA, the most severe is stroke, an acute neurological event secondary to the occlusion of an artery or hemorrhage that might cause subsequent tissue ischemia and/or neurological signs and symptoms (Sarnaik *et al*, 2001; Kato *et al*, 2018). It has been shown that several molecules might be evolved in the pathogenesis of ischemic brain injury (Chen *et al*, 2011; Domingos *et al*, 2020). Mannose Binding Lectin (MBL) acts by regulating inflammation and activating the complement system (CS) (Dogru *et al*, 2020), and although previous studies have demonstrated that low MBL levels are associated with protection against stroke, (Calvo-alén *et al*, 2006) no consensus was reached so far and little is known about the role of MBL in SCA patients.

This study has as objective to verify whether *MBL2* gene polymorphisms are related to the occurrence of cerebrovascular disease (CD) in sickle cell anemia (SCA) patients. Here, we report our data concerning *MBL2* genotyping and the possible association of polymorphisms at -221 (Y/X), -550 (H/L) and exon 1 (A/O) regions of *MBL2* with cerebrovascular disease (CD) in a specific cohort of patients with SCA from the Northeast region of Brazil.

2. Methods

The research aims to bring new knowledge to society as recommended by Pereira et al. (2018). In the present study, a quantitative study is carried out in Sickle Cell Anemia patients.

Between August 2011 and November 2012, 259 eligible SCA patients were enrolled. The diagnosis of SCA (presence of Hemoglobin S homozigosity) was performed following standard molecular procedures (Sanchaisuriya *et al*, 2004). The patients with stroke and/or presenting altered Transcranial Doppler (time-averaged mean velocities higher than 200 cm/s) were considered with CD (Adams *et al*, 1998; Flanagan *et al*, 2011). Then, patients were divided into three groups: control group (n=141) with patients presenting normal Transcranial Doppler (TCD), no stroke, median age of 37 years (25 to 64) and 55% males; range risk group (n=54) with patients presenting no stroke episode, altered TCD, median age of 16 years (12 to 28), 39% males; and stroke group (n=64), patients with diagnosed stroke episode, median age 26 (11 to 71), 36% males.

The study was approved by the local Research Ethics Board of the HEMOPE Foundation, Recife, Pernambuco (number 014/2010) and, in accordance with the Declaration

of Helsinki, informed consent was obtained from all patients, or, when applicable, their parents, prior to study commencement. DNA was extracted from peripheral blood, as previously described (Davis *et al*, 1988). All laboratory data (hemoglobin levels, platelet counts, reticulocyte counts, leukocyte counts and β^S globin gene haplotype) were obtained from medical records during steady state (Table 1). Real Time PCR was used to determine the polymorphisms of the promoter regions and exon 1 of the *MBL2* gene, using Rotor Gene 6000TM (Corbett Research Mortlake, Sydney, Australia). SYBR GREEN® was used for the detection of exon 1 polymorphism (A/O) and the primers were: forward 5′AGGCATCAACGGCTTCCCA 3′ and reverse 5′ CAGAACAGCCCAACACGTACCT 3′ (Calvo-alén *et al*, 2006). The promoter regions -550 (H/L) and −221 (X/Y) polymorphisms were detected by Taqman® SNP (Life Technologies, CA, USA). Primer and probe sequences were obtained from NCBI website http://snp500cancer.nci.nih.gov.

The GraphPad Prism program (version 6.0) for Windows (GraphPad Software, San Diego, California, USA) was used for association analysis. For the tests of relationship between categorical variables, the chi-square test ($\chi 2$) or Fischer's exact with Yates correction was used. The association between the studied variables was estimated by the Odds Ratio (OR) with a 95% confidence interval and it was considered significant when $p < 0.05$. To compare continuous variables, the t-Student test or Mann-Whitney non-parametric test were applied, and the ANOVA or Kruskal Wallis test, when appropriate.

3. Results

All primary strokes were confirmed by baseline brain magnetic resonance imaging. All patients were fully genotyped for the variants of promoter regions -221 (Y/X) and -550 (H/L), and exon 1 (A/O) region of *MBL2*, along with β-globin gene haplotypes. The population was in Hardy-Weinberg equilibrium. The clinical and laboratory data were last updated in December 2019, and they are presented in Table 1.

Table 1: Clinical and laboratory features of SCA patients according to the groups.

	Controls (N=141)	Range of risk (N=54)	Stroke (N=64)	P Value*
Age Median (Min-Max)	37 (25-64)	16 (12-28)	26 (11-71)	<0.0001
Gender (Male/Female)	78/63	21/33	23/41	0.0144
Hemoglobin Median (Min-Max)	7.9 (3.9-10.7)	7.3 (5.6-8.8)	7.5 (6-9.4)	<0.0001
Platelets Median (Min-Max)	386000 (55000-670000)	405000 (135000-610000)	423000 (173000-689000)	0.1114
Reticulocytes Median (Min-Max)	8.4 (2.6-21.0)	10.2 (4.8-19.9)	10.1 (3.7-22.7)	0.0003
Leukocytes Median (Min-Max)	10800 (5400-21900)	14450 (6000-25600)	12600 (8760-19500)	<0.0001
β^S globin gene haplotype				
CAR/CAR	77 (55%)	35 (65%)	47 (73%)	-
non CAR/CAR	64 (45%)	19 (35%)	17 (27%)	0.0314

* Kruskal Wallis test

The frequencies of genotypes and alleles of exon 1 and the promoter regions -221 and -550 of the *MBL2* gene are in Table 2, according to the groups (control, range of risk and stroke).

Table 2: Frequencies of alleles and genotypes of *MBL2* SNPs in SCA patients according to the groups.

	Controls (N=141)	Range of risk (N=54)	Stroke (N=64)	p Value, OR (95% CI)
Promoter (-550)				
Allele				
L	209 (0.74)	77 (0.71)	104 (0.81)	*L vs H *p*= 0.6635, 1.153 (0.7027 – 1.891)
H	73 (0.26)	31 (0.29)	24 (0.19)	**L vs H *p*= 0.147, 0.6607 (0.3937 – 1.109)
Genotype				
LL	75 (0.53)	28 (0.52)	41 (0.64)	*LL vs HH *p*= 0.4762, 1.913 (0.5608 – 6.528)
HL	59 (0.42)	21 (0.39)	22 (0.34)	*LL vs LH/HH *p*= 0.9941, 1.055 (0.5630 - 1.978)
HH	7 (0.05)	5 (0.09)	1 (0.02)	**LL vs HH *p*= 0.3501, 0.2613 (0.03105 – 2.199)
HH+HL	66 (0.47)	26 (0.48)	23 (0.36)	**LL vs LH/HH *p*= 0.1925, 0.6375 (0.3469 – 1.172)
Promoter (-221)				
Allele				
Y	228 (0.81)	88 (0.81)	105 (0.82)	*Y vs X *p*= 0.9982, 0.9596 (0.5432 – 1.695)
X	54 (0.19)	20 (0.19)	23 (0.18)	**Y vs X *p*= 0.8831, 0.9249 (0.5389 – 1.587)
Genotype				
YY	92 (0.65)	37 (0,68)	42 (0.66)	*YY vs XX *p*= 0.8953, 1.492 (0.3390 – 6.565)
YX	44 (0.31)	14 (0.26)	21 (0.33)	*YY vs YX/XX *p*= 0.7927, 0.8627 (0.4410 – 1.687)
XX	5 (0.04)	3 (0.06)	1 (0.01)	**YY vs XX *p*= 0.7565, 0.4381 (0.04960 – 3.869)
XX+YX	49 (0.35)	17 (0.32)	22 (0.34)	**YY vs YX/XX *p*= 0.9157, 0.9835 (0.5282 – 1.831)
Exon 1				
Allele				
A	221 (0.78)	93 (0.86)	92 (0.72)	*A vs O *p*= 0.1131, 0.5843 (0.3160 – 1.081)
O	61 (0.22)	15 (0.14)	36 (0.28)	**A vs O *p*= 0.1908
Genotype				
AA	87 (0.62)	40 (0.74)	31 (0.48)	*AA vs OO *p*= 0.4612, 0.3107 (0.03696 – 2.612)
AO	47 (0.33)	13 (0.24)	30 (0.47)	*AA vs AO/OO *p*= 0.1459, 0.5639 (0.2808 – 1.132)
OO	7 (0.05)	1 (0.02)	3 (0.05)	**AA vs OO *p*= 0.9072, 1.203 (0.2926 – 4.944)
AO+OO	54 (0.38)	14 (0.26)	33 (0.52)	**AA vs AO/OO *p*= 0.1035, 1.715 (0.9446 – 3.114)

* Controls vs Range of risk
**Controls vs Stroke

Concerning the genotyping of the *MBL2*, there was no difference in the frequency of allelic and genotypic variants of the exon 1 and the promoter regions -221 and -550 of the *MBL2* gene among the studied groups.

4. Discussion

MBL activates the complement system, which acts as a defense against pathogens, but it may also cause self-damage following adverse activation (Song *et al*, 2014). It is known that MBL modulates proinflammatory cytokine production (Zhou *et al*, 2019), and it may aggravate local and systemic inflammation via complement system activation (Fiane *et al*, 2003; Neglia *et al*, 2019). Therefore, high MBL levels may mediate harmful inflammation through excessive complement activation (Bąk-Romaniszyn *et al*, 2020).

It has been shown that *MBL2* polymorphisms are associated with low functional MBL levels (Medeiros *et al*, 2017; El-Behedy *et al*, 2019). In our study, we did not find an association between *MBL2* polymorphisms and the occurrence of CD. In a study evolving 248 Chinese patients, in which 148 were diagnosed with acute ischemic stroke, elevated levels of MBL were associated with an increased risk of stroke (Wang *et al*, 2014).

In a cohort study with 415 individuals, including Africans, Amerindians, and Caucasians, it was found association between genotypes related to low MBL production and protection against CD only in the Caucasian population (Calvo-alén *et al*, 2006). It is possible to suggest that the protection against stroke found in Caucasian patients with low MBL levels (Calvo-alén *et al*, 2006) could be different in the Brazilian population due to the miscegenation.

The mechanism evolving MBL levels with the occurrence of CD is not yet established. In a study evolving a rat model of acute myocardial infarction, it was shown that inhibition of MBL reduces postischemic reperfusion injury (Jordan *et al*, 2001). Therefore, MBL is more likely to be considered a contributing factor to the outcomes rather than a simple marker.

5. Conclusion

Despite the small number of patients, and the lack of association between *MBL2* polymorphisms and CD, our study represents an effort to understand the impact of *MBL2* polymorphisms in the clinical outcome of patients with SCA, which is a public health issue in Brazil. Nevertheless, we highlight here the importance of further studies with a larger sample and with different populations to confirm our data. Then, it will be possible to understand better the physiopathology of stroke in SCA and the identification of new markers for this clinical event.

Conflict of interest

The authors declare no conflict of interest.

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