HLA-DRB1*11 is a strong risk factor for acquired thrombotic thrombocytopenic purpura in children

by Bérangère S. Joly, Pascale Loiseau, Michael Darmon, Thierry Leblanc, Hervé Chambost, Fanny Fouyssac, Vincent Guigonis, Jérôme Harambat, Alain Stepanian, Paul Coppo, and Agnès Veyradier

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HLA-DRB1*11 is a strong risk factor for acquired thrombotic thrombocytopenic purpura in children.

Running head: HLA-DRB1*11 and acquired TTP in children.

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16 references
1 Table
2 figures
Child-onset thrombotic thrombocytopenic purpura (TTP; age <18 years old) is a very rare (prevalence of one case per million children, annual incidence of 0.2 new case per million children), heterogeneous, relapsing and life-threatening thrombotic microangiopathy (TMA). Pediatric TTP represents less than 10% of all TTPs which mostly remains an adult-onset disease (peak of incidence: 30-40 years old). At the acute phase, TTP is defined by a microangiopathic hemolytic anemia and a severe thrombocytopenia, associated with multivisceral ischemic disorders. TTP pathophysiology is based on a severe deficiency of ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats, member 13), the specific von Willebrand factor (VWF)-cleaving protease. ADAMTS13 deficiency induces the accumulation of platelet-hyperadhesive ultralarge VWF multimers, leading to the spontaneous formation of platelet-rich microthrombi in the microcirculation and subsequent multivisceral organ ischemia. ADAMTS13 deficiency may be either congenital (linked to biallelic mutations of ADAMTS13 gene) or acquired (mostly linked to the presence of anti-ADAMTS13 autoantibodies). Characterization of anti-ADAMTS13 autoantibodies showed that the immune response against ADAMTS13 is polyclonal and mostly targets the spacer domain of ADAMTS13 with predominant immunoglobulin G (IgG) 1 and IgG4 subclasses. Pathophysiological mechanisms involved in the loss of tolerance of the immune system towards ADAMTS13 are still unknown.

The major histocompatibility complex (MHC), or Human Leukocyte Antigen (HLA) region, encompasses 7.6 Mb on chromosome 6p21, and encodes key immune response genes. The HLA region contains the largest degree of polymorphism within the genome and the most dense linkage disequilibrium. HLA class II encodes HLA-DR and -DQ molecules which present exogenous antigen on antigen-presenting cells to CD4+ T cells. The interaction of T-cell receptor (TCR) with a peptide/HLA class II complex on professional antigen-presenting cells is required for CD4+ T-cells activation. A strong association between the HLA region, the generation of autoantibodies against self-antigen and autoimmune diseases has been described. Four European independent groups identified HLA-DRB1*11 antigen as a strong risk factor for autoimmune TTP in Caucasian people suggesting that CD4+ T helper (Th) cells contribute to the pathogenesis of the disease, but this risk factor was not found in Black people. HLA-DQB1*03 and HLA-DQB1*03:01 were also reported as a risk factor for autoimmune TTP. A recent Italian study also suggested an association of rs6903608 variant and HLA-DQB1*05:03 with acquired TTP. Moreover, HLA-DRB1*04 was found underrepresented in acquired TTP patients, and is likely to protect against the development of the
disease.\textsuperscript{6,9–12} Also, peptides derived from the CUB1 and CUB2 domains of ADAMTS13 are potential immunodominant T-cell epitopes in TTP patients.\textsuperscript{14}

All studies devoted to the investigation of the HLA system in acquired TTP were conducted exclusively in adult patients.\textsuperscript{6,9–13} For this study, we hypothesized that particular HLA alleles may be involved in the process leading to a loss of tolerance of the immune system against ADAMTS13 in child-onset acquired TTP patients and we focused on the allele frequencies of HLA-DRB1*11, -DRB1*04 and -DQB1*03.

From January 2000 to June 2019, 1296 children with a clinical suspicion of TMA were enrolled prospectively and consecutively in the Registry of the French Reference Center for TMA. We identified 99 consecutive children with a diagnosis of TTP (ADAMTS13 activity <10 IU/dL at presentation). Fifty-two of them (53\%) presented an acquired TTP defined by positive anti-ADAMTS13 IgG during an acute episode and/or a recovery of ADAMTS13 activity in remission (Figure 1).\textsuperscript{1} ADAMTS13 phenotypic investigations were performed as previously described.\textsuperscript{1} Twenty-six patients (26/52, 50\%) had DNA available for HLA class II typing (DRB1 and DQB1 loci). We specifically considered these alleles as candidate risk factors for autoimmunity in acquired TTP.\textsuperscript{6,9–13} The blood samples for HLA typing were analyzed for HLA-DRB1 and -DQB1 alleles as described previously.\textsuperscript{9,15} Both HLA-DRB1 and -DQB1 allele and phenotype frequencies were calculated in all cases. HLA phenotype and allele frequencies were compared to those of the Allele Frequency Net Database (AFND) (\url{http://www.allelefrequencies.net}) reported in France.\textsuperscript{16} Allele and phenotype frequencies of HLA-DRB1*11, -DQB1*03 and -DRB1*04 observed in child-onset acquired TTP were compared to those observed in the French population (AFND), using a probabilistic approach. Moreover, these frequencies observed in our cohort were compared to those reported by Coppo and collaborators in Caucasian people, including 172 healthy volunteers and 61 adult-onset acquired TTP patients.\textsuperscript{9} A two-sided \textit{p-value} less than 0.025 was considered as significant, \textit{p-value} above 0.025 was considered as non-significant (NS). The study was approved by the institutional review board of Pitié-Salpêtrière hospital, registered with ClinicalTrials.gov, number NCT00426686, and informed consent was obtained from all patients or their parents.

Twenty-six children with a diagnosis of acquired TTP were included in the current study: 18 patients had an idiopathic presentation and 8 patients had an associated clinical context (autoimmune
diseases, infections, nephropathy and liver insufficiency). The sex ratio was 1.9F/M (17 girls and 9 boys) and 18 patients were Caucasian. Anti-ADAMTS13 IgG were positive at diagnosis in all but two patients. The latter had either an inherited nephropathy or an immunosuppressive treatment for lupus nephritis both diagnosed prior to TTP.

The comparison of HLA-DRB1 and -DQB1 phenotypes between acquired TTP patients and French healthy individuals revealed a significant difference for the three alleles (HLA-DRB1*04, -DRB1*11 and DQB1*03) (Table 1). The most striking difference involved HLA-DRB1*11 that was found positive in 54% of our patients (n=14/26) although this phenotype is reported in 27% of French healthy individuals (http://www.allelefrequencies.net) (p<0.001) and 23% of Caucasian healthy individuals (p<0.001) (Table 1, Figure 2A). In our patients, the positivity of HLA-DRB1*11 was further emphasized when considering either Caucasian patients (72%), idiopathic TTP (61%) or both idiopathic TTP and Caucasian ethnicity together (71%) (Table 1). Although HLA-DRB1*11 was carried with an homozygous status in only 2 patients (2/26, 8%), the frequency of HLA-DRB1*11 alleles (31%) remained significantly higher than that of French healthy individuals (13%, p<0.01) and Caucasian healthy individuals (12%, p<0.01) (Table 1, Figure 2B). Interestingly, HLA-DRB1*11 phenotype was systematically associated with HLA-DQB1*03 in all our patients. HLA-DQB1*03 phenotype was found positive in 81% of our patients, higher than the ones reported in French healthy individuals (72%, NS) and Caucasian healthy individuals (54%, p<0.001) (Table 1). HLA-DQB1*03:01 was present in 16 patients (62%) (versus 48% of French healthy individuals). As expected, considering its high association with HLA-DRB1*11, HLA-DQB1*03 was further positive in pediatric Caucasian patients with idiopathic TTP (86%) (Table 1). Five patients (5/26, 19%) were homozygous for HLA-DQB1*03 but the frequency for HLA-DQB1*03 allele was not found significantly different between our pediatric TTP patients (50%) and French healthy individuals (47%) or Caucasian healthy individuals (34%) (Table 1). In contrast to HLA-DRB1*11 and HLA-DQB1*03, the HLA-DRB1*04 phenotype was lower, although not significantly, in our patients (2/26, 8%) as compared to French healthy individuals (24%, NS), and to Caucasian healthy individuals (28%, p<0.025) (Table 1). Both these children were heterozygous for HLA-DRB1*04 and also carried an HLA-DQB1*03 allele.

Among French patients with acquired TTP, HLA-DRB1*11 phenotype was present in 54% of children (vs 27% of French healthy individuals; p<0.001), 62% of Caucasian adults (vs 23% of controls; p<0.001) (Table 1, Figure 2A) and in only 24% of Black adults (vs 31% of controls). Other
European studies focused on Caucasian people have also reported higher proportion of HLA-DRB1*11 phenotype in TTP patients, as compared to controls (44-48% vs 12-24%, respectively).\textsuperscript{10,11} When compared to healthy individuals, HLA-DQB1*03 phenotype was also more common in French patients with acquired TTP including 81% of children (vs 72% of healthy controls; \textit{NS}), 77% of Caucasian adults (vs 54% of controls) (Table 1) and in 58% of Black adults (vs 25% of controls).\textsuperscript{9,12} Other European studies dedicated to HLA phenotype in Caucasian people however reported no significant difference of HLA-DQB1*03 between TTP patients and healthy individuals (62-72% vs 53-65%, respectively).\textsuperscript{10,11} Among French patients with acquired TTP, HLA-DRB1*04 phenotype was found in only 8% of children (vs 24% of French healthy individuals), in 10% of Caucasian adults (vs 28% of controls) (Table 1) and in 6% of Black adults (vs 8% of controls).\textsuperscript{9,12} In Caucasian people reported in Europe, HLA-DRB1*04 was also less represented when compared to healthy individuals (7-10% vs 25-35%, respectively).\textsuperscript{10,11} Also, the low natural frequency of HLA-DRB1*04 in Black people may be associated with the greater risk of TTP in this population.\textsuperscript{12} The profile of HLA-DRB1*11, -DQB1*03 and -DRB1*04 phenotypes in our child-onset TTP cohort is finally very similar to that reported in adult TTP patients.\textsuperscript{9-12}

In conclusion, our study further emphasizes that child-onset acquired TTP is very similar to adult-onset acquired TTP\textsuperscript{1} as HLA-DRB1*11 appears as a susceptibility factor for TTP while HLA-DRB1*04, when not associated to HLA-DQB1*03, may be protective. Also, as previous reports have shown the association between HLA-DRB1*11 and other autoimmune diseases, like systemic sclerosis, early-onset juvenile chronic arthritis, sarcoidosis, our study highlights the importance of long-term follow-up of patients with child-onset acquired TTP, to early detect the occurrence of another autoimmune disease.

\textbf{Conflict of interest.} The authors declare no conflict of interest related to this study.

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Contributions: BSJ wrote the manuscript, collected, analyzed and summarized the clinical and biological data. PL analyzed HLA typing. MD performed the statistical analysis. PL, MD and AS critically reviewed the manuscript. TL, HC, FF, VG, JH and PC enrolled the patients, provided blood samples for HLA typing and access to clinical and biological data, and critically reviewed the manuscript. AV supervised the study, co-wrote and critically reviewed the manuscript.
References


Table 1. Phenotype and allele frequencies of HLA-DRB1*11, -DRB1*04 and -DQB1*03 in the French cohort of 26 child-onset acquired TTP as compared to those reported in 61 French Caucasian adult-onset acquired TTP, in 172 French Caucasian healthy individuals and in French healthy individuals (Allele Frequency Net Database).^9,16

<table>
<thead>
<tr>
<th>HLA</th>
<th>Acquired TTP</th>
<th>Controls 1</th>
<th>Controls 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children (cohort of interest)</td>
<td>Adults</td>
<td>French healthy individuals</td>
</tr>
<tr>
<td></td>
<td>All patients</td>
<td>Caucasian and idiopathic TTP</td>
<td>Caucasian healthy Individuals</td>
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<tr>
<td></td>
<td>n=26 patients n=52 alleles</td>
<td>n=61 patients n=122 alleles</td>
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<td>n=14 patients n=28 alleles</td>
<td>n=18 patients n=36 alleles</td>
</tr>
<tr>
<td>DRB1*11</td>
<td>14 54% 16 31%</td>
<td>13 72% 15 42%</td>
<td>11 61% 13 36%</td>
</tr>
<tr>
<td></td>
<td>2 8% 2 4%</td>
<td>2 11% 2 6%</td>
<td>1 6% 1 3%</td>
</tr>
<tr>
<td></td>
<td>21 81% 26 50%</td>
<td>16 89% 20 56%</td>
<td>14 78% 18 50%</td>
</tr>
<tr>
<td>DRB1*04</td>
<td>2 8% 2 4%</td>
<td>1 6% 1 3%</td>
<td>1 7% 1 4%</td>
</tr>
<tr>
<td></td>
<td>2 8% 2 4%</td>
<td>1 6% 1 3%</td>
<td>1 7% 1 4%</td>
</tr>
<tr>
<td>DQB1*03</td>
<td>21 81% 26 50%</td>
<td>16 89% 20 56%</td>
<td>14 78% 18 50%</td>
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<tr>
<td></td>
<td>2 8% 2 4%</td>
<td>1 6% 1 3%</td>
<td>1 7% 1 4%</td>
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Legends to figures

Figure 1. Flowchart of children inclusion in the French Thrombotic Microangiopathy Registry. TMA: thrombotic microangiopathy; TTP: thrombotic thrombocytopenic purpura.

Figure 2. Phenotype (A) and allele (B) frequencies of HLA-DRB1*11 in the French cohort of child-onset acquired TTP as compared to those in adult-onset acquired TTP patients and in healthy individuals. Phenotype and allele frequencies of HLA-DRB1*11 were significantly higher in the French cohort of child-onset acquired TTP (red line) as compared to French healthy individuals (Allele Frequency Net Database, blue area) \( p < 0.001 \) and \( p < 0.01 \), respectively) and to Caucasian healthy individuals previously reported by Coppo and collaborators (red area) \( p < 0.001 \) and \( p < 0.01 \), respectively). Moreover, phenotype and allele frequencies of HLA-DRB1*11 were not different between our cohort of child-onset acquired TTP and the cohort of acquired TTP previously reported in Caucasian adults (green area).
French TMA Registry
(January 2000 – June 2019)
1296 child-onset TMA

1197 child-onset other TMA
(ADAMTS13 activity ≥10% at presentation)

99 child-onset TTP
(ADAMTS13 activity <10% at presentation)

35 child-onset congenital TTP

12 child-onset TTP
(unavailable follow-up or genotypic investigations in progress)

52 child-onset acquired TTP patients
Figure 2

A

B

DRB1*11 phenotype frequency

DRB1*11 allele frequency

- Child-onset acquired TTP (French cohort)
- French healthy individuals (World database, Allele Frequency Net Database)
- French control group in Caucasian healthy individuals (Coppo et al, J Thromb Haemost 2010)
- Adult-onset acquired TTP in Caucasian patients (Coppo et al, J Thromb Haemost 2010)
Appendix

The members of the Reference Center for Thrombotic Microangiopathies (CNR-MAT) are:

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