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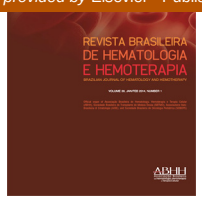

Letter to the Editor

Klotho gene polymorphisms and their association with sickle cell disease phenotypes

Dear Editor,

We read with great interest the recent review presented by de Souza Pacheco and Goncalves,[1] and we would like to briefly report our experience with *Klotho* (*KL*) single nucleotide polymorphisms (SNPs) and sickle cell disease (SCD).

SCD presents a phenotypic heterogeneity that has not been fully elucidated yet. Genetic modifiers and environmental effects may account for the different clinical outcomes observed. Some genetic modulators of SCD are well known, however, they do not explain all the phenotypic variations.[2,3]

Genetic association studies have tried to elucidate this variability by evaluating SNPs in genes other than the *beta globin* gene. However, these data still need to be validated.[2–4]

Association between *KL* SNPs and clinical manifestations of SCD have been observed, especially related with priapism and leg ulcers.[5–9] Despite the existence of some data about this topic, these associations have not been confirmed worldwide. Thus, we aimed to study *KL* SNPs in 109 steady state SCD patients, 74 (68%) with SCA, 10 (9%) with Hemoglobin (Hb) S/beta-thalassemia, and 25 (23%) with Hb SC. The mean age was 32.6 ± 11.3 years with predominance of females (62.3%). The patients were followed in the Outpatient Clinics of the Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), Brazil. The Institutional Ethics Committee approved this study and informed consent was obtained. We studied the correlation between the rs211234 and rs2249358 SNPs and priapism[9]; and between rs516306 and rs685417 and leg ulcers.[8] The DNA was obtained from peripheral blood leukocytes using a standard kit (QIAamp DNA minikit, QIAGEN). The rs2249358 and rs516306 SNPs were identified by polymerase chain reaction (PCR) followed by digestion with restriction enzymes (RFLP), as described before.[8,9] The rs2111234 and rs685417 SNPs were identified by the allele-specific oligonucleotide-PCR (ASO-PCR) method. Statistical analysis was performed using GraphPad Prism® (San Diego, CA, USA); and differences with a p-value < 0.05 were considered statistically significant.

Of the 41 male patients in this study, fourteen (34%) had a history of priapism, 11 with SCA and 3 with Hb SC. The median age of the patients with priapism was significantly higher than the age of the individuals without this manifestation [32.5 years (range: 25–68) and 27.5 years (range: 20–56), respectively; p-value = 0.03].

We did not find any association between priapism and rs211234 [p-value = 0.4; Odds Ratio (OR): 2.20; 95% confidence interval (95% CI): 0.45–10.63] or rs2249358 (p-value = 0.72; OR: 1.50; 95% CI: 0.37–6.08). Our results were similar to Elliot et al. who did not confirm any association between rs211234 and priapism.[6] However, these results were discordant with the data of Nolan, who described a significant association between priapism and these SNPs.[9] The small number of patients studied could have influenced these results.

The frequency of leg ulcers (19%) was significantly more frequent in males than females (29.2% vs. 13.2% respectively, p-value = 0.047). Regarding the genotype, 20 (95%) patients were diagnosed as SCA and one (5%) as Hb SC.

A previous study about *KL* SNPs and leg ulcers reported an association.[8] Nevertheless, we did not find any association between rs516306 (p-value = 0.29; OR: 2.19 95% CI: 0.67–7.12) or rs685417 (p-value = 1.00; OR: 0.95; 95% CI: 0.33–2.73) and leg ulcers.

Although we did not confirm any relationship between *KL* SNPs and clinical manifestations, the importance of these SNPs in SCD needs to be better elucidated. Given the relevance of this gene, as highlighted by de Souza Pacheco and Goncalves,[1] we believe that more research is needed to clarify this association.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. de Souza Pacheco AP, Goncalves M. Klotho: its various functions and association with sickle cell disease subphenotypes. Rev Bras Hematol Hemoter. 2014;36(6):430–6.
2. Steinberg MH, Rodgers GP. Pathophysiology of sickle cell disease: role of cellular and genetic modifiers. Semin Hematol. 2001;38(4):299–306.
3. Steinberg MH. Predicting clinical severity in sickle cell anaemia. Br J Haematol. 2005;129(4):465–81.
4. Fertrin KY, Costa FF. Genomic polymorphisms in sickle cell disease: implications for clinical diversity and treatment. Expert Rev Hematol. 2010;3(4):443–58.
5. Baldwin C, Nolan VG, Wyszynski DF, Ma QL, Sebastiani P, Embury SH, et al. Association of klotho, bone morphogenic protein 6, and annexin A2 polymorphisms with sickle cell osteonecrosis. Blood. 2005;106(1):372–5.
6. Elliott L, Ashley-Koch AE, De Castro L, Jonassaint J, Price J, Ataga KI, et al. Genetic polymorphisms associated with priapism in sickle cell disease. Br J Haematol. 2007;137(3):262–7.
7. Driss A, Asare KO, Hibbert JM, Gee BE, Adamkiewicz TV, Stiles JK. Sickle cell disease in the post genomic era: a monogenic disease with a polygenic phenotype. Genomics Insights. 2009;2009(2):23–48.
8. Nolan VG, Adewoye A, Baldwin C, Wang L, Ma Q, Wyszynski DF, et al. Sickle cell leg ulcers: associations with haemolysis and SNPs in Klotho, TEK and genes of the TGF-beta/BMP pathway. Br J Haematol. 2006;133(5):570–8.
9. Nolan VG, Baldwin C, Ma Q, Wyszynski DF, Amirault Y, Farrell JJ, et al. Association of single nucleotide polymorphisms in klotho with priapism in sickle cell anaemia. Br J Haematol. 2005;128(2):266–72.

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