Sickle Cell Anemia – Nitric Oxide related genetic modifiers of hematological and biochemical parameters

Laura Aguiar1, Andreia Matos1, Ângela Gil5, Conceição Afonso5, Lígia Braga2, João Lavinha5, Paula Kjollerstrom2, Paula Faustino5, Manuel Bicho5, Ângela Inácio4,1

1Laboratório de Genética, Faculdade de Medicina da Universidade de Lisboa (FMUL), Lisboa
2Unidade de Hematologia do Hospital de Dona Estefânia (HDE), Lisboa
3Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge (INS), Lisboa
4Instituto De Investigação Científica Bento Da Rocha Cabral (IICBRC), Lisboa

INTRODUCTION

Sickle Cell Anemia (SCA) is an inherited blood disorder characterized by the presence of hemoglobin S (Hb S). This disease is caused by a single mutation in the beta-globin gene with a corresponding amino acid substitution at the sixth position of the beta-globin chain. The easy ability of Hb S to polymerize in deoxygenated conditions gives rise to abnormal sickled red blood cells (Figure 1) (Rees et al, 2010). Vaso-occlusion and hemolytic anemia are the major features of this disease, however SCA patients present clinical and hematologic variability that cannot be only explained by the single mutation in the beta-globin gene. Others genetic modifiers and environmental effects are important in the clinical phenotype (Steinberg & Sebastiani, 2012). SCA patients present arginine deficiency that contributes to a lower nitric oxide (NO) bioactivity. The amino acid citrulline increases arginine levels and promotes NO production (Kare et al, 2013).

The aim of this work was to determine the association between hematological or biochemical parameters and genetic variants from candidate genes, in SCA patients. Effects of oral citrulline supplementation in SCA were also considered.

METHODS

Subjects: 26 paediatric SCA patients (mean age of 8.58 years) followed-up in Hospital de Dona Estefânia, in Lisbon.

Hematological or biochemical parameters: Hb S, total Hb, red cell distribution width (RDW), levels of leukocytes, levels of neutrophils, transmembrane reductase, methemoglobin reductase, myeloperoxidase (MPO), serum lactate dehydrogenase (LDH), total bilirubin and reticulocyte count.

Candidate genes: BCL11A, HBA, HBB cluster, HMOX1, eNOS, MTHFR and MPO.

Citrulline supplementation: The citrulline was given orally in daily doses of approximately 0.1 g/kg for 6 months.

Statistical analysis: Association studies were performed using T test/ ANOVA parametric tests or Mann-Whitney/Kuskal-Wallis non-parametric tests. ANOVA, Kuskal-Wallis and Spearman’s correlation were used to measure differences between control (before citrulline intake) and months 1, 3 and 6 of citrulline supplementation. All tests were performed with SPSS 22.0 software.

RESULTS

The following significant associations were observed (Table 1, 2 and 3, Figure 2, 3 and 4).

Results from this study show a significant statistical association between some parameters and genetic variants at eNOS gene. An increased reticulocyte count and high serum lactate dehydrogenase levels were associated with both the rs2070744 TT and the rs1799983 GG genotypes at eNOS gene and high levels of neutrophils were associated with the eNOS4a allele.

A symptomatic improvement was observed in patients with citrulline supplementation.

Table 1 - Association between the parameters reticulocyte count and LDH and rs2070744 genotypes (TT and CT) at eNOS gene

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TT</th>
<th>CT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulocyte</td>
<td>0.54 ± 3.40 (11)</td>
<td>6.12 ± 2.50 (10)</td>
<td>0.005*</td>
</tr>
<tr>
<td>LDH (u/L)</td>
<td>490.00; 410-790 (7)</td>
<td>375.30; 128-451 (8)</td>
<td>0.047*</td>
</tr>
</tbody>
</table>

Table 2 - Association between the parameters reticulocyte count and LDH and rs1799983 genotypes (GG and GT/TT) at eNOS gene

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GG</th>
<th>GT/TT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulocyte</td>
<td>9.20 ± 2.31 (17)</td>
<td>4.53 ± 1.79 (5)</td>
<td>0.006*</td>
</tr>
<tr>
<td>LDH (u/L)</td>
<td>490.00; 410-790 (7)</td>
<td>375.30; 128-451 (8)</td>
<td>0.062*</td>
</tr>
</tbody>
</table>

Table 3 - Association between the parameter levels of neutrophils and the presence or absence of eNOS4a allele at eNOS gene

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Presence of eNOS allele</th>
<th>Absence of eNOS allele</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels of neutrophils (10e06/uL)</td>
<td>5.43±1.60; 1.890 (7)</td>
<td>3.61±0.0; 1.147,70 (5)</td>
<td>0.045*</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Our findings suggest that polymorphisms in the eNOS gene may act as genetic modifiers of the haemolysis, which could provide utility for the prediction of increased susceptibility to haemolysis-related complications.

Furthermore, our results reinforce the importance of nitric oxide (NO) bioactivity in SCA. We presume that NO, and possible its precursors such as arginine or citrulline, might be used as pharmacological tools to improve the quality of life of these patients.

References