Gene Abnormalities in Transplant Associated-Thrombotic Microangiopathy: Comparison between Recipient and Donor’s DNA

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Gavrilak et al.,1 in their very interesting analysis on the development of transplant associated-thrombotic microangiopathies (TA-TMAs), suggested that genetic susceptibility was evident in transplant recipients, but not in controls or in donors. These results are in contrast with our experience that instead suggests that abnormalities in the donor’s genes, usually involved in TMAs,2–4 such as C3, CFB, CFH and related, CFI, CD46, THBD, and DGKE, could be transferred from the donor to the recipient after full bone marrow chimerism is achieved following allogeneic hematopoietic stem cell transplantation (HSCT). In our previous study,5 we described 6 out of 16 patients having variants received from their respective donors. Based on these preliminary results, we extended the cohort of investigated TA-TMA patients to include referrals to our center between 2008 and 2021. Methods of genetic analysis are provided in detail elsewhere6 and patients (or both parents in case of minors) gave their written consent to use the results of the genetic analysis for research purposes.

Our population was made up of 80 patients with a median age of 30 years (interquartile range [IQR]: 17–50 years): 46% were females, 39% were pediatric patients; the median time between HSCT and TA-TMA was 77 days (IQR: 50.3–174.5 days), 88% had an allogenic transplant, 64% from a related donor, 33% from an HLA (human leucocyte antigen) fully matched donor, 52% received peripheral blood stem cell as a graft source, and 70% underwent myeloablative conditioning.

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regimen. In 37 patients (out of 80) both pretransplant and donor DNAs were available for the analysis. Amongst these patients, in four cases both the patient (pretransplant DNA) and the related donor had the same variants; these patients were excluded from the statistical analysis since these alterations could not be attributed to either the recipient or the donor. In the remaining 33 patients, the proportion of subjects with variants in the investigated genes increased from 12% in the pretransplant DNA to 33% in the posttransplant DNA (the donor’s DNA), corresponding to a threefold relative risk (RR = 2.75; 95% confidence interval: 0.95–7.98; matched analysis = 0.333/0.121 = 2.75). Additionally, five patients had multiple variants in their posttransplant DNA (Table 1).

The distribution of TMA-related gene variants in the population is reported to be 5 to 12%,7-10 therefore the increased frequency (>30%) among donors of patients who subsequently develop TA-TMA needs to find an explanation.

The present study confirms our previous hypothesis that genetic risk may be transferred from donors to the recipients of HSCT transplant. These findings are in marked contrast with those of Gavriilaki et al, who described a significantly increased frequency of mutations in pretransplant DNA of TA-TMA patients. Furthermore, in our study all identified variants were either benign or of unknown significance (Table 1), while Gavriilaki et al found a surprising high number of pathogenic mutations. The discrepancies among these two studies are astonishing and according to our experience may be only explained either by a fundamentally different population (ethnicity, diagnostic criteria, etc.) or by the different approach used to classify the variants (Fh HUS database supplemented by six others in our study and ClinVar database and three others in Gavriilaki et al).

### Table 1 Variants identified in the DNA of TA-TMA patients: recipient versus donor

<table>
<thead>
<tr>
<th>Gene/LGR</th>
<th>cDNA</th>
<th>Protein</th>
<th>ACMG classification</th>
<th>Recipient (n)</th>
<th>Donor (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td>c.1548T &gt; A</td>
<td>p.Asns516Lys&lt;sup&gt;b&lt;/sup&gt;</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>c.741C &gt; T</td>
<td>p.Asns516Lys</td>
<td>VUS</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>CFH</td>
<td>c.3236C &gt; G</td>
<td>p.Thr1079Ser</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFI</td>
<td>c.4645C &gt; A</td>
<td>p.Leu1549Met&lt;sup&gt;c&lt;/sup&gt;</td>
<td>B</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFH</td>
<td>c.4855A &gt; C</td>
<td>p.Ser1619Arg</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFI</td>
<td>c.1217G &gt; A</td>
<td>p.Arg406His&lt;sup&gt;d&lt;/sup&gt;</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFI</td>
<td>c.1246A &gt; C</td>
<td>p.Ile416Leu&lt;sup&gt;e&lt;/sup&gt;</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MCP/CD46</td>
<td>c.38C &gt; T</td>
<td>p.Ser13Phe&lt;sup&gt;d&lt;/sup&gt;</td>
<td>B</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFB</td>
<td>c.502A &gt; C</td>
<td>p.Arg168Val</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFHR1</td>
<td>c.615G &gt; A</td>
<td>p.Thr205Thr</td>
<td>B</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFHR3</td>
<td>c.50A &gt; C</td>
<td>p.Thr205Thr</td>
<td>B</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFHR5</td>
<td>c.434G &gt; A</td>
<td>p.Arg127His&lt;sup&gt;b&lt;/sup&gt;</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CFB</td>
<td>c.485_486dupAA</td>
<td>p.Glu163Lysfs&lt;sup&gt;10&lt;/sup&gt;</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DGKE</td>
<td>c.59G &gt; A</td>
<td>p.Glu20Glu&lt;sup&gt;c&lt;/sup&gt;</td>
<td>B</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>THBD</td>
<td>c.584G &gt; T</td>
<td>p.Arg195Ile&lt;sup&gt;e&lt;/sup&gt;</td>
<td>VUS</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LGR</td>
<td>CFHR3-CFHR1 homozygous deletion</td>
<td>p.?</td>
<td>VUS</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** ACMG, American College of Medical Genetics; B, benign; LGR, large genomic rearrangement; VUS, variant of unknown significance.

<sup>a</sup>CFH: NM_000186, NP_000177; C3: NM_000064, NP_000055; CF: NM_000204, NP_000195; CD46: NM_002389, NP_002380; CFB: NM_001710, NP_001701; CFHR1: NM_002113, NP_002104; CFHR3: NM_021023, NP_066303; CFHR5: NM_030787, NP_110414; DGKE: NM_003647, NP_003638; THBD: NM_003631, NP_003632.<br>
<sup>b</sup>-<sup>g</sup>Variants marked with the same letter were identified in the posttransplant DNA of the same patient.
One of the implications of our results is that the donors, who exhibited most of the identified variant of unknown significance (12 vs. 3), may have the same potential to develop a complement dysregulation as the TA-TMA patients. However, unlike the donors, the recipients are exposed to triggers that could explain the onset of clinical signs.

In terms of pathophysiology, other complement-related TMAs, such as aHUS, are explained by a “second and third hit” hypothesis, where the genetic predisposition needs to be triggered in the patient to initiate complement overactivation.11 If this is the case, and the recipient's DNA was the source of the TMA-related gene variant as postulated by Gavrillaki et al, we assume that the patient should have already exhibited the disease in one of the many insults occurring before HSCT. These include their severe underlying primary disease, previous treatments, treatment complications, and, last but not least, the conditioning regimen given for transplantation and the administration of immunosuppressive drugs for GVHD (graft-versus-host disease) prophylaxis. Since TA-TMA patients have not experienced a thrombotic microangiopathy before transplant, a clearly pathogenic variant is unlikely. Therefore, a possible additional factor is the donor's hematopoietic stem cells that colonize the bone marrow and give rise to their progeny including donor-derived monocytes, part of the reticuloendothelial system (Kupffer cells), which also produce regulatory proteins in the body.12

Based on these findings and reasoning, we believe that more studies are needed in this area to clarify the pathogenic role of the identified variant to understand the genetic susceptibility to TA-TMA, to potentially screen both the donor and the patient for TMA-related gene variants.

A final point is that, while Gavrillaki et al reported an overall transplant-related case-fatality rate of 65%, and a similar rate in those treated with C5 inhibition (Soliris, Alexion Pharma Inc., Boston, Massachusetts, United States), in our cohort 30 patients had been exposed to C5 inhibition, with a case-fatality rate in the treated versus untreated group of 33.3 and 48.1%, respectively. Finally, the fact that in patients carrying a variant on the donor DNA and being treated with C5 inhibition (n = 9), the case-fatality rate was as low as 0% versus 50% of those who were treated without variants (n = 16) may be related to an underlying complement activation linked to the received variant.

If our results are confirmed in larger series and supported by functional tests, the screening of donors for TMA-related gene variants might be useful to better define the risk for TA-TMA development in patients addressed to transplantation.

Conflict of Interest
G.A. is a member of the SAB Scientific Advisory Board of the HUS Global Registry supported by Alexion Pharma INC.

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References
8 Fakhouri F, Frémoux-Bacchi V. Thrombotic microangiopathy in aHUS and beyond: clinical clues from complement genetics. Nat Rev Nephrol 2021;17(08):543–553
11 Khosla J, Yeh AC, Spitzer TR, Dey BR. Hematopoietic stem cell transplant-associated thrombotic microangiopathy: current paradigm and novel therapies. Bone Marrow Transplant 2018;53(02):129–137