



**Scientific Letter**

**Association of the *SOD2* Polymorphism (Val16Ala) and SOD Activity with Vaso-occlusive Crisis and Acute Splenic Sequestration in Children with Sickle Cell Anemia**

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*The *SOD2* polymorphism Val16Ala T→C influences the antioxidative response. This study investigated the association of the *SOD2* polymorphism and superoxide dismutase (SOD) activity with the vaso-occlusive crisis (VOC) and acute splenic sequestration (ASS) in children with sickle cell anemia (SCA). One hundred ninety-five children with SCA aged 1-9 years old were analyzed. The TC and CC genotypes were associated with lower SOD activity compared with the TT genotype ( $p=0.0321$ ;  $p=0.0253$ , respectively). Furthermore, TC and CC were more frequent in patients with VOC or ASS ( $p=0.0285$ ;  $p=0.0090$ , respectively). These results suggest that the *SOD2* polymorphism associated with low SOD activity could be a susceptibility factor for the occurrence of VOC and ASS.*

**Introduction.** Sickle erythrocytes trigger vaso-occlusive events such as vaso-occlusive crisis (VOC) and acute splenic sequestration (ASS), mainly in sinuous circulation where there is a limited terminal arterial blood supply.<sup>1</sup> Recurrent ischemia-reperfusion, subsequent activation of the endothelium and vascular injury induce continuous inflammatory responses in sickle cell anemia (SCA).<sup>2</sup> Reactive oxygen species (ROS) can cause significant damage to erythrocytes, reducing their lifespan, especially in patients with SCA.<sup>3</sup>

Superoxide dismutase (SOD) is responsible for the first major cellular antioxidant defense, and it catalyzes the dismutation of the superoxide anion

into hydrogen peroxide.<sup>4</sup> Manganese superoxide dismutase (MnSOD) isoform is encoded by the *SOD2* gene, which is addressed to the mitochondria. Its role in the cell's defense against the deleterious effects of ROS is evident. The polymorphism -9 T→C (V16A) in the mitochondrial targeting sequence of the gene results in the substitution of valine by alanine.<sup>5</sup> Studies in different diseases and populations revealed that the variant allele C of *SOD2* reduces MnSOD catalytic activity.<sup>4,6</sup>

Patients with SCA (HbSS) presented reduced SOD activity when compared to the control (HbAA).<sup>7</sup> In addition, Schacter et al.<sup>7</sup> observed that patients with SCA presenting severe manifestations had lower SOD activity.

Because *SOD2* has a wide range of allele frequencies depending on ethnicity,<sup>8</sup> and the Brazilian population has mixed ancestry, our study aimed to investigate the frequency of *SOD2* polymorphisms in the controls and in patients with SCA.

**Material and Methods.** The study included 173 children with SCA (HbSS), aged 1-9 years old with a median age of 4 years; 52% were male. These patients were followed up at neonatal screening, from 2002 until December 2012, and diagnosed at the Hematology Hospital of the HEMOPE Foundation. Clinical data were collected from the records in the medical files. The clinical events considered were ASS and VOC, which consisted of painful crisis (severe pain in

the abdomen and/or chest), dactylitis (severe pain that affects the bones of the hands and/or the feet) and acute thoracic syndrome (which was confirmed by radiographic pulmonary infiltrates after the occurrence of thoracic pain). The control group included 172 blood donor volunteers without SCA and sickle cell trait (HbAS), aged 18 to 56 years, with a median age of 33 years; 60% were male.

The patients selected for SOD determination were included randomly during the routine consultations at HEMOPE, and they were clinically stable, without the use of erythropoietin or hydroxyurea and/or without transfusion history in the last three months.

*SOD2* polymorphism analysis (rs4880) was performed by real time PCR using the Rotor Gene 6000™ apparatus (Corbett Research Mortlake, Sydney, Australia) and Taqman Genotyping Assays (ID: C\_\_8709053\_10). SOD catalytic activity was determined in the plasma of patients using the Superoxide Dismutase Assay KIT according to the manufacturer's protocol (Cayman Chemical, Ann Arbor, MI, USA).

The Hardy-Weinberg equilibrium test was performed using ARLEQUIN software (Geneva, Switzerland), and the differences of frequencies were analyzed using the Chi-square test with Yates' correction using 2×2 contingency tables. The T-Student was used to see differences in SOD activity. The statistical analyses were performed using EPinfo (CDC, Atlanta, USA), with  $p < 0.05$  considered significant.

**Results.** Genotypic frequencies of the *SOD2* polymorphism in **Table 1** showed no difference between the control group and the patients ( $p=0.7915$ ). The populations were in Hardy-Weinberg equilibrium.

SOD activity was associated with the *SOD2* polymorphism. TC ( $p=0.0321$ ) and CC ( $p=0.0253$ ) genotypes were related to decreased activity compared with TT (**Figure 1**). However, the SOD activity was not associated with VOC or ASS ( $p=0.7603$ ;  $p=0.6909$ , respectively). On the other hand, a positive association was found between the genotypes (TC+CC) of *SOD2*, related to lower SOD activity, and the presence of one or more than one VOC or ASS episodes ( $p=0.0285$ ;  $p=0.0090$ , respectively) (**Table 1**).

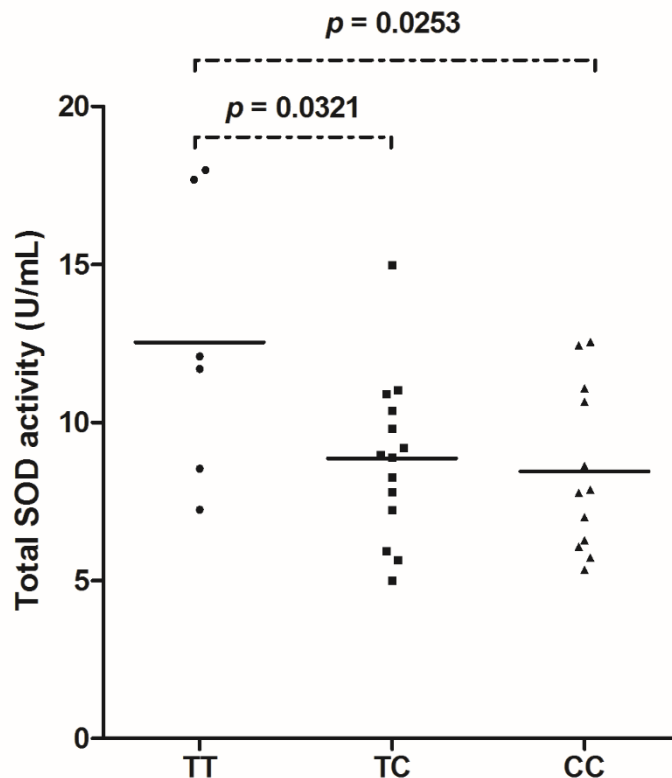
**Discussion.** The frequency of *SOD2* genotypes was associated with VOC and ASS in children with SCA. Our study determined, for the first time, the frequency of the *SOD2* polymorphism in Brazilian blood donor volunteers as well as in patients with SCA. The C allele frequency in the blood donors was 0.53, similar to healthy Caucasians (0.50);<sup>8</sup> however, it was higher than the Japanese population (0.015).<sup>9</sup> In contrast, the allele C frequency in patients with SCA was 0.35 in the Turkish population,<sup>10</sup> while it was 0.52 in our study. The Turkish study, as well as ours, showed no difference in *SOD2* frequency when comparing patients with SCA to the healthy individuals.

The present study found an association between *SOD2* genotypes (TC+CC) and the presence of VOC and ASS. The association between the

**Table 1.** Genotypic frequencies of *SOD2* polymorphism and its association with clinical events in children with sickle cell anemia treated in Hemope Foundation, Recife/Brazil.

<i>SOD2</i>	Controls (N=172)	SCA (N=173)	VOC (+) (N=153)	VOC (-) (N=20)	ASS (+) (N=64)	ASS (-) (N=60)
Genotypes						
TT	42 (0.25)	42 (0.24)	33 (0.22)	09 (0.45)	08 (0.12)	22 (0.33)
TC	76 (0.44)	82 (0.48)	75 (0.49)	07 (0.35)	39 (0.61)	24 (0.37)
CC	54 (0.31)	49 (0.28)	45 (0.29)	04 (0.20)	17 (0.27)	20 (0.30)
TC+CC	130 (0.75)	131 (0.76)	120 (0.72)	11 (0.55)	56 (0.88)	44 (0.67)
Statistical Analysis (TT vs TC+CC)				$p^*$	OR	CI
VOC (+) vs VOC (-)				0.0285	2.98	1.03 – 8.58
ASS (+) vs ASS (-)				0.0090	3.50	1.32 – 9.53

SCA= sickle cell anemia; VOC(+)= patients with SCA and presence of vasoocclusive crisis; VOC(-)= patients with SCA and absence of vasoocclusive crisis; ASS= patients with SCA and presence of acute splenic sequestration; ASS= patients with SCA and absence of acute splenic sequestration. \*Chi-squared test with the Yates correction (OR CI 95%).



**Figure 1.** Total superoxide dismutase (SOD) activity with *SOD2* genotypes in patients with sickle cell anemia. TT (n=6, mean=12.54±1.84); TC (n=14, mean=8.87±0.69); CC (n=12, mean=8.45±0.75). TTxCC,  $p=0.0253$ ; TTxTC,  $p=0.0321$ . T test. T–Wild-type Allele; C –Variant Allele.

genotypes of decreased SOD activity (TC+CC) with ASS could be explained by the fact that macrophages in the spleen of patients with SCA would be highly activated promoting the phagocytosis of sickled cells, however, presenting diminished ability to control oxidative stress. Consequently, these macrophages could deregulate the homeostasis, contributing to the production of pro-inflammatory cytokines such as TNF- $\alpha$  found during VOC episodes,<sup>11</sup> that induces *SOD2* expression to protect cells from the TNF- $\alpha$  pro-inflammatory effects.<sup>12</sup>

Human MnSOD precursor variants imported into rat liver mitochondria showed that the C allele precursor generated higher activity when compared to the T allele.<sup>5</sup> It was demonstrated that the C allele seems to allow efficient import of MnSOD into the mitochondrial matrix, while the variant T allele appears to cause a partial arrest of the precursor within the inner membrane and a decreased formation of the active MnSOD homotetramer in the mitochondrial matrix.<sup>5</sup>

On the other hand, Martin et al.<sup>4</sup> showed that the C allele of the *SOD2* polymorphism was associated with lower catalytic activity of MnSOD

in cryopreserved hepatocytes. Bastaki et al.<sup>6</sup> also found an association between the allele C and low activity of MnSOD in erythrocyte isolates of 231 healthy volunteers. In part, these reports are in accordance with our results, except that we determined the total SOD activity in the present work. The divergent effects of the allelic variants of *SOD2* could be related to the experimental conditions of each study, indicating that the impact of the polymorphism could differ in regards to the species, tissue and organ compartment, such as plasma, spleen or liver.

Studies about the effects of *SOD2* as marker of oxidative stress suggest a protective role of the T allele. Hong et al.<sup>13</sup> observed increased formation of 8-OHdG, a common biomarker of DNA damage induced by ROS, in individuals carrying MnSOD TC/CC genotypes. Accordingly, Park et al.<sup>14</sup> found that TC/CC genotypes modulate the effect of 1-OHP (a biomarker of exposure to PAHs), resulting in increased oxidative damage compared with the TT genotype. Thus, although these studies did not measure the functional enzyme activity, they support the idea that the T allele could be a marker for ROS protection.

Manfredini et al.<sup>15</sup> showed increased SOD activity in hemolysates of patients with SCA when compared to the healthy individuals, indicating the activation of chronic oxidative stress in the patients with SCA. However, Schacter et al.<sup>7</sup> showed association of lower SOD activity with severe manifestations in patients with SCA. Therefore, the individuals presenting genetic predisposition to have lower SOD activity would have difficulties to compensate the ROS triggered by sickling cells.

Our findings indicate that the *SOD2* C allele may affect the SOD activity hampering innate cell response to oxidative stress, which plays an essential role in the occurrence of VOC and ASS. However, further functional studies should be

conducted to clarify the role of the *SOD2* polymorphism and its influence in SCA.

Therefore, the polymorphism of *SOD2* could be considered as a genetic marker for predisposition of VOC and ASS. However, the genetic frequencies of SNPs should be limited to the studied population due to the small sample size. In addition, the combined analysis of SNPs and serum levels strongly suggests that these SNPs could have a significant influence in the variation of serum levels, which could influence the occurrence of VOC and ASS in SCA.

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