ADAMTS13 gene mutations influence ADAMTS13 conformation and disease age-onset in the French cohort of Upshaw-Schulman syndrome.

Running head: French cohort of Upshaw-Schulman syndrome

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Extra Table

What is known on this topic

1. Upshaw-Schulman syndrome (USS) is the inherited form of thrombotic thrombocytopenic purpura (TTP) linked to *ADAMTS13* gene sequence variations
2. USS is characterized by a great clinical, phenotypic and genotypic heterogeneity.
3. In healthy individuals, ADAMTS13 circulates in a folded conformation where CUB domains interact with the spacer domain. The spacer-CUB interaction is abrogated when ADAMTS13 is conformationally activated.

What this paper adds

1. We performed a cohort study of 56 patients with a child-onset or an adult-onset USS from the French TTP registry.
2. Age-onset of USS defines two distinct clinical, biological and molecular entities.
3. USS-related *ADAMTS13* sequence variations modify both ADAMTS13 conformation and ADAMTS13 allosteric activation.

Abstract

Background. Congenital thrombotic thrombocytopenic purpura (TTP) or Upshaw-Schulman syndrome (USS) is a rare, life-threatening, inherited thrombotic microangiopathy (TMA). USS is mostly due to bi-allelic recessive sequence variations of *ADAMTS13* gene inducing a severe ADAMTS13 deficiency (activity <10 IU/dL). In healthy individuals, ADAMTS13 circulates in a folded conformation where CUB domains interact with the spacer domain. The spacer-CUB interaction is abrogated when ADAMTS13 is conformationally activated.

Objectives. To evaluate the influence of *ADAMTS13* sequence variations on both clinical/biological phenotype and ADAMTS13 conformation in USS.

Patients/Methods: All USS patients from the French registry for TMAs (January 1st 2000 to June 1st 2017) were investigated for ADAMTS13 genotype, phenotype (activity, antigen and autoantibodies) and conformation. Clinical records were analyzed (inaugural acute TTP and follow-up). Child-onset USS was compared to adult-onset USS.

Results. 56 USS patients from 51 families (34 child-onset and 22 adult-onset cases) were enrolled. Child-onset USS was characterized by a large panel of *ADAMTS13* sequence variations (n=43), spread all over *ADAMTS13* gene and not correlated with either clinical features or plasmatic ADAMTS13 parameters. In contrast, adult-onset USS, consisting exclusively in pregnancy-induced TTP, included a smaller and distinct panel of *ADAMTS13* sequence variations (n=20) because of one mutation (p.Arg1060Trp) present in 82% of patients. ADAMTS13 conformation was studied in 16 USS patients (5 child-onset and 11 adult-onset USS, encompassing 16 distinct *ADAMTS13* sequence variations) whose ADAMTS13 antigen levels were detectable: 14 patients/16 (87.5%) exhibited abnormalities of ADAMTS13 conformation.

Conclusion. In USS, age-onset defines two entities and *ADAMTS13* sequence variations modify ADAMTS13 conformation.

Key words

ADAMTS13 – Thrombotic thrombocytopenic purpura – Upshaw-Schulman syndrome – Von Willebrand factor – Rare disease

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare (annual prevalence ~10 cases per million), relapsing and life-threatening thrombotic microangiopathy (TMA) occurring mostly during adulthood (~90% of all TTP cases).[1–3] Clinically, acute phases of TTP are defined by a microangiopathic mechanical hemolytic anemia, a severe thrombocytopenia that may be associated with systemic visceral ischemia. TTP pathophysiology is based on a severe functional deficiency (activity <10 IU/dL) of ADAMTS13 (*A Disintegrin and Metalloprotease with ThromboSpondin type 1 repeats, member 13*), the specific von Willebrand factor- (VWF) cleaving protease.[4,5] ADAMTS13 severe deficiency leads to the accumulation of hyperadhesive ultralarge VWF multimers inducing the spontaneous formation of platelet-rich microthrombi within arterioles and capillaries.[4,5] The mechanism for severe ADAMTS13 deficiency is mostly acquired *via* ADAMTS13 autoantibodies and leads to the autoimmune TTP representing ~95% of cases.[2,5] More rarely, ADAMTS13 severe deficiency is genetically inherited, mostly *via* biallelic mutations of *ADAMTS13* gene or, in very rare cases, *via* monoallelic mutations associated with a cluster of single nucleotid polymorphism (SNPs), and leads to the congenital TTP also named Upshaw-Schulman syndrome (USS, OMIM #274150) and representing ~5% of cases.[3,6]

So far, about 200 USS cases involving ~180 distinct *ADAMTS13* sequence variations have been described worldwide thanks to the first report of *ADAMTS13* sequence variations,[7] to the international hereditary TTP registry (www.ttpregistry.net) and to national USS registries.[8–15] USS is more frequent among child-onset TTP when compared to adult-onset TTP (about one third of cases *versus* less than 5% of cases, respectively)[6,16] and its clinical presentation is significantly different as a function of its age-onset. Child-onset USS usually starts in the neonatal period with hematological features and severe jaundice.[12,17] In contrast, almost all cases of adult-onset USS are unmasked during the first pregnancy of childbearing age women whose disease was silent during childhood.[14,18–22] These two peaks of age-onset of USS reflect a variable penetrance of the different *ADAMTS13* sequence variations. Moreover, *in vitro* and *in silico* studies of some *ADAMTS13* sequence variations identified in USS patients have confirmed their deleterious effect on the function, the synthesis or the secretion of ADAMTS13.[11,23–28] More recently, ADAMTS13 structure/function relationship was further elucidated as ADAMTS13 was shown to undergo a physiological conformational change inducing its functional activation: upon interaction with VWF D4-CK domains, ADAMTS13 switches from a folded “closed” conformation (based on an auto-inhibitory CUB-spacer domain interaction and defining a basic state) to an unfolded “open”

conformation.[29–34] In physiologic conditions, this conformation shift is transitory and allows the unmasking of exosites of ADAMTS13 (active state) and consequently, the enhancing of ADAMTS13 activity towards its VWF substrate. In a very recent work, Roose and collaborators developed an original ELISA to distinguish between folded *versus* open ADAMTS13 conformation.[35] However, it is still unknown if sequence variations of *ADAMTS13* gene observed in USS may also influence ADAMTS13 conformation. Identifying such mutations might shed more light on the mechanism for conformational activation of ADAMTS13.

In the present study based on our 18-year experience of the French Reference Center for TMA, we focused on 56 French patients with either child- or adult-onset USS. The aim of this study was to evaluate the influence of *ADAMTS13* sequence variations on both USS clinical/biological phenotype and ADAMTS13 conformation using two main objectives: firstly, to provide a demographic and a clinical picture of USS at presentation and during the long-term follow-up*;* secondly, to analyze both ADAMTS13 genotype and phenotype including ADAMTS13 conformation. We show that age-onset defines two distinct entities of USS and that *ADAMTS13* sequence variations may modify ADAMTS13 conformation.

Patients, materials and methods

Patients

Since 2000 in France, all patients with a presumptive diagnosis of TMA (microangiopathic hemolytic anemia, severe thrombocytopenia and organ ischemia) have been enrolled prospectively and consecutively in the national Registry of the French Reference Center for TMA, qualified by the National Plan for Rare Diseases of the French Health Ministry.[36] First, a cross-sectional analysis of the French TMA registry was performed retrospectively from January 1st 2000 to June 1st 2017 to identify patients with USS using the following inclusion criteria: i) a first TMA episode occurring before age 18 for child-onset USS and after age 18 for adult-onset USS; ii) the absence of anti-ADAMTS13 autoantibodies associated with both an ADAMTS13 activity <10 IU/dL during acute TTP episodes and an ADAMTS13 activity <20 IU/dL during remission phases; iii) the presence of two *ADAMTS13* gene sequence variations different from SNPs, or the presence of one *ADAMTS13* gene sequence variation associated with several SNPs. Then, medical records were extensively reviewed to collect clinical and biological data, during both the initial acute phase and the follow-up, with a standardized form. Remission was defined by a complete response to treatment with no further thrombocytopenia or clinical worsening

occurred during at least 30 days after the first day of platelet count recovery. Relapse was defined as the reappearance of clinical manifestations or thrombocytopenia with no other identifiable cause after achieving a durable remission, or both.[37] The ADAMTS13 phenotypic/genotypic data of the child-onset USS patients were compared to those of the adult-onset USS patients.

Written informed consent was obtained from each patient and/or his/her parents according to the Declaration of Helsinki; the study was approved by the Ethics Committee of Hospital Pitié-Salpêtrière and Hospital Saint-Antoine (Paris, France). No patient refused enrolment in the study, registered with ClinicalTrials.gov, number NCT00426686.

Blood collection

Venous blood was collected at time of enrolment (inaugural TTP episode) before any treatment, and during follow-up, into 1:10 final volume of 3.8% sodium citrate; platelet-poor plasma was obtained as previously described.[38] Plasma was frozen at -20°C until transport to the central laboratory.

ADAMTS13 phenotypic assays

ADAMTS13 activity was measured using 3 reference methods: FRETS-VWF73 (Peptide Institute Inc, Osaka, Japan),[39] full-length VWF ELISA[40] and full-length VWF CBA.[41] Calibration curves were performed using a normal pooled plasma (NPP) from 50 healthy individuals adjusted to the WHO 1st International Standard ADAMTS13 plasma (NIBSC, Potters Bar, Hert Fordshire, EN6 3QG, UK Official Medicines Control Laboratory, UK).[42] For all methods, the lower limit of detection (LLD) was 10 IU/dL and normal range was between 50 IU/dL and 100 IU/dL. ADAMTS13 activity was also measured using a chromogenic assay providing a LLD of 3 IU/dL,[43] Technozym ADAMTS13 activity ELISA® (Chr-VWF73, Technoclone, Vienna, Austria) according to the manufacturer's instructions.

ADAMTS13 autoantibodies were screened using both a home-made functional semi-quantitative assay testing a circulating inhibitor against ADAMTS13 as previously described[20] and an ELISA measuring anti-ADAMTS13 IgG titer using the Technozym ADAMTS13-INH ELISA® assay (Technoclone, Vienna, Austria; positive threshold >15 IU/mL) according to the manufacturer's instructions.[22]

ADAMTS13 antigen was measured using 3 assays: the first assay was the commercial IMUBIND ADAMTS13 ELISA® assay (Sekisui Diagnostics, Stamford, CT, USA) used according to the manufacturer's instructions: briefly, ADAMTS13 was captured by a polyclonal anti-ADAMTS13 antibody and stained by a biotinylated polyclonal antibody against ADAMTS13 TSP1-5/TSP1-7 domains; normal range was between 0.63 µg/mL and 0.85 µg/mL, and the LLD was 0.065 µg/mL, as indicated by the manufacturer. The second assay was a home-made ELISA using a mouse anti-hADAMTS13 capture antibody 3H9 directed against ADAMTS13 metalloprotease domain and a pool of 2 staining biotinylated mouse anti-hADAMTS13 antibodies 17G2 and 19H4, as previously described.[32,35,44] Normal range determined from 40 healthy donors (normal human plasma, NHP) was between 0.93 µg/mL and 1.35 µg/mL, and the LLD was 0.02 µg/mL.[35] To further check the presence of ADAMTS13 antigen in patients with detectable ADAMTS13 antigen levels found with the 3H9-based ELISA, we controlled ADAMTS13 antigen levels using a third assay consisting in a home-made ELISA using a mouse anti-hADAMTS13 capture antibody 4B9 directed against ADAMTS13 TSP1-4-5 domains and a pool of 2 staining biotinylated mouse anti-hADAMTS13 antibodies 17G2 and 19H4, as previously described.[32]

ADAMTS13 genetic analysis

Patients' genomic DNA was screened for sequence variations by direct sequencing of the coding sequence of the *ADAMTS13* gene (NM_139025.4) with Big Dye version 3.1, ABI3130XL capillary sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed with SeqScape version 2.5 (Applied Biosystems). We used nomenclature of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/) for the sequence variations. All novel missense mutations and SNPs defined as sequence variations absent from both the dbSNP (built 147), 1000 Genomes, or gnomAD databases were studied *in silico* using Poly-Phen-2 (http://genetics.bwh.harvard.edu/pph2/), AlignGVD (https://agvgd.iarc.fr/), Mutation Taster (http://mutationtaster.org) and SIFT (http://sift-.jcvi.org/) softwares for the prediction on structure and functional effect of protein changes. Ensembl Compara (http://www.ensembl.org/info/genome/compara/index.html) was used for sequence conservation among the species. *ADAMTS13* gene mutations were screened using both the international literature (www.pubmed.com) and the databases for *ADAMTS13* gene variations (Clinvar: https://www.ncbi.nlm.nuh.gov/clinvar) to determine their deleterious or candidate status.

ADAMTS13 conformation

The determination of ADAMTS13 conformation (folded *vs* open) was performed using a home-made ELISA as previously described.[35] Briefly, 96-well microtitrated plates were coated with the anti-ADAMTS13 spacer domain murine monoclonal antibody 1C4 (capture antibody) which exclusively binds to a cryptic epitope in ADAMTS13 spacer domain when in a so-called open conformation.[35] Only plasma samples which ADAMTS13 antigen level was higher than 0.03 µg/mL with the 3H9-based ELISA, were added both in the absence (-17G2) and in the presence (+17G2) of the anti-ADAMTS13 CUB1 domain murine monoclonal antibody 17G2 which induces an open conformation in ADAMTS13, exposing the cryptic epitope of 1C4.[30–32] Finally, bound ADAMTS13 was detected using biotinylated antibody 3H9 followed by HRP labeled Pierce high sensitivity streptavidin (1/10.000, Invitrogen). Binding of ADAMTS13 to 1C4 antibody reflected an open conformation of ADAMTS13 with an exposed cryptic epitope in the spacer domain, while no binding of ADAMTS13 to 1C4 reflected a folded conformation of ADAMTS13. In each series, intra-assay controls consisted in both NHP (folded conformation of ADAMTS13) and one patient with acute acquired TTP (previously established with an open conformation of ADAMTS13). The OD_{490nm} values obtained by the 1C4 ELISA were first corrected for ADAMTS13 antigen concentrations and then normalized with the OD_{490nm} value of the intra-assay control (NHP) to express data as a "Conformation index" (CI). A CI above 0.5 defined an open conformation of ADAMTS13 while a CI below 0.5 defined a folded conformation of ADAMTS13.[35]

Statistical analysis

Quantitative parameters were reported as median and interquartile range (IQR, 25_{th}-75_{th} percentile) and qualitative parameters as number and percentage. Graphpad Prism v5.03 software (GraphPad Software Inc., San Diego, CA, USA) was used to analyze statistics (one-way ANOVA). A *p*-value <0.05 was considered as statistically significant.

Results

Demographic and clinical presentation (inaugural acute phase and follow-up) of USS patients

Over the 18-year study period, 8421 consecutive patients with either an adult-onset (n=7309) or a child-

onset (n=1112) TMA suspicion were prospectively enrolled in our registry (Fig. 1). Out of adult-onset TTP patients (n=1608), 22 patients (from 20 families) were diagnosed with USS and partially previously reported (Fig. 1).[22] Of the 1112 patients with child-onset TMA, 87 patients presented with TTP of which 34 cases were identified as USS. Accurate clinical data and plasma samples were available at presentation and during follow-up (median of 11 years [IQR 7; 16]) in the 34 child-onset USS patients from 31 families (Fig. 1) including the 6 princeps French families.[9] Demographic and clinical features of this child-onset USS cohort are reported in Table 1: the sex ratio was 1.1F/1M, comorbidities and familial consanguinity were both identified in about one-third of patients (Table S1). Familial inquiry revealed likely affected members (fetal loss and/or neonatal death of siblings) or affected siblings in 10 (31%) families. Fourteen patients were Caucasian, 14 were Africans (Maghreb, Central Africa, Mauritius, Reunion) or Afro-caribbean (West Indies, Haiti), and 6 were Leibanish, Turkish, Asians (India, Sri Lanka, Pakistan) or had an Asian ancestry (Guyana).

Twenty-six (76%) patients exhibited symptoms suggestive of TMA immediately at birth (microangiopathic anemia, thrombocytopenia and/or severe icterus), including 14 patients (54%) who required exchange blood transfusion (Table S2). The first acute TTP episode occurred during the neonatal and the post-neonatal period in 15 patients (44%) and during childhood in 17 patients (50%). In two patients (6%) who exhibited TMA symptoms requiring exchange blood transfusion at birth and an unexplained fluctuant thrombocytopenia in childhood, the first TTP episode was reported in adulthood during a first pregnancy (patients 12 and 24, Table S2). During acute TTP episodes, all patients had a microangiopathic hemolytic anemia with schistocytes and a severe thrombocytopenia (platelet count usually < 30 $x10^9$/L) and 22 of them (65%) also exhibited one or several visceral ischemia affecting either the kidneys, the brain or the heart (Table S2). A trigger for acute TTP (mainly viral infections and vaccinations) was identified in about two thirds of patients (Table 1). Acute TTP episodes were successfully treated with plasmatherapy in all patients (fresh frozen plasma infusion 10-15 mL/kg/day until remission) (Table S2). During the long-term follow-up (median of 11 years [IQR: 8-16]), no patient died but 7 (21%) patients exhibited more than 10 relapses. TTP sequelae (from renal, neurological and/or cardiac ischemia) were reported in 16 (47%) patients mainly because of initial misdiagnosis (Evans syndrome, immune thrombocytopenic purpura (ITP) and hemolytic uremic syndrome (HUS)).

Prophylactic plasmatherapy (10 mL/kg every 2 or 3 weeks) was initiated on the physician's discretion in 28 (82%) patients to prevent relapses and sequelae (Table 1). No patient developed an allo-immune

response against ADAMTS13.

Adult-onset USS patients were exclusively pregnancy-triggered USS with no TMA symptoms neither at birth, during childhood nor outside of an obstetrical context.[20,22] After the diagnosis of USS, all women were actively monitored and successfully treated with prophylactic plasmatherapy throughout subsequent pregnancies and the postpartum period (live birth rate of 100%).

Genotypic analysis of *ADAMTS13*

In our 34 child-onset USS patients, we identified 5 distinct SNPs (p.Arg7Trp, p.Gln448Glu, p.Ala618Pro, p.Ala732Val and p.Ala900Val) and 43 distinct sequence variations of *ADAMTS13* gene different from common SNPs, including 24 (56%) so far unreported sequence variations. These sequence variations were spread all over *ADAMTS13* gene without any hot spot (Fig. 2A, Table 2). In most cases, each sequence variation was found in only one family except each of the following 5 sequence variations - p.Arg507Glu; p.Ala596Val; p.Glu735*; p.Cys1024Gly and p.Arg1206* - that were found in 2 unrelated families (Table 2). In all patients, at least 2 *ADAMTS13* sequence variations were identified, including 3 patients with 3 sequence variations. Fifty-six percent (19/34) of our child-onset USS patients were compound heterozygous for *ADAMTS13* sequence variations whereas 44% (15 patients from 12 families/34 patients) were homozygous for *ADAMTS13* mutations. The combination of 2 missense mutations (either 2 N-tem, 2 C-term or 1 N-term + 1 C-term) was present in 38% of patients (Fig. 3A and 3B).The presence of at least one truncating sequence variation, either associated with another truncating sequence variation (32%) or with a missense mutation (30%), was the most frequently observed (62%) (Fig. 3A and 3B).

The genotypic comparison of the current child-onset USS cohort with our adult-onset USS cohort showed some significant differences. *Firstly*, the panel of *ADAMTS13* sequence variations in adult-onset USS was smaller (20 *vs* 43) and did not overlap with the one of child-onset USS (Fig.2A and 2B). Indeed, there were no shared mutations between both our age onset–dependent sub-cohorts. It also included one hotspot within the TSP1-7 domain consisting in one specific missense mutation, p.Arg1060Trp, found in 82% (18/22) of patients (15 heterozygous and 3 homozygous) and systematically associated with the p.Ala1033Thr SNP (Table S3). *Secondly*, the frequency of patients with truncating sequence variations was much lower in adult-onset USS when compared to child-onset USS (36% *vs* 62%) (Fig. 3A), especially when considering the association of 2 truncating sequence variations (5% *vs* 32%).

Thirdly, in contrast to all child-onset USS patients whose both *ADAMTS13* alleles were affected with deleterious sequence variations, 2 adult-onset USS patients (9%, 2/22) were found with only one ADAMTS13 missense mutation (p.Arg1060Trp) combined with several SNP (Fig. 3A and 3B, patients A02 and A20 from Table 4) i.e. p.Arg7Trp, p.Gln448Glu, p.Pro618Ala, p.Ala732Val and p.Ala1033Thr (Table 4 and Table S3). *Fourthly,* the rate of homozygous patients was much lower in adult-onset USS when compared to child-onset USS (18%, 4/22 *vs* 44%, 15/34) (data not shown).

Phenotypic ADAMTS13 characterization

ADAMTS13 antigen levels were found undetectable with the commercial assay (<0.065 µg/mL LLD) in all child-onset USS patients (Table S1) but detectable although very low (between 0.050 µg/mL and 0.068 µg/mL) using the 3H9-based ELISA in 5 child-onset USS patients (5/33 samples tested, 15%) (Fig.4A, Table S1). All patients had a persistent and severe ADAMTS13 deficiency in plasma with all home-made reference methods (ADAMTS13 activity <10 IU/dL) and also with the Chr-VWF73 assay (ADAMTS13 activity <3 IU/dL) (Fig. 4B). No patient had a detectable inhibitor against ADAMTS13 or anti-ADAMTS13 IgG during the acute phase and the follow-up.

In adult-onset USS patients, the proportion of ADAMTS13 antigen higher than 0.03 µg/mL (using the 3H9-based ELISA) was much higher when compared to pediatric patients ($p < 0.005$) as 11 adult patients (11/20 samples tested, 55%) had detectable although very low ADAMTS13 antigen levels (ranging from 0.034 µg/mL to 0.201 µg/mL) (Fig. 4A, Table 4). Interestingly, ADAMTS13 activity (Chr-VWF73 assay) was measurable (between 3 and 10 IU/dL) in 10 adult-onset USS patients (10/17 samples tested, 59%) (Fig. 4B). Also, patients A02, A14 and A20 (Table 4) recovered a detectable ADAMTS13 activity ranging from 12 to 20 IU/dL in remission phase studied several years after the inaugural pregnancy-induced acute TTP episode.

ADAMTS13 folded/open conformation

Finally, 16 USS patients (5 child-onset and 11 adult-onset) exhibited ADAMTS13 antigen levels above 0.030 µg/mL allowing the study of ADAMTS13 conformation (Table 4). In healthy individuals, ADAMTS13 conformation is folded under normal conditions and changes to an open conformation when antibody 17G2 is added.[35] Interestingly, native ADAMTS13 conformation (without addition of 17G2 antibody) exhibited variable profiles in child-onset USS patients (Fig. 4C): 3 patients had a folded

ADAMTS13 (children 08, 10 and 33, Table 4) while 2 patients had an abnormally open ADAMTS13 (children 13 and 30, Table 4). After addition of 17G2 antibody, ADAMTS13 remained folded in the 3 patients whose native ADAMTS13 was folded. Surprisingly, in one patient whose native ADAMTS13 was open, ADAMTS13 folded again after addition of 17G2 (child 30, Table 4). In the other patient with an open ADAMTS13, ADAMTS13 remained open after addition of 17G2 (child 13, Table 4).

In contrast to the child-onset USS patients, ADAMTS13 was folded in all 11 adult-onset USS (Fig. 4D). However, when adding 17G2 antibody, ADAMTS13 remained folded in 9 patients with bi-allelic mutations of *ADAMTS13* gene (adults 01, 03, 04, 08, 10, 11, 14, 15, 19, Table 4) but opened in 2 patients with one heterozygous mutation of *ADAMTS13* gene associated with several SNPs (adults 02 and 20, Table 4) (Fig.4D).

Correlation between *ADAMTS13* genotype, USS clinical/biological phenotype and ADAMTS13 conformation

In our child-onset USS patients, no obvious correlation was found between the clinical features (age-onset of the first TTP episode, presence of visceral ischemia, relapses, ischemic sequelae, prophylactic plasmatherapy and delay between 2 FFP infusions), the biological phenotype (ADAMTS13 antigen using the 3H9-based ELISA, ADAMTS13 conformation) and the genotype (features of ADAMTS13 sequence variations i.e. localization within N-terminal part versus C-terminal part domains and missense versus truncating mutations) (Suppl. Fig.1). Whatever their genotype, ADAMTS13 activity was lower than 3 IU/dL in all pediatric patients. Correlation between *ADAMTS13* genotype and ADAMTS13 conformation was difficult to establish considering the small number of tested patients. However, interestingly, all 5 patients exhibited abnormal ADAMTS13 conformation profile when compared to NHP and all of them had ADAMTS13 sequence variations affecting some domains of ADAMTS13 (spacer, TSP1-5, TSP1-7, CUB1, CUB2) that may interfere either with the physiologic inhibitory CUB-spacer interaction defining ADAMTS13 native conformation or with the access of 17G2 antibody to ADAMTS13 CUB1 domain (Fig. 4C, Table 4). In that regard, the most paradoxical abnormality of ADAMTS13 conformation (open in native state and closed after addition of 17G2 antibody) was interestingly observed in the one patient who exhibited a homozygous mutation of ADAMTS13 CUB1 domain (p.Arg1206*) (Fig. 4C).

In contrast to child-onset USS, adult-onset USS showed a better correlation between clinical features and *ADAMTS13* genotype considering the homogeneity of both clinical presentation (pregnancy-onset TTP) and genetics (hotspot of p.Arg1060Trp mutation). However, the N-terminal/C-terminal localization and the missense/truncating feature of *ADAMTS13* sequence variation did not significantly influence ADAMTS13 antigen levels (Suppl. Fig.1). Also, the miscellaneous ADAMTS13 sequence variations did not influence ADAMTS13 native conformation, found similar to that of NHP (Fig.4D).

Discussion

Our study provides a global picture of USS in France with 56 patients from 51 families (prevalence ~0.86 cases/million on June 1, 2017) exhibiting 63 distinct sequence variations of *ADAMTS13* gene. Comparing children and adult patients, we show that the age-onset of USS defines two distinct entities and that *ADAMTS13* sequence variations have an impact on ADAMTS13 conformation.

The clinical and biological features of our USS cohort are globally in line with those reported in the international literature encompassing ~200 USS patients worldwide with ~180 distinct sequence variations of *ADAMTS13* gene.[17,27,45,46] Child-onset and adult-onset USS are totally distinct entities, especially in terms of clinical presentation and prophylactic treatment requirement. Our child-onset USS patients exhibited a severe disease characterized by a 47% rate of ischemic sequelae and a high frequency of relapses requiring efficient prophylactic plasma therapy in 82% of cases. In contrast, our adult-onset USS patients were exclusively pregnancy-induced TTP with no relapse and no requirement for plasma prophylaxis out of an obstetrical context. Although mean ADAMTS13 antigen levels were significantly lower in children when compared to adults, they were not correlated with the features of *ADAMTS13* sequence variations. Therefore, the age-onset dependent clinical presentation of USS is likely to be directly related to the penetrance spectrum of *ADAMTS13* sequence variations which shows no overlap between children and adult patients. These results are contrasting with some previous studies performed in smaller series of USS patients where a correlation between *ADAMTS13* mutations and ADAMTS13 antigen levels was suggested.[11,12] Interestingly, none of our USS patients died during the short- and long-term follow-up. However, the mortality rate could be underestimated because 8 families reported fetal loss, intrauterine fetal or neonatal death in siblings whose DNA was not available for further investigations (data not shown).

The majority of our USS patients (~65%) are compound heterozygous for *ADAMTS13* sequence variations, making it difficult to establish strong phenotype/genotype correlations. Moreover, additional factors like SNPs may play a role in the phenotype variability.[47] About half of the mutations reported in the current study were previously described.[8–15] Two sequence variations observed in European patients deserve specific attention: firstly, the c.4143_4144dupA resulting in a frameshift in exon 29 and exclusively reported in patients with an ancestry from central and northern Europe[8,13,48] was found in 4.4% of the alleles of our child-onset USS patients whose familial inquiry interestingly identified ancestries from central Europe; secondly, the c.3178C>T mutation (p.Arg1060Trp) in exon 24 leading to a severely impaired ADAMTS13 synthesis and secretion[18,49] and reported with a high prevalence in patients from Europe, Scandinavia, North America and Turkey[15] was present in 48% of the alleles of our adult-onset USS patients. Moreover, as previously reported by Camilleri and collaborators,[18] two patients from our cohort with an adult-onset USS acute presentation exhibited only one identified mutation, the heterozygous p.Arg1060Trp, associated with a cluster of *ADAMTS13* SNPs; interestingly, they recovered a very moderate ADAMTS13 activity (fluctuating between 12 and 20 IU/dL) in remission. As expected, the overlap between ADAMTS13 sequence variations found in our patients and Japanese patients[46] is weak but two *ADAMTS13* sequence variations published in the Japanese Registry[10] were however found in two of our families with Asian ancestry, one from Guyana (p.Arg268Pro) and one from Sri Lanka (p.Arg1206*). Also, in contrast to the Japanese USS cohort[46] and to the worldwide USS patients carrying the c.4143_4144dupA,[8,13,48] the dichotomy of USS age onset looks very strict in French USS patients as there were no shared mutations between the child-onset and the adult-onset sub-cohorts.

In addition to this clinical and biological study of the French patients with USS, we decided to investigate the influence of the *ADAMTS13* sequence variations on the ADAMTS13 conformation using a very recently developed ELISA.[35] Using this new tool, an open conformation of ADAMTS13 was shown to be a hallmark of acute autoimmune TTP.[35] Because ADAMTS13 autoantibodies developed in patients with autoimmune TTP are polyclonal,[3,6,50] they may interfere with the physiologic binding of the N-term Metalloprotease/Disintegrin/Thrombospondin1-1/Cystein-rich/Spacer (MDTCS) domains to the C-term CUB domains that normally maintains the closed native ADAMTS13 conformation. As a complementary approach, studying the influence of USS-related *ADAMTS13* sequence variations on ADAMTS13 conformation may bring new insights in the structure related-mechanisms that govern the auto-

interaction of ADAMTS13 domains. The current study shows that ADAMTS13 adopts several profiles of conformation in USS.

Overall, patients considered as cross reactive material positive (CRM+) cases for ADAMTS13 represented ~29% (16/56) of our USS cohort and thus, could be tested for ADAMTS13 conformation. The native conformation of ADAMTS13 was not modified by *ADAMTS13* sequence variations in 14/16 USS patients (Fig. 4C and 4D; Table 4). In other terms, their combinations of sequence variations involving the TSP1-7 domain in 12 patients (p.Arg1060Trp and p.Cys1067Serfs*30), the CUB2 domain in one patient (c.988-2A>G/p.Asp1362Val) and the spacer domain (p.Ala596Val) in another patient, did not alter the auto-inhibitory spacer-CUB interaction. Among those mutations, only mutations p.Ala596Val and p.Arg1060Trp were present at the homozygous state and then, may be concluded with no consequence on ADAMTS13 native conformation. In contrast, 2 patients (with c.1309-1G>A/p.Cys908Ser and p.Arg1206*/p.Arg1206* genotype, respectively) / 16 exhibited an abnormally open ADAMTS13 native conformation (Fig.4C). These results show that the Cys908 residue (TSP1-5 domain) may play a role in the looped organization of the TSP1-2 to 7 domains enabling the proximity between the N- and C-terminal regions of native ADAMTS13. Also, the Arg1206 residue itself or the following residues of CUB1-CUB2 domains may be important for the auto-inhibitory binding of the CUB1 domain to the spacer domain.[33]

The switch of ADAMTS13 conformation expected after addition of 17G2 antibody was disturbed in 13/16 patients. In 12 patients, ADAMTS13 remained folded suggesting that their *ADAMTS13* sequence variations interfere with the conformational activation induced by 17G2 antibody. In one unique patient with p.Arg1206*/p.Arg1206* genotype, it seems that ADAMTS13 paradoxically switched from open to folded (Fig. 4B and 4C, Table 4). We hypothesize that the truncating mutation p.Arg1206* (truncating a big part of the CUB1 domain) interrupts the spacer-CUB interaction leading to an open ADAMTS13. In addition, ADAMTS13 of this specific patient has a closed conformation in the presence of the 17G2 antibody. Whether 17G2 antibody really closes the conformation of ADAMTS13 or whether the p. Arg1206* modifies the epitope of the 17G2 antibody (anti-CUB1) and hence interferes with the ELISA, remains to be determined. Studies with recombinant ADAMTS13 variants are currently ongoing to study the effect of the different mutations on ADAMTS13 conformation and allosteric activation. Anti-ADAMTS13 antibodies are usually screened and titrated in patients benefitting from regular prophylactic

plasma therapy. However, no case of alloimmunization against ADAMTS13 after plasma therapy has been reported yet.

This study has some strength i.e. the large number and the long-term follow-up of patients, the presentation of both pediatric and adult patients and the innovative data about ADAMTS13 conformation but it also has some limitations: firstly, we cannot guarantee a perfect exhaustiveness of enrollment in spite of an efficient national organization for rare diseases; secondly, we did not express mutated recombinant ADAMTS13 to prove *in vitro* the deleterious effect of the 63 *ADAMTS13* sequence variations found in our patients; thirdly, the study of ADAMTS13 conformation is of limited benefit for patients because a quantitative deficiency of plasma ADAMTS13 is the predominant mechanism for their functional ADAMTS13 defect.

In conclusion, USS is a rare entity within a rare disease, characterized by a great molecular heterogeneity limiting the phenotype/genotype correlation. Further studies should be performed to characterize the effect of *ADAMTS13* gene variations on ADAMTS13 conformation. While curative and prophylactic plasmatherapy is the only available and efficient therapy in USS today, the innovating recombinant ADAMTS13 appears very promising and should help to both improve clinical management and establish therapeutic guidelines.[14,51,52]

Authors' Contributions

Contribution: B.S.J. collected, analyzed, interpreted data, and wrote the manuscript; A.V. and P.C. designed and supervised the study and co-wrote the manuscript; B.S.J. and E.R. performed and interpreted ADAMTS13 phenotypic experiments; P.B. and C.G. performed genetic analysis and critically reviewed the manuscript; K.V. designed and supervised research, interpreted data and reviewed the manuscript for scientific content; NB, JH, FP and YD included patients; AS, NB, JH, FP and YD co-analyzed data and critically reviewed the manuscript. The final version of the manuscript was read and approved by all authors.

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Supporting information

Additional Supporting Information may be found on the online version of this article.

Table S1. Demographic data and ADAMTS13 phenotype investigations in 34 patients (31 families) with child-onset Upschaw-Schulman syndrome.

Table S2. Clinical features of 34 patients with child-onset Upschaw-Schulman syndrome.

Table S3. Genetic analysis of *ADAMTS13* gene polymorphisms in 56 patients with Upschaw-Schulman syndrome.

Figure S1. Correlation between ADAMTS13 genotype and ADAMTS13 antigen in 53/56 patients with Upshaw-Schulman syndrome (USS).

Appendix :

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Legends to figures

Figure 1: Design of the study.

Analysis of the French Thrombotic MicroAngiopathies (TMA) Registry from January 1st 2000 to June 1st 2017 reported 7309 adults and 1112 children (age <18 at inclusion) with a TMA suspicion. Of these, 1608 adults (22%) and 87 children (7.8%) exhibited a first TMA episode related to a severe ADAMTS13 functional deficiency (activity <10 IU/dL) defined as thrombotic thrombocytopenic purpura (TTP). Acquired TTP, unavailable biological sample or missing data during follow-up and genotypic investigations in progress were exclusion criteria. Our cohort of interest consisted in 22 patients with an adult-onset and 34 patients with a child-onset congenital TTP (Upshaw-Schulman syndrome) from 20 and 31 families, respectively.

Figure 2: Distribution of ADAMTS13 mutations in 51 French families with child-onset or adult-onset Upshaw-Schulman syndrome (USS).

ADAMTS13 consists of N-terminal (signal peptide, propeptide, metalloprotease, disintegrin, first thrombospondin type 1 repeat, cys-rich and spacer) and C-terminal (seven thrombospondin type 1 repeats, two CUB domains) domains.

Panel **2A** presents 43 distinct sequence variations in 31 French families (34 patients) with child-onset USS. Missense mutations (top) were the most frequent (56%, 24/43) while truncating sequence variations (bottom) including nonsense mutations (9%), frameshift/splice mutations (30%) and deletions (5%) represented 44% (19/43) of all sequence variations. Overall, 56% (24/43) and 42% (18/43) of these sequence variations were located in the part of *ADAMTS13* gene coding for the N-terminal part and the C-terminal part of the protein, respectively; only 2% (1/43) consisted in one large deletion of *ADAMTS13* gene.

Panel **2B** presents 20 distinct sequence variations in 20 French families (22 patients) with adult-onset USS. Missense mutations (top) represented 60% (12/20) while truncating sequence variations (bottom) were 40% (8/20). Sixty percent (12/20) of *ADAMTS13* sequence variations were located in the N-term part of ADAMTS13.

All missense mutations were predicted *in silico* to be deleterious. Truncating sequence variations were predicted *in silico* to be certainly deleterious, inducing the destruction of mRNA by nonsense-mediated mRNA decay and thus a non expression of *ADAMTS13* gene.

Abbreviations. int: intron, del: deletion, ins: insertion, acc splice: acceptor splice, *: stop codon.

Figure 3. ADAMTS13 genotypic analysis in 56 patients with Upshaw-Schulman syndrome (USS).

Sequence variations of *ADAMTS13* in child-onset and adult-onset USS are represented in black and white, respectively.

3A. Proportion of patients with miscellaneous combinations of *ADAMTS13* gene sequence variations as a function of the localization of the amino-acid change on the protein (N-term or C-term domain for missense mutations, “other” for unpredictable localization linked to truncating mutation).

3B. Proportion of patients with miscellaneous combinations of *ADAMTS13* gene sequence variations as a function of missense or truncating features.

Figure 4. ADAMTS13 phenotypic analysis in 56 patients with Upshaw-Schulman syndrome (USS).

ADAMTS13 antigen (**4A**) and activity (**4B**) distributions were represented in child-onset (black circle) and adult-onset (white circle) patients with USS. The low limit of detection of ADAMTS13 antigen (3H9-based ELISA) was 0.03 µg/mL (dotted black line). The low limit of detection of ADAMTS13 activity (chromogenic VWF73 ELISA) was 3 IU/dL (dotted black line). The threshold of ADAMTS13 activity for diagnosis of thrombotic thrombocytopenic purpura (TTP) was 10 IU/dL (dotted grey line).

ADAMTS13 conformation was studied in 16 patients with either child-onset (**4C**) or adult-onset (**4D**) USS, before and after addition of anti-CUB1 antibody 17G2. A conformation index <0.5 corresponds to a folded ADAMTS13; a conformation index >0.5 corresponds to an open ADAMTS13. ADAMTS13 genotype in 5 child-onset and 11 adult-onset USS patients are indicated on the right side of panels 4C and 4D, respectively. NHP: Normal Human Plasma.

Table 1. Demographic and clinical features of the first thrombotic thrombocytopenic purpura (TTP) episode and long-term follow-up in 34 patients with child-onset Upshaw-Schulman syndrome.

Demographic features of the inaugural TTP episode	n (%) (n total: 34)
Female	18 (53%)
Male	16 (47%)
Presence of comorbidities	11 (32%)
Familial consanguinity	11 (32%)
TMA symptoms at birth	26 (76%)
Age at first TTP episode (acute phase)	
- neonatal period (before day 28)	3 (9%)
- post-neonatal period (day 28 – 1 year)	12 (35%)
- childhood (>1 year – <18 years)	17 (50%)
- adulthood (>18 years) *	2 (6%)
Follow-up (median of 11 years)	
Death	0 (0%)
<10 TTP relapses	27 (79%)
Chronic disease (≥10 TTP relapses)	7 (21%)
Trigger for acute phase of TTP	
- infection / vaccination	23 (68%)
- pregnancy (transition child/adult)	4 (12%)
- none	10 (29%)
TTP sequelae	16 (47%)
- renal	7 (21%)
- neurological	7 (21%)
- cardiac	8 (24%)
Treatment	
Prophylactic plasmatherapy	28 (82%)

(TTP: thrombotic thrombocytopenic purpura, TMA: thrombotic microangiopathy)

* Two patients exhibited a first identified TTP episode in adulthood during a first pregnancy but they had TMA symptoms requiring exchange blood transfusion at birth and an unexplained fluctuant thrombocytopenia in childhood (explaining the child-onset classification).

Table 2. Genetic analysis of *ADAMTS13* gene in 34 patients (31 families) with child-onset Usphaw-Schulman syndrome.

Sequence change	Exon/ Intron	Amino acid change	Domain of ADAMTS13	Sequence variation	Status	N of alleles§ (%)	Reported in the literature
c.330+3G>C	Intron 3	p.?		splice site	HTZ	1 (1.5%)	NO
c.687-7C>A	Intron 6	p.?		splice site	HTZ	1 (1.5%)	NO
c.988-2A>G	Intron 8	p.?		splice site	HTZ	1 (1.5%)	NO
c.1308+2_1308+5del	Intron 11	p.?		splice site	HTZ	1 (1.5%)	YES
c.1309-1G>A	Intron 11	p.?		splice site	HTZ	1 (1.5%)	YES
c.1584+2T>A	Intron 13	p.?		splice site	HTZ	1 (1.5%)	NO
c.1585-1G>C	Intron 13	p.?		splice site	HTZ	1 (1.5%)	NO
c.2104+1G>A	Intron 17	p.?		splice site	HMZ	2 (2.9%)	NO
c.3892+1G>A	Intron 27	p.?		splice site	HMZ	2 (2.9%)	NO
Large deletion	Ex1–5'UTR	p.?		deletion	HTZ	1 (1.5%)	NO
c.22dupG	Exon 1	p.Ala8Glyfs*131		frameshift	HTZ	1 (1.5%)	NO
c.237C>G	Exon 3	p.Ile79Met	metalloprotease	missense	HTZ	1 (1.5%)	YES
c.607T>C	Exon 6	p.Ser203Pro	metalloprotease	missense	HTZ	1 (1.5%)	YES
c.655G>C	Exon 6	p.Gly219Arg	metalloprotease	missense	HTZ	1 (1.5%)	NO
c.803G>C	Exon 7	p.Arg268Pro	metalloprotease	missense	HTZ	1 (1.5%)	YES
c.825-10_843del29	Exon 8	p.?		deletion	HTZ	2 (2.9%)	YES
c.844G>C	Exon 8	p.Val282Leu	metalloprotease	missense	HTZ	2 (2.9%)	NO
c.964T>G	Exon 8	p.Cys322Gly	disintegrine-like	missense	HTZ	1 (1.5%)	YES
c.1193G>A	Exon 10	p.Arg398His	TSP1-1	missense	HTZ	1 (1.5%)	YES
c.1308G>C	Exon 11	p.Gln436His	TSP1-1	missense	HMZ	2 (2.9%)	YES
c.1312T>C	Exon 12	p.Cys438Arg	TSP1-1	missense	HMZ	2 (2.9%)	NO
c.1328T>C	Exon 12	p.Leu443Pro	CYS rich	missense	HTZ	2 (2.9%)	NO
c.1409G>A	Exon 12	p.Trp470*		nonsense	HTZ	2 (2.9%)	NO
c.1520G>A	Exon 13	p.Arg507Gln	CYS rich	missense	HTZ/HMZ	3 (4.4%)	YES
c.1643G>T	Exon 14	p.Cys548Phe	CYS rich	missense	HTZ	1 (1.5%)	NO
c.1787C>T	Exon 16	p.Ala596Val	spacer	missense	HTZ/HMZ	3 (4.4%)	YES
c.1908dupG	Exon 16	p.Leu637Alafs*18		frameshift	HTZ	1 (1.5%)	NO
c.2074C>T	Exon 17	p.Arg692Cys	TSP1-2	missense	HMZ	2 (2.9%)	YES
c.2085C>G	Exon 17	p.Cys695Trp	TSP1-2	missense	HTZ	1 (1.5%)	NO
c.2203G>T	Exon 18	p.Glu735*		nonsense	HMZ	4 (5.8%)	YES
c.2260T>C	Exon 19	p.Cys754Arg	TSP1-3	missense	HMZ	2 (2.9%)	YES
c.2272T>C	Exon 19	p.Cys758Arg	TSP1-3	missense	HTZ	2 (2.9%)	YES
c.2404C>T	Exon 19	p.Gln802*		nonsense	HTZ	1 (1.5%)	NO
c.2723G>C	Exon 21	p.Cys908Ser	TSP1-5	missense	HTZ	1 (1.5%)	YES
c.2836T>C	Exon 22	p.Cys946Arg	TSP1-5	missense	HTZ	1 (1.5%)	NO
c.2929T>C	Exon 23	p.Cys977Arg	TSP1-6	missense	HTZ	1 (1.5%)	NO
c.3070T>G	Exon 24	p.Cys1024Gly	TSP1-7	missense	HMZ	4 (5.8%)	YES
c.3254_3255delCT	Exon 25	p.Ser1085Cysfs*12		frameshift	HTZ	1 (1.5%)	YES
c.3390T>G	Exon 25	p.Cys1130Trp	TSP1-8	missense	HMZ	2 (2.9%)	NO
c.3616C>T	Exon 26	p.Arg1206*		nonsense	HTZ/HMZ	5 (7.4%)	YES
c.3655C>T	Exon 26	p.Arg1219Trp	CUB-1	missense	HTZ	1 (1.5%)	YES
c.4085A>T	Exon 29	p.Asp1362Val	CUB-2	missense	HTZ	1 (1.5%)	YES
c.4143dupA	Exon 29	p.Glu1382Argfs*6		frameshift	HTZ	3 (4.4%)	YES

§ total number of alleles = 68
(HMZ: homozygous; HTZ: heterozygous)

Table 3. Genetic analysis of *ADAMTS13* gene in 22 patients (20 families) with adult-onset Usphaw-Schulman syndrome.

Sequence change	Exon/Intron	Amino acid change	Domain of ADAMTS13	Sequence variation	Status	N of alleles§ (%)	Reported in the literature
c.173-14_173-2del12	Intron 2	p.?		splice site	HTZ	1 (2.3%)	NO
c.988-2A>C	Intron 8	p.?		splice site	HTZ	1 (2.3%)	YES
c.1309-25C>A	Intron 11	p.?		splice site	HTZ	1 (2.3%)	YES
c.262G>C	Exon 3	p.Val88Leu	metalloprotease	missense	HTZ	1 (2.3%)	YES
c.283G>C	Exon 3	p.Ala95Pro	metalloprotease	missense	HTZ	2 (4.5%)	YES
c.460G>A	Exon 5	p.Val154Ile	metalloprotease	missense	HTZ	1 (2.3%)	NO
c.559G>C	Exon 6	p.Asp187His	metalloprotease	missense	HTZ	1 (2.3%)	YES
c.706G>T	Exon 7	p.Gly236Cys	metalloprotease	missense	HMZ	2 (4.5%)	YES
c.1058C>T	Exon 9	p.Pro353Leu	disintegrin	missense	HTZ	1 (2.3%)	YES
c.1408dup	Exon 12	p.Trp470Leufs*64		frameshift	HTZ	1 (2.3%)	YES
c.1651G>T	Exon 14	p.Asp551Tyr	Cys-rich	missense	HTZ	1 (2.3%)	NO
c.1892C>T	Exon 16	p.Ala631Val	spacer	missense	HTZ	2 (4.5%)	YES
c.2434G>T	Exon 20	p.Glu812*		nonsense	HTZ	2 (4.5%)	YES
c.2455delG	Exon 20	p.Ala819Leufs*24		frameshift	HTZ	1 (2.3%)	YES
c.2746C>T	Exon 22	p.Arg916Cys	TSP1-5	missense	HTZ	1 (2.3%)	YES
c.2890G>A	Exon 23	p.Val964Met	TSP1-5	missense	HTZ	1 (2.3%)	
c.3178C>T	Exon 24	p.Arg1060Trp	TSP1-7	missense	HTZ/HMZ	21 (48%)	YES
c.3198_3199del	Exon 24	p.Cys1067Serfs*30		frameshift	HTZ	1 (2.3%)	YES
c.3313C>T	Exon 25	p.Gln1105*		nonsense	HTZ	1 (2.3%)	YES
c.4135T>C	Exon 29	p.Trp1379Arg	CUB-2	missense	HTZ	1 (2.3%)	YES

§ Total number of alleles: 44.
(HMZ: homozygous; HTZ: heterozygous)

Table 4. ADAMTS13 conformation according to *ADAMTS13* gene sequence variations in 16 patients with Upshaw-Schulman syndrome.

Patients N°	Age-onset of USS	ADAMTS13 genotype seq. variation / ADAMTS13 domain (allele 1 and allele 2)	ADAMTS13 antigen (3H9-based ELISA (µg/mL)*	Basal ADAMTS13 conformation (-17G2)	ADAMTS13 conformation after pre-incubation with 17G2
Child 10	child	p. Ala596Val / spacer p. Ala596Val / spacer	0.068	folded	folded
Child 13	child	c.1309-1G>A p.Cys908Ser / TSP1-5	0.050	open	open
Child 32	child	p.Cys1024Gly / TSP1-7 p.Cys1024Gly / TSP1-7	0.058	folded	folded
Adult 02	adult	SNP (p.Arg7Trp, p.Gln448Glu, p.Pro618Ala, p.Ala732Val, p.Ala1033Thr) p.Arg1060Trp / TSP1-7	0.158	folded	open
Adult 20	adult	SNP (p.Arg7Trp, p.Gln448Glu, p.Pro618Ala, p.Ala732Val, p.Ala1033Thr) p.Arg1060Trp / TSP1-7	0.201	folded	open
Adult 08	adult	p.Val88Leu / Metalloprotease p.Arg1060Trp / TSP1-7	0.143	folded	folded
Adult 04	adult	p.Pro353Leu / Disintegrin p.Arg1060Trp / TSP1-7	0.043	folded	folded
Adult 19	adult	p.Trp470Leufs*64 p.Arg1060Trp / TSP1-7	0.054	folded	folded
Adult 10	adult	p.Asp551Tyr / Cys-rich p.Arg1060Trp / TSP1-7	0.051	folded	folded
Adult 01	adult	p.Glu812* p.Arg1060Trp / TSP1-7	0.122	folded	folded
Adult 15	adult	p.Arg916Cys / TSP1-5 p.Arg1060Trp / TSP1-7	0.034	folded	folded
Adult 11	adult	p.Arg1060Trp / TSP1-7 p.Arg1060Trp / TSP1-7	0.053	folded	folded
Adult 03	adult	p.Arg1060Trp / TSP1-7 p.Arg1060Trp / TSP1-7	0.054	folded	folded
Adult 14	adult	c.173-14_173-2del12 p.Cys1067Serfs*30	0.109	folded	folded
Child 30	child	p. Arg1206* p. Arg1206*	0.053	open	folded
Child 08	child	c.988-2A>G p. Asp1362Val / CUB-2	0.057	folded	folded
NHP	-	WT WT	1.000	folded	open

USS: Upshaw-Schulman syndrome, NHP: normal human plasma, WT: wild type.

*All patients exhibited similar ADAMTS13 antigen levels when re-checked with the 4B9-based ELISA.

ADAMTS13 gene mutations influence ADAMTS13 conformation and disease age-onset in the French cohort of Upshaw-Schulman syndrome.

Running head: French cohort of Upshaw-Schulman syndrome

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SUPPLEMENTAL SECTION

Suppl. Table 1. Demographic data and ADAMTS13 phenotypic investigations in 34 patients (31 families) with child-onset Usphaw-Schulman syndrome.

	Demographic features				Phenotypic investigations of ADAMTS13				
Patients	**Sex**	**Age at time of the study (2017) (years)**	**Comorbidities**	**Family**	**ADAMTS13 activity (reference methods*) (IU/dL)**	**ADAMTS13 activity (Chr-VWF73) (IU/dL)**	**Anti-ADAMTS13 autoantibodies**	**ADAMTS13 antigen (µg/mL)**	
								Commer-cial assay	**3H9 based ELISA**
Child 01	M	22	obesity	none	<10	<3	negative	<0.065	<LLD
Child 02	M	11	none	none	<10	<3	negative	<0.065	<LLD
Child 03	F	10	none	consanguinity	<10	<3	negative	<0.065	<LLD
Child 04	M	8	none	none	<10	<3	negative	<0.065	<LLD
Child 05	M	24	G6PD deficiency	consanguinity	<10	<3	negative	<0.065	<LLD
Child 06	F	36	none	none	<10	<3	negative	<0.065	<LLD
Child 07	F	11	MCP mutation	none	<10	<3	negative	<0.065	<LLD
Child 08	F	34	none	none	<10	<3	negative	<0.065	0.057
Child 09	F	19	mutation in *CFH*	none	<10	<3	negative	<0.065	<LLD
Child 10	F	22	none	none	<10	<3	negative	<0.065	0.068
Child 11	M	31	dialysis	none	<10	<3	negative	<0.065	<LLD
Child 12	F	34	none	none	<10	<3	negative	<0.065	<LLD
Child 13	M	40	kidney transplantation	consanguinity	<10	N/A	negative	<0.065	0.050
Child 14	F	23	hypothyroidism (Hashimoto)	none	<10	<3	negative	<0.065	<LLD
Child 15	M	43	none	none	<10	<3	negative	<0.065	<LLD
Child 16	F	5	none	consanguinity	<10	<3	negative	<0.065	<LLD
Child 17	F	25	mutation in *CFH*	none	<10	<3	negative	<0.065	<LLD
Child 18	M	12	none	none	<10	<3	negative	<0.065	<LLD
Child 19	F	1	none	consanguinity	<10	<3	negative	<0.065	<LLD
Child 20	M	3	none	none	<10	<3	negative	<0.065	<LLD
Child 21	F	34	none	consanguinity	<10	<3	negative	<0.065	<LLD
Child 22	M	43	none	none	<10	<3	negative	<0.065	<LLD
Child 23	M	22	none	consanguinity	<10	<3	negative	<0.065	<LLD
Child 24	F	42	none	none	<10	<3	negative	<0.065	<LLD
Child 25	F	8	none	consanguinity	<10	<3	negative	<0.065	<LLD
Child 26	F	46	none	none	<10	<3	negative	<0.065	<LLD
Child 27	M	8	none	none	<10	<3	negative	<0.065	<LLD
Child 28	F	9	Moyamoya disease	none	<10	<3	negative	<0.065	<LLD
Child 29	F	16	hypothyroidism	consanguinity	<10	<3	negative	<0.065	<LLD
Child 30	M	12	minor alpha thalassemia	consanguinity	<10	<3	negative	<0.065	0.053
Child 31	M	15	none	none	<10	<3	negative	<0.065	<LLD
Child 32	F	16	none	none	<10	<3	negative	<0.065	0.058
Child 33	M	49	none	none	<10	N/A	negative	N/A	N/A
Child 34	M	32	none	consanguinity	<10	<3	negative	<0.065	<LLD

CFH: complement factor H; MCP: membrane cofactor protein; N/A: not available; LLD: low limit of detection.

* Reference methods for ADAMTS13 activity measurement: FRETS-VWF73 (Kokame et al, BJH 2005), full-length VWF ELISA (Veyradier et al, Blood 2001), full-length VWF CBA (Gerritsen et al, Thromb Haemost 1999).

Suppl. Table 2. Clinical features of 34 patients with child-onset Usphaw-Schulman syndrome.

Patients	USS symptoms at birth *	Age at first USS acute phase	Follow-up (years)	Number of TTP relapses***	Triggering factor of acute phase of TTP	Clinical features of TTP acute phases		TTP sequelae	Plasmatherapy****
						Hematological features	Visceral ischemia		
Child 01	TMA	postneonatal period (10 months)	22	1	none	hemolytic anemia thrombocytopenia	none	none	curative and prophylactic
Child 02	anemia	childhood (20 months)	8	[0-5] (2)	infection	hemolytic anemia thrombocytopenia	none	none	curative
Child 03	none	postneonatal period (7 months)	9	[5-10] (6)	infection and vaccine	hemolytic anemia thrombocytopenia	renal (proteinuria), hypertension	cardiac disorder	curative and prophylactic
Child 04	exchange blood transfusion	childhood (4 years, prophylactic FFP infusions)	7	[0-5] (3)	infection	hemolytic anemia thrombocytopenia	none	none	curative and prophylactic
Child 05	none	childhood (3 years)	13	[5-10] (7)	infection	hemolytic anemia thrombocytopenia	renal (proteinuria)	none	curative
Child 06	exchange blood transfusion	postneonatal period (6 months)	17	[5-10] (8)	infection	hemolytic anemia thrombocytopenia	neurological (intracranial hypertension), renal (AKI), hypertension	cardiac disorder	curative and prophylactic
Child 07	TMA	postneonatal period (10 months)	10	[0-5] (4)	infection	hemolytic anemia thrombocytopenia	renal (proteinuria)	none	curative and prophylactic
Child 08	none	childhood (18 months)	17	chronic disease (13)	infection	hemolytic anemia thrombocytopenia	renal (AKI), neurological (headaches, confusion)	none	curative and prophylactic
Child 09	exchange blood transfusion	postneonatal period (9 months)	17	[5-10] (5)	infection	hemolytic anemia thrombocytopenia	renal (AKI)	none	curative and prophylactic
Child 10	TMA (aHUS)	postneonatal period (11 months)	17	chronic disease (11)	infection	hemolytic anemia thrombocytopenia	renal (AKI), neurological (headaches, coma, transient focal defects)	none	curative and prophylactic
Child 11	exchange blood transfusion	childhood (5 years)	16	chronic disease (>10)	infection	hemolytic anemia thrombocytopenia	renal (chronic renal failure), cardiac (impaired left ventricular function)	renal and cardiac disorders	curative and prophylactic

Child 12	exchange blood transfusion	adulthood (24 years)	16	[0-5] (4)	pregnancy and infection	hemolytic anemia thrombocytopenia	neurological (headaches, transient focal defects), intrauterine fetal death	neurological disorder	curative and prophylactic (pregnancy)
Child 13	exchange blood transfusion	childhood (5 years)	8	chronic disease (>14)	infection	hemolytic anemia thrombocytopenia	renal (chronic renal failure requiring dialysis and kidney transplantation), neurological (strokes, seizures)	renal, neurological, cardiac and eye disorders	curative and prophylactic
Child 14	none	Adolescent-hood (17 years)	4	2	none	hemolytic anemia thrombocytopenia	neurological (stroke)	neurological disorder	prophylactic
Child 15	none	childhood (8 years)	14	[0-5] (2)	none	hemolytic anemia thrombocytopenia	neurological (transient focal defect)	none	curative and prophylactic
Child 16	exchange blood transfusion	childhood (1 year)	2	2	none	hemolytic anemia thrombocytopenia	none	none	curative and prophylactic
Child 17	exchange blood transfusion	postneonatal period (6 months)	16	chronic disease (>20)	infection	hemolytic anemia thrombocytopenia	renal (TMA)	renal disorder	curative and prophylactic (and during pregnancy)
Child 18	TMA	none (prophylactic FFP infusions)	11	[0-5] (0)	none	hemolytic anemia thrombocytopenia	renal (AKI), neurological (cerebral haemorrhage)	none	curative and prophylactic
Child 19	exchange blood transfusion	postneonatal period (4 months)	<1	1	infection	hemolytic anemia thrombocytopenia	none	none	curative and prophylactic
Child 20	disseminated intravascular coagulation	childhood (1 year)	<1	1	infection	hemolytic anemia thrombocytopenia	none	neurological and cardiac disorders	?
Child 21	exchange blood transfusion	postneonatal period (10 months)	15	[0-5] (>2)	infection and pregnancy	hemolytic anemia thrombocytopenia	renal (AKI), neurological (cerebral vasculitis), cardiac (hypertension, hypertrophy)	renal, neurological and cardiac disorders	curative and prophylactic
Child 22	icterus	childhood (5 years)	9	chronic disease (>15)	infection	hemolytic anemia thrombocytopenia	none	none	curative
Child 23	anemia thrombocytopenia	neonatal period (3 weeks)	15	[0-5] (>2)	none	hemolytic anemia thrombocytopenia	none	none	curative and prophylactic
Child 24	exchange blood transfusion	adulthood (40 years)	1	[0-5] (>2)	pregnancy	hemolytic anemia thrombocytopenia	renal (TMA, AKI)	renal disorder	curative and prophylactic

Child 25	TMA (TTP)	none (prophylactic FFP infusions)	7	[0-5] (0)	infection	hemolytic anemia thrombocytopenia	none	renal disorder	curative and prophylactic
Child 26	exchange blood transfusion	childhood (4 years)	16	[5-10] (5)	infection and pregnancy	hemolytic anemia thrombocytopenia	renal (TMA, AKI), neurological (confusion, transient focal defects), placental hematoma	none	curative and prophylactic
Child 27	none	postneonatal period	6	0	none	hemolytic anemia thrombocytopenia	none	none	curative and prophylactic
Child 28	none	childhood (2 years)	6	[0-5] (1)	none	hemolytic anemia thrombocytopenia	neurological (transient focal defect, strokes)	neurological disorder	curative and prophylactic
Child 29	hemolytic anemia thrombocytopenia	childhood (4 years)	12	[0-5] (4)	infection	hemolytic anemia thrombocytopenia	renal (proteinuria), neurological (headaches)	renal disorder	curative and prophylactic
Child 30	exchange blood transfusion	postneonatal period (9 months)	12	[0-5] (1)	infection	hemolytic anemia thrombocytopenia	renal (AKI, proteinuria)	cardiac disorder	curative and prophylactic
Child 31	icterus	postneonatal period (9 months)	9	[0-5] (1)	none	hemolytic anemia thrombocytopenia	none	none	curative and prophylactic
Child 32	TMA	childhood (2 years)	11	[5-10] (6)	infection	hemolytic anemia thrombocytopenia	renal (AKI), neurological (confusion)	neurological disorder	curative and prophylactic
Child 33	exchange blood transfusion	childhood (1 year)	40	chronic disease (>23)	infection	hemolytic anemia thrombocytopenia	none	none	curative plasmatherapy of acute phase
Child 34	none	childhood (1 year)	11	[0-5] (1)	infection	hemolytic anemia thrombocytopenia	renal (AKI), cardiac (atrial fibrillation)	cardiac disorder	curative

aHUS: atypical hemolytic uremic syndrome; AKI: acute kidney injury; TMA: thrombotic microangiopathy; TTP: thrombotic thrombocytopenic purpura;

* USS symptoms:

- exchange transfusion
- or anemia
- or thrombocytopenia
- or icterus
- or several items together

** neonatal period from 0 to 28 days, postneonatal period from 28 days to 1 year, childhood ≥1 year

*** chronic disease defined USS course characterized by no real acute phase or remission phase but chronic bicytopenia requiring prophylactic plasmatherapy.

**** plasmatherapy: curative plasmatherapy of acute phases, or prophylactic FFP infusions, or both.

Suppl. Table 3. Genetic analysis of *ADAMTS13* polymorphisms (SNPs) in 56 patients with Upshaw-Schulman syndrome.

SNPs	Child-onset USS		Adult-onset USS		gnomAD population
	N alleles	Allele frequency	N alleles	Allele frequency	Allele frequency
c.19C>T (p.Arg7Trp)	3	9%	16	73%	9.1%
c.1342C>G (p.Gln448Glu)	20	59%	5	23%	37.5%
c.1852C>G (p.Pro618Ala)	4	12%	5	23%	6%
c.2195C>T (p.Ala732Val)	3	9%	4	18%	1%
c.2699C>T (p.Ala900Val)	3	9%	0	0%	8.4%
c.3097G>A (p.Ala1033Thr)	1	3%	21	95%	3%

(USS: Upshaw-Schulman syndrome)

All SNPs found in our patients have been previously reported in the literature (Levy et al, Nature 2001): p.Arg7Trp, p.Gln448Glu and p.Ala900Val were predicted to be non-deleterious, whereas p.Ala732Val, p.Ala618Pro and p.Ala1033Thr were predicted to be deleterious (Moatti et al, Blood 2012).

Legend to suppl. Figure 1. Correlation between ADAMTS13 genotype and ADAMTS13 antigen in 53/56 patients with Upshaw-Schulman syndrome (USS).

ADAMTS13 antigen levels measured by 3H9-based ELISA assay (0.03 ug/mL limit of detection for use in ADAMTS13 conformation ELISA, indicated by dotted black line) is represented as a function of the localization of the amino acid change on ADAMTS13 protein (N-term or C-term domain for missense mutations, “other” for unpredictable localization linked to truncating mutations) in child-onset (**1A**) and adult-onset (**1B**) USS patients.

Similarly, ADAMTS13 antigen is also represented as a function of missense or truncating features of ADAMTS13 mutations in child-onset (**1C**) and adult-onset (**1D**) USS patients

Suppl. Figure 1

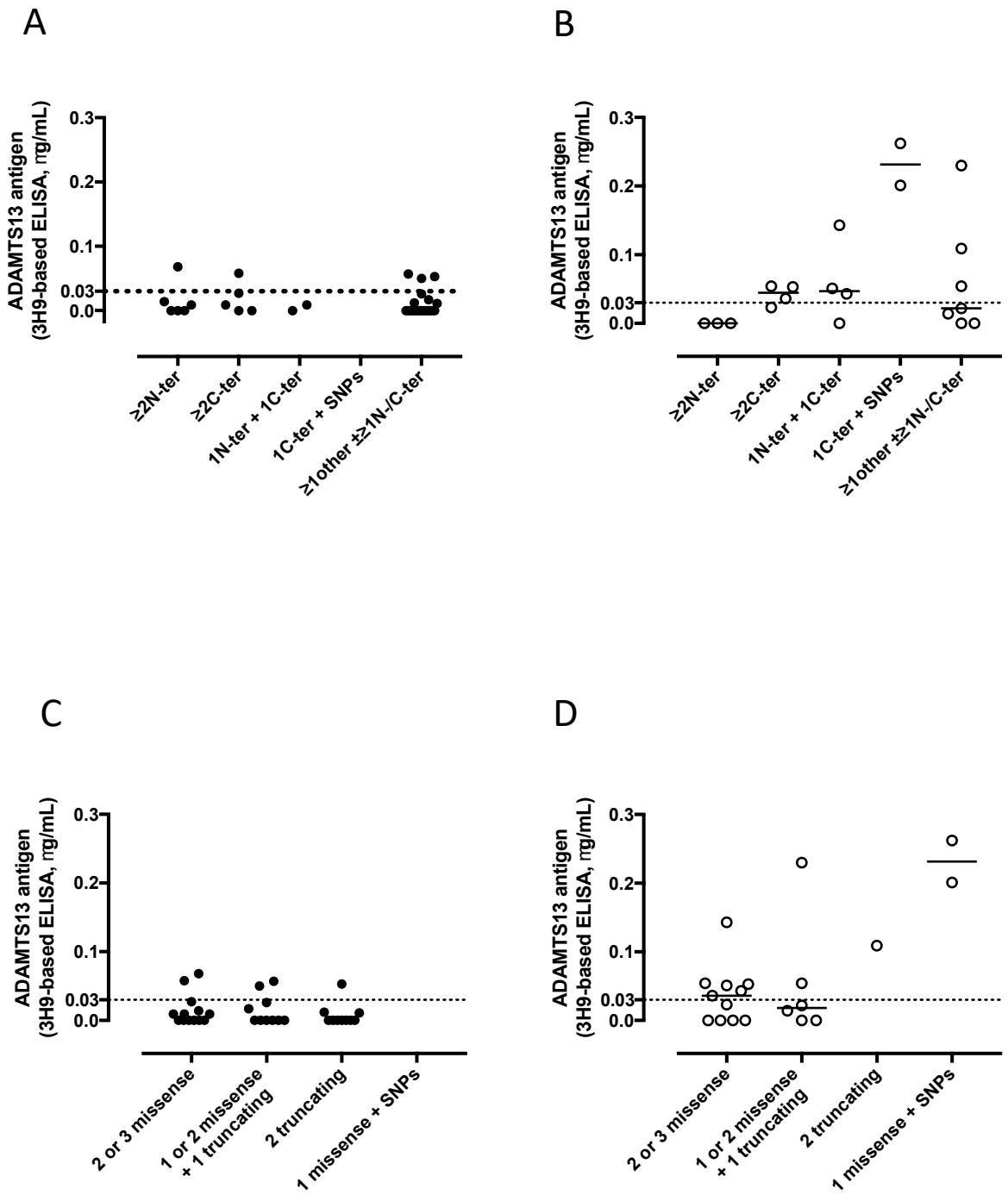
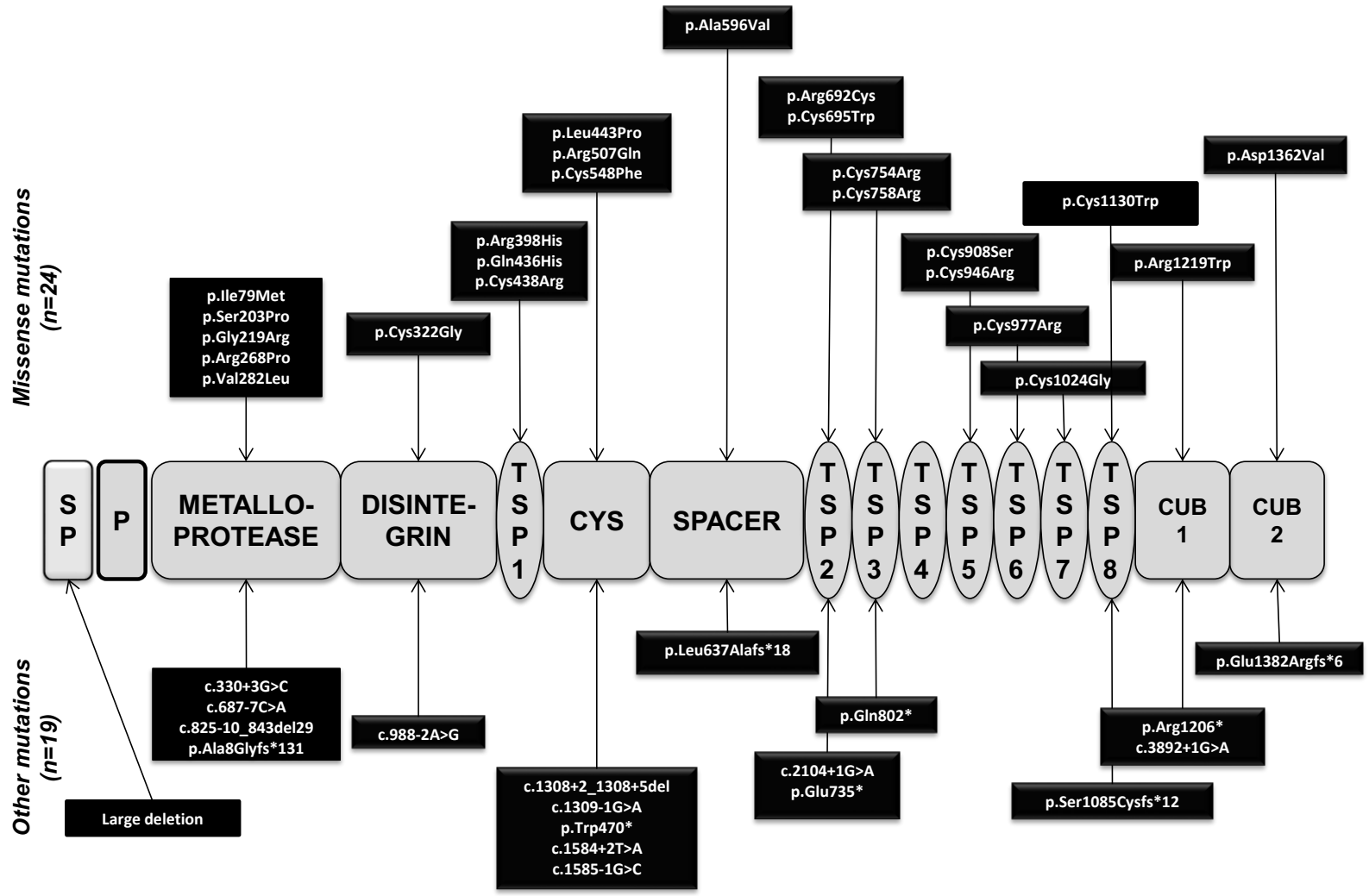


Figure 2


A. Child-onset USS
Missense mutations (n=24)
Other mutations (n=19)
p.Ala596Val
p.Arg692Cys
p.Cys695Trp
p.Leu443Pro
p.Arg507Gln
p.Cys548Phe
p.Cys754Arg
p.Cys758Arg
p.Asp1362Val
p.Cys1130Trp
p.Arg398His
p.Gln436His
p.Cys438Arg
p.Cys908Ser
p.Cys946Arg
p.Arg1219Trp
p.Ile79Met
p.Ser203Pro
p.Gly219Arg
p.Arg268Pro
p.Val282Leu
p.Cys322Gly
p.Cys977Arg
p.Cys1024Gly
SP
P
METALLO-PROTEASE
DISINTE-GRIN
TSP1
CYS
SPACER
TSP2
TSP3
TSP4
TSP5
TSP6
TSP7
TSP8
CUB 1
CUB 2
p.Leu637Alafs*18
p.Glu1382Argfs*6
c.330+3G>C
c.687-7C>A
c.825-10_843del29
p.Ala8Glyfs*131
c.988-2A>G
p.Gln802*
c.2104+1G>A
p.Glu735*
p.Arg1206*
c.3892+1G>A
p.Ser1085Cysfs*12
Large deletion
c.1308+2_1308+5del
c.1309-1G>A
p.Trp470*
c.1584+2T>A
c.1585-1G>C

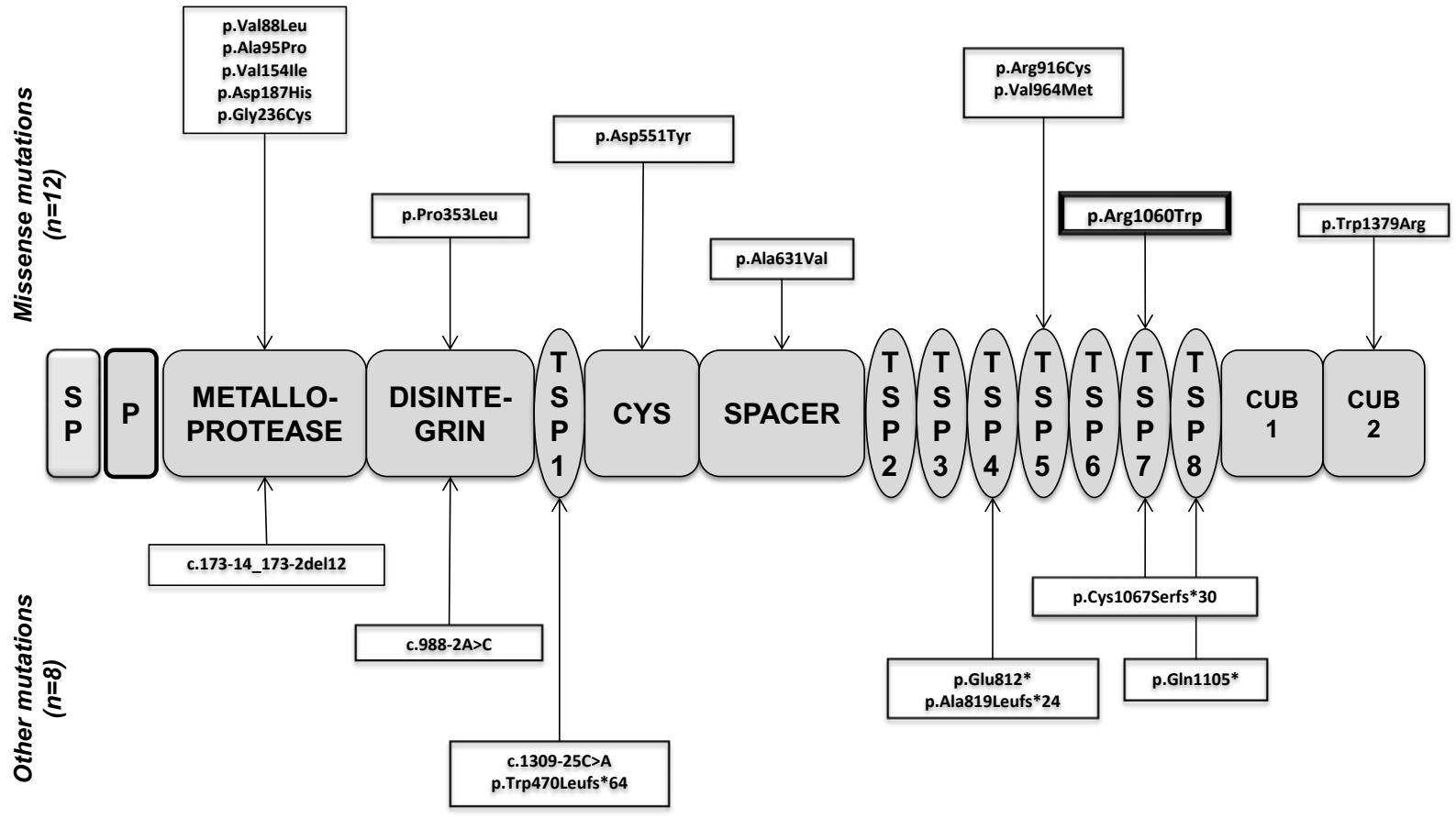
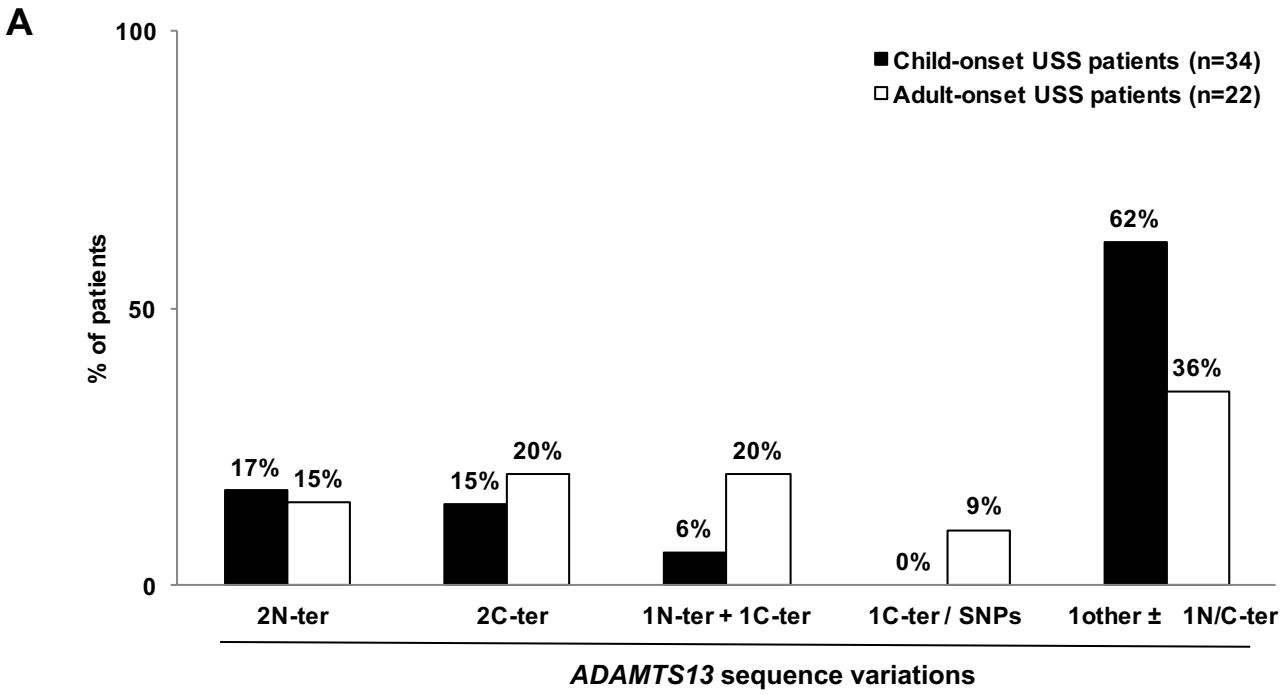

B. Adult-onset USS
Missense mutations (n=12)
Other mutations (n=8)
p.Val88Leu
p.Ala95Pro
p.Val154Ile
p.Asp187His
p.Gly236Cys
p.Arg916Cys
p.Val964Met
p.Asp551Tyr
p.Pro353Leu
p.Arg1060Trp
p.Trp1379Arg
p.Ala631Val
SP
P
METALLO-PROTEASE
DISINTE-GRIN
TSP1
CYS
SPACER
TSP2
TSP3
TSP4
TSP5
TSP6
TSP7
TSP8
CUB 1
CUB 2
c.173-14_173-2del12
p.Cys1067Serfs*30
c.988-2A>C
p.Glu812*
p.Ala819Leufs*24
p.Gln1105*
c.1309-25C>A
p.Trp470Leufs*64

Figure 3


A
100
50
0
% of patients
Child-onset USS patients (n=34)
Adult-onset USS patients (n=22)
17%
15%
15%
20%
6%
20%
0%
9%
62%
36%
2N-ter
2C-ter
1N-ter + 1C-ter
1C-ter / SNPs
1other ± 1N/C-ter
ADAMTS13 sequence variations

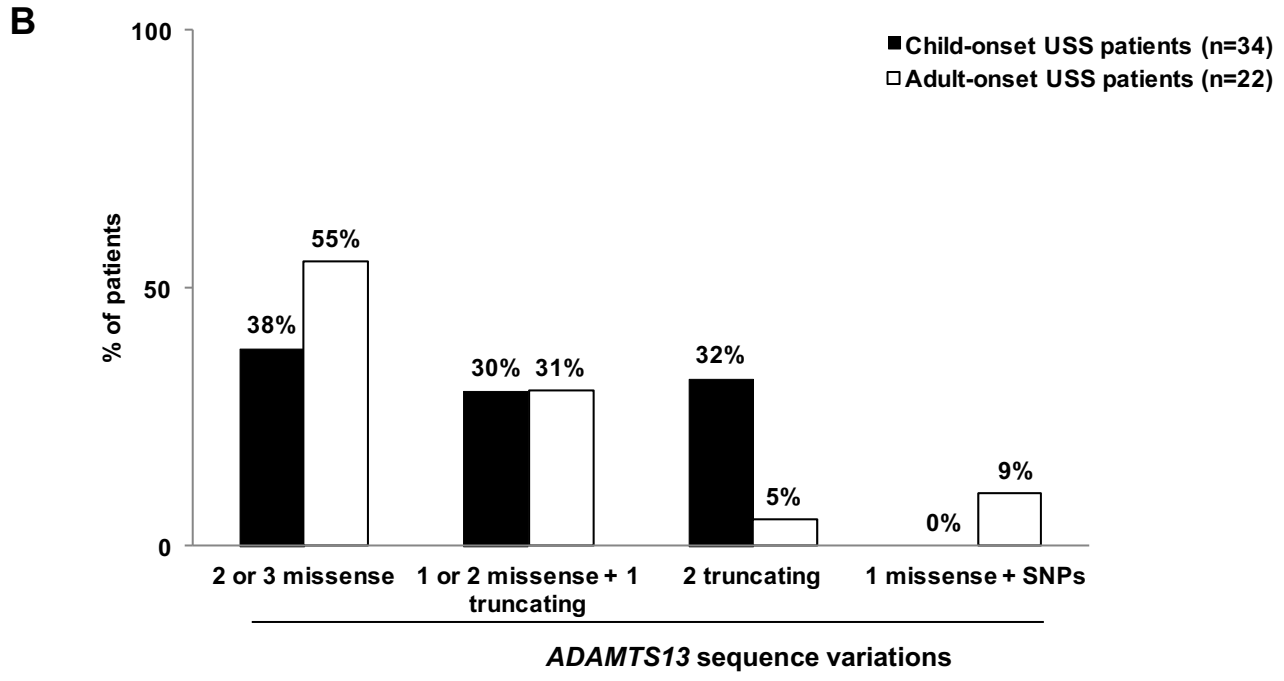
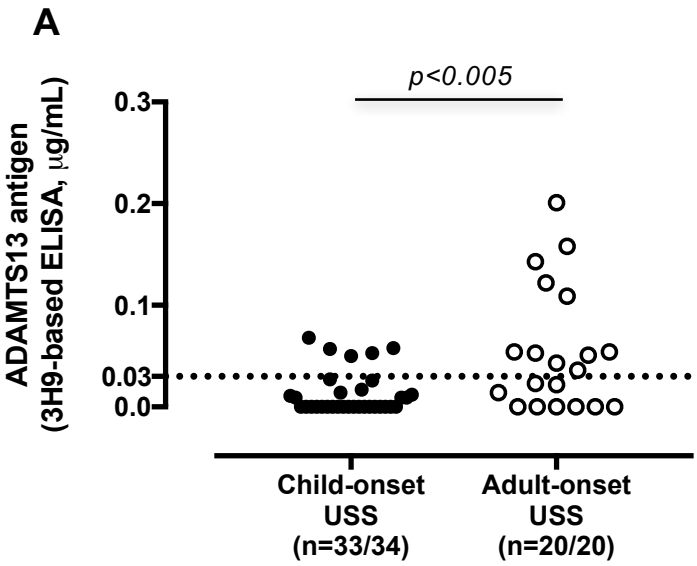
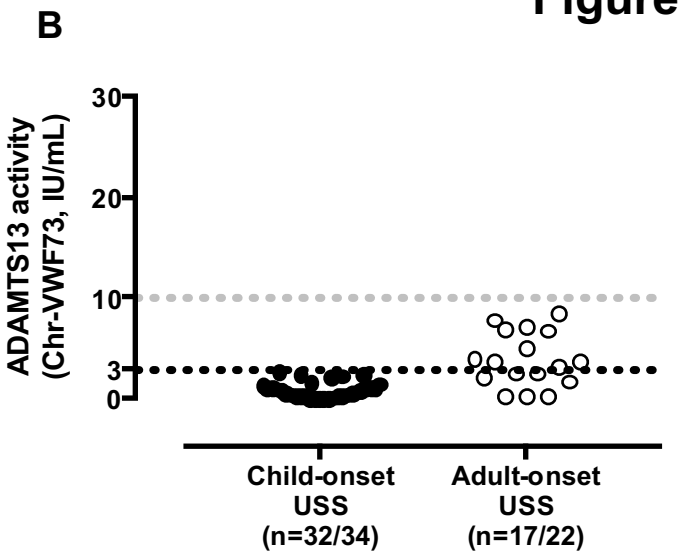
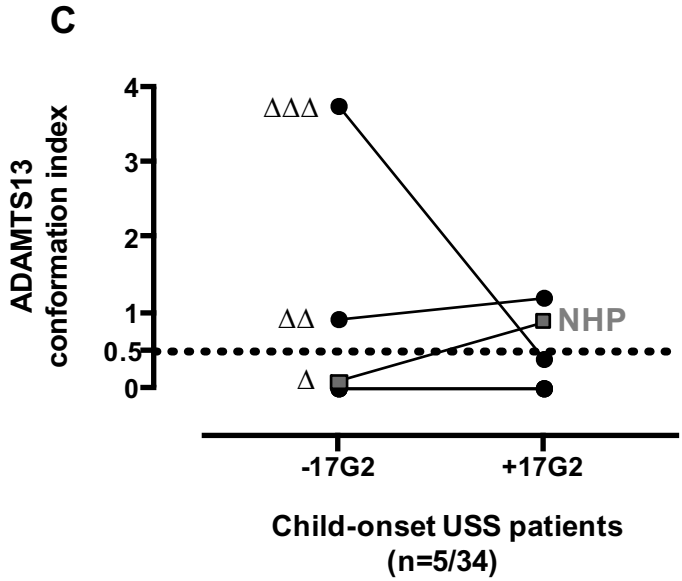

B
100
50
0
% of patients
Child-onset USS patients (n=34)
Adult-onset USS patients (n=22)
38%
55%
30%
31%
32%
5%
0%
9%
2 or 3 missense
1 or 2 missense + 1 truncating
2 truncating
1 missense + SNPs
ADAMTS13 sequence variations

Figure 4





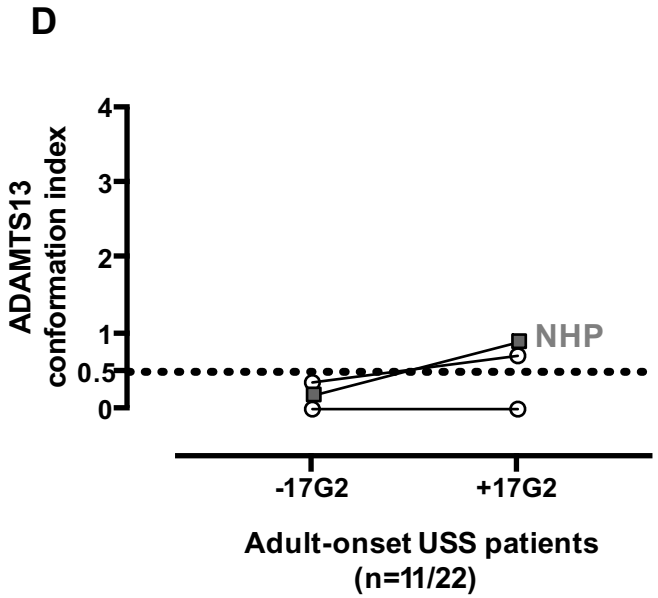


Child-onset USS patients (n=5)
***ADAMTS13* genotype**

ΔΔΔ **p.Arg1206*/p.Arg1206*** (CUB1/CUB1)

ΔΔ **c.1309-1G>A/p.Cys908Ser** (./TSP1-5)

Δ **c.988-2A>G/p.Asp1362Val** (./CUB2)
Δ **p.Ala596Val/p.Ala596Val** (spacer/spacer)
Δ **p.Cys1024Gly/p.Cys1024Gly** (TSP1-7/TSP1-7)

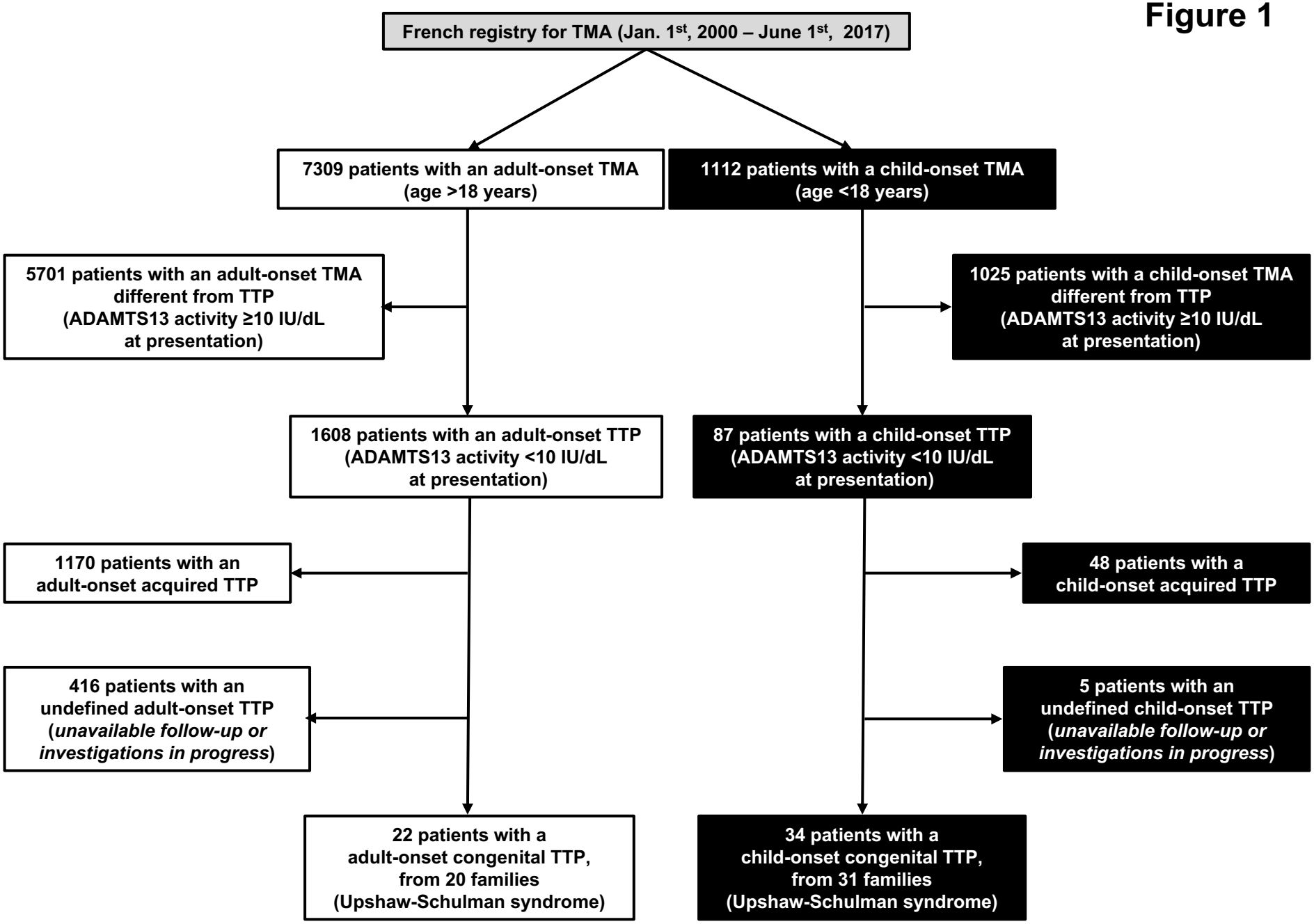


Adult-onset USS patients (n=11)
***ADAMTS13* genotype**

ΔΔ **p.Arg1060Trp/SNPs (n=2)** (TSP1-7/.)

Δ **p.Glu812*/p.Arg1060Trp** (./TSP1-7)
Δ **p.Pro353Leu/p.Arg1060Trp** (Disintegrin/TSP1-7)
Δ **p.Val88Leu/p.Arg1060Trp** (Metalloprotease/TSP1-7)
Δ **p.Asp551Tyr/p.Arg1060Trp** (Cys-rich/TSP1-7)
Δ **p.Arg1060Trp/p.Arg1060Trp (n=2)** (TSP1-7/TSP1-7)
Δ **c.173-14_173-2del12/p.Cys1067Serfs*30** (./TSP1-7)
Δ **p.Arg916Cys/p.Arg1060Trp** (TSP1-5/TSP1-7)
Δ **p.Trp470Leufs*64 / p.Arg1060Trp** (./TSP1-7)

Figure 1


French registry for TMA (Jan. 1st, 2000 – June 1st, 2017)
7309 patients with an adult-onset TMA (age >18 years)
1112 patients with a child-onset TMA (age <18 years)
5701 patients with an adult-onset TMA different from TTP (ADAMTS13 activity ≥10 IU/dL at presentation)
1025 patients with a child-onset TMA different from TTP (ADAMTS13 activity ≥10 IU/dL at presentation)
1608 patients with an adult-onset TTP (ADAMTS13 activity <10 IU/dL at presentation)
87 patients with a child-onset TTP (ADAMTS13 activity <10 IU/dL at presentation)
1170 patients with an adult-onset acquired TTP
48 patients with a child-onset acquired TTP
416 patients with an undefined adult-onset TTP (unavailable follow-up or investigations in progress)
5 patients with an undefined child-onset TTP (unavailable follow-up or investigations in progress)
22 patients with a adult-onset congenital TTP, from 20 families (Upshaw-Schulman syndrome)
34 patients with a child-onset congenital TTP, from 31 families (Upshaw-Schulman syndrome)