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

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 cases per million children), heterogeneous, relapsing and life-threatening thrombotic microangiopathy (TMA).[1] Pediatric TTP represents less than 10% of all TTP 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.[2] 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.[2] ADAMTS13 deficiency may be either congenital (linked to biallelic mutations of *ADAMTS13* gene) or acquired (mostly linked to the presence of anti-ADAMTS13 autoantibodies).[1] 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.[3–6] Pathophysiological mechanisms involved in the loss of tolerance of the immune system towards ADAMTS13 are still unknown.

The major histocompatibility complex (MHC), or the 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 the T-cell receptor (TCR) with a peptide/HLA class II complex on professional antigen-presenting cells is required for $CD4^+$ T-cells activation.[7] A strong association between the HLA region, the generation of autoantibodies against self-antigen and autoimmune diseases has been described.[8] Four European independent groups identified HLA-DRB1*11 antigen as a strong risk factor for autoimmune TTP in Caucasian people[6,9–11] suggesting that $CD4^+$ T-helper (Th) cells contribute to the pathogenesis of the disease, but this risk factor was not found in Black people.[12] HLA-DQB1*03 and HLA-DQB1*03:01 were also reported as a risk factor for autoimmune TTP.[6,9–11,13] A recent Italian study also suggested an association of the rs6903608 variant and HLA-DQB1*05:03 with acquired TTP.[13] Moreover, HLA-DRB1*04 was found underrepresented in acquired TTP patients, and is likely to protect against the development of the disease.[6,9–12] Also, peptides derived from the CUB1 and CUB2 domains of ADAMTS13 are potential immunodominant T-cell epitopes in TTP patients.[14]

All studies devoted to the investigation of the HLA system in acquired TTP were conducted exclusively in adult patients.[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).[1] ADAMTS13 phenotypic investigations were performed as previously described.[1] Twenty-six patients (26 of 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.[6,9–13]. The blood samples for HLA typing were analyzed for *HLA-DRB1* and *-DQB1* alleles as described previously.[9,15] Both *HLA-DRB1* and *-DQB1* alleles and phenotype frequencies were calculated in all cases. The HLA phenotype and allele frequencies were compared to those of the Allele Frequency Net Database (AFND) (*http://www.allelefrequencies.net*) reported in France.[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.[9] A

**Table 1.** Phenotype and allele frequencies of *HLA-DRB1*11, -DRB1*04* and *-DQB1*03* in the French cohort of 26 child-onset acquired trombothic thrombocytopenic purpura (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]

| HLA | Child-onset acquired TTP (cohort of interest) | | Child-onset acquired TTP: Caucasian patients | | Child-onset acquired TTP: Idiopathic TTP | | Child-onset acquired TTP: Idiopathic TTP in Caucasian patients | | Adult-onset acquired TTP: Idiopathic TTP in Caucasian patients[9] | | Caucasian healthy individuals[9] | | French healthy individuals[15] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n=26 patients | n=52 alleles | n=18 patients | n=36 alleles | n=18 patients | n=36 alleles | n=14 patients | n=28 alleles | n=61 patients | n=122 alleles | n=172 subjects | n=344 alleles | subjects | alleles |
| DRB1*11 | 14<br>*54%* | 16<br>*31%* | 13<br>*72%* | 15<br>*42%* | 11<br>*61%* | 13<br>*36%* | 10<br>*71%* | 12<br>*43%* | 38<br>*62%* | 41<br>*34%* | 39<br>*23%* | 41<br>*12%* | *27%* | *13%* |
| DRB1*04 | 2<br>*8%* | 2<br>*4%* | 2<br>*11%* | 2<br>*6%* | 1<br>*6%* | 1<br>*3%* | 1<br>*7%* | 1<br>*4%* | 6<br>*10%* | 6<br>*5%* | 48<br>*28%* | 55<br>*16%* | *24%* | *13%* |
| DQB1*03 | 21<br>*81%* | 26<br>*50%* | 16<br>*89%* | 20<br>*56%* | 14<br>*78%* | 18<br>*50%* | 12<br>*86%* | 15<br>*54%* | 46<br>*77%* | 56<br>*46%* | 92<br>*54%* | 117<br>*34%* | *72%* | *47%* |

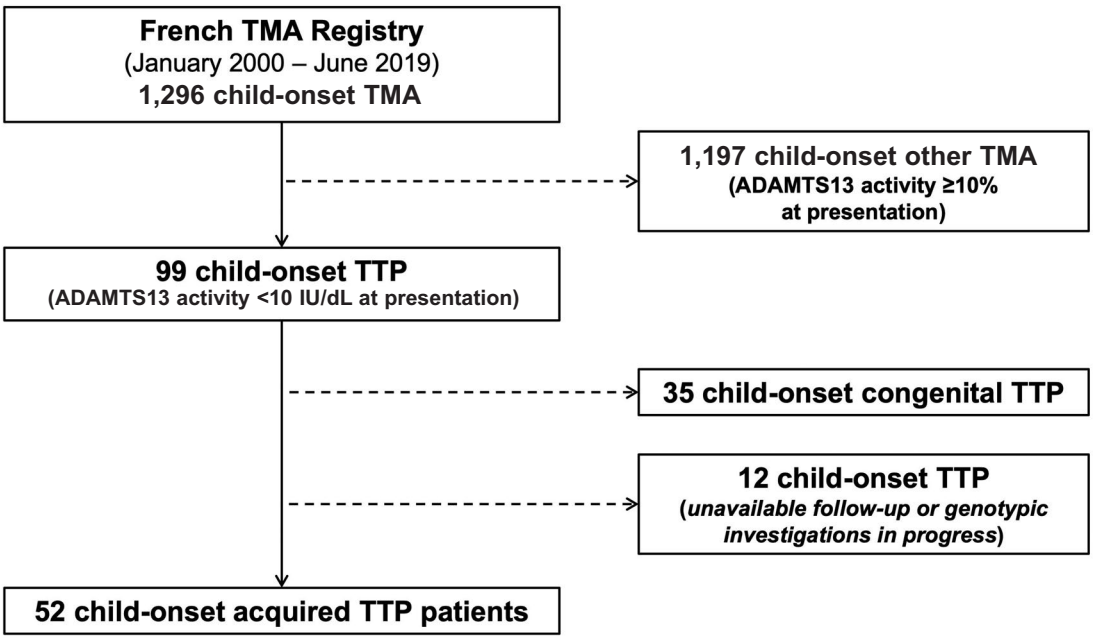


**Figure 1. Flowchart of children inclusion in the French Thrombotic Microangiopathy Registry.** TMA: thrombotic microangiopathy; TTP: thrombotic thrombocytopenic purpura.

two-sided *P*-value less than 0.025 was considered significant, a *P*-value above 0.025 was considered non-significant (NS). The study was approved by the Institutional Review Board of Pitié-Salpétrière Hospital, registered with *clinicaltrials.gov. NCT002426686*, 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 1.9 F/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 and Figure 2A).[9,16] 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 two 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 and Figure 2B).[9,16] Interestingly, the *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).[9,16] *HLA-DQB1*03:01* was present in 16 patients (62% *vs.* 48% of French healthy individuals).[16] 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 of 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).[9,16] In contrast to *HLA-DRB1*11* and *HLA-DQB1*03*, the *HLA-DRB1*04* phenotype was lower, although not significantly, in our patients (2 of 26, 8%) compared to French healthy individuals (24%, NS), and to Caucasian healthy individuals (28%, *P*<0.025) (Table 1).[9,16] Both of these children were heterozygous for *HLA-DRB1*04* and also carried an *HLA-DQB1*03* allele.

Among French patients with acquired TTP, the *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 and Figure 2A) and in only 24% of Black adults (*vs.* 31% of controls).[9,12] Other European studies focused on Caucasian people have also reported higher proportion of the *HLA-DRB1*11* phenotype in TTP patients, compared to controls (44-48% *vs.* 12-24%, respectively).[10,11] When compared to healthy individuals, the HLA-DQB1*03 phenotype was also more common in French patients with acquired TTP including 81% of children (*vs.* 72% of healthy controls; NS), 77% of Caucasian adults (*vs.* 54% of controls) (Table 1) and in 58% of Black adults (*vs.* 25% of controls).[9,12] Other European studies dedicated to the 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%,

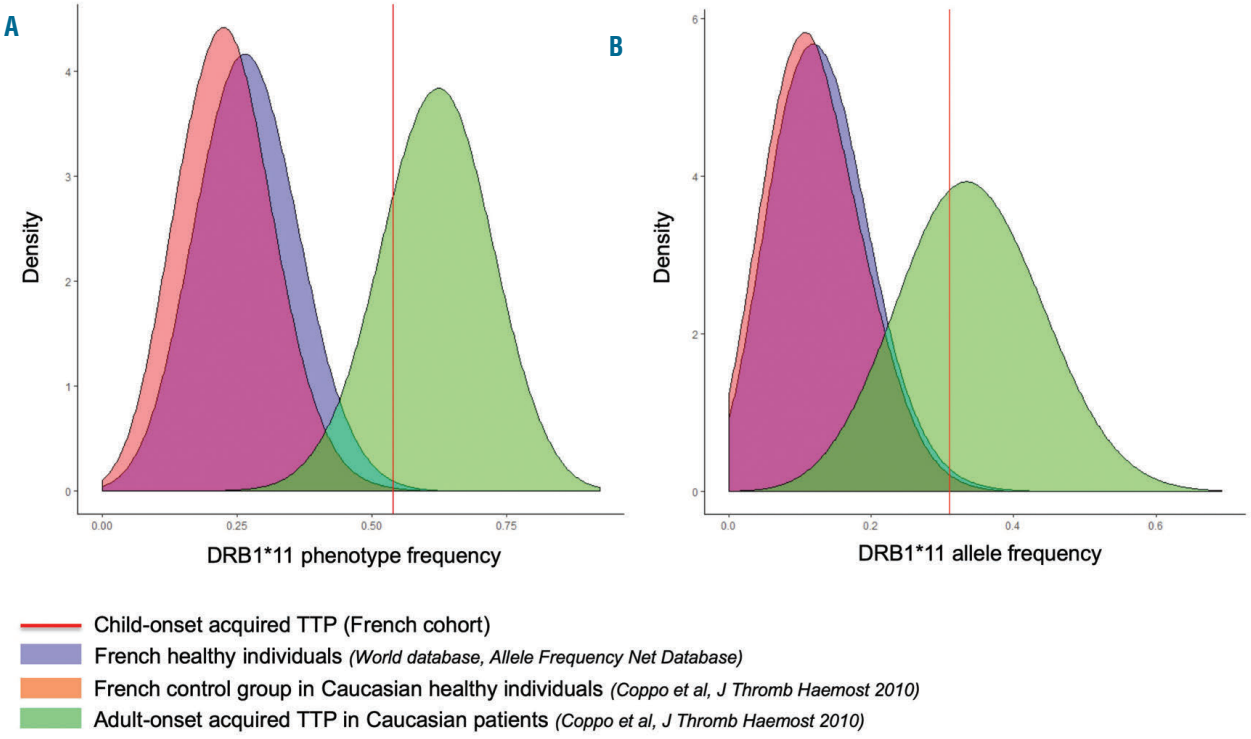


**Figure 2. Phenotype (A) and allele (B) frequencies of *HLA-DRB1*11* in the French cohort of child-onset acquired thrombotic thrombocytopenic purpura (TTP) compared to those in adult-onset acquired TTP patients and in healthy individuals.** Phenotype and allele frequencies of *HLA-DRB1*11* were significantly higher in the French cohort of child-onset acquired TTP (red line) 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).[9,16] 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).[9]

respectively).[10,11] Among French patients with acquired TTP, the *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).[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).[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.[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.[9–12]

In conclusion, our study further emphasizes that child-onset acquired TTP is very similar to adult-onset acquired TTP[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 detect the occurrence of another autoimmune disease early.

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*doi:10.3324/haematol.2019.241968*

*Funding. this study was partially supported by the National Plan for Rare Diseases of the French Ministry of Health (qualification of the French Reference Centre for Thrombotic Micro-Angiopathies) and the Programme Hospitalier Recherche Clinique AOM 05012 (DRCD Assistance Publique – Hôpitaux de Paris) and a grant from CSL-Behring (AP-HP-2017-47-26).*

*Acknowledgments: the authors wish to thank Sandrine Benghezal, Sophie Capdenat, Sandrine Malot, Sylvaine Savigny, Isabelle Turquois for expert assistance; Gilles Blondin, Fadi Fakhouri, Pascal*

*Godmer, Ferielle Louillet, Jean-Michel Rebibou, Laurent Salomon, Tim Ulinski of the Competence Centers networking with the Reference Center for Thrombotic Microangiopathies for their help in providing blood samples for HLA typing; and all the paediatricians of the French Reference Center for Thrombotic Microangiopathies listed in the appendix.*

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