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Novel cryptic ADAMTS13 epitopes uncover a distinct open ADAMTS13 conformation in immune-mediated TTP

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Title

Novel cryptic ADAMTS13 epitopes uncover a distinct open ADAMTS13 conformation in immune-mediated TTP

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Running title

Distinct open ADAMTS13 conformation in iTTP

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Authorship Contribution

Q. Bonnez conceptualized the study, designed and performed experiments, analyzed and discussed data, prepared figures and wrote the manuscript. F. Boudry, L. De Waele, L. Vermeersch and I. Pareyn performed experiments and assisted in data analysis. K. Kangro designed and produced proteins. E. Tellier and G. Kaplanski provided samples. C. Tersteeg, S. F. De Meyer, B. S. Joly, A. Veyradier and P. Coppo discussed data and edited the manuscript. K. Vanhoorelbeke conceptualized the study, performed data analysis, data discussion and manuscript writing. All authors discussed results, supplied critical feedback and approved the final version of the manuscript.

Conflicts of Interest Disclosures

B. S. Joly participated in advisory boards for Sanofi, Takeda, Alexion and Werfen. P. Coppo is a member of the clinical advisory board for Alexion, Sanofi-Genzyme, Takeda and Janssen Pharmaceutica. He received fees from Alexion, Sanofi-Genzyme, Takeda, SOBI and Janssen Pharmaceutica. A. Veyradier is a member of the French advisory board for caplacizumab (Sanofi-Genzyme) and for recombinant human ADAMTS13 (Takeda). K. Vanhoorelbeke participated in advisory boards for Takeda and Werfen. All other authors declare to have no conflicts of interest.

Data Sharing Statement

All data is available upon reasonable request, please contact the corresponding author.

Key points:

- Novel cryptic epitopes were identified after antibody-induced opening of closed plasma ADAMTS13.
- All novel cryptic epitopes are consistently exposed in ADAMTS13 of iTTP patients, indicating a distinct open ADAMTS13 conformation.

Abstract

Open ADAMTS13 conformation is gaining clinical interest as a biomarker for diagnosing immune-mediated thrombotic thrombocytopenic purpura (iTTP) and monitoring remission patients for increased relapse risks. Yet, little is known on how open the structure of ADAMTS13 exactly is in iTTP patients. In this study, we aimed to assess the uniformity of open ADAMTS13 across iTTP patients. Hereto, we identified four monoclonal antibodies (mAbs) that recognize epitopes cryptic in closed ADAMTS13 from healthy donors but accessible upon antibody-mediated ADAMTS13 opening. Distributed across its D, T7, T8 and CUB1 domains, these cryptic epitopes indicate ADAMTS13 closure through multiple interdomain contacts extending beyond the well-described S-CUB interaction. Interestingly, all acute iTTP patients consistently present one distinct open ADAMTS13 in which all novel cryptic epitopes are accessible. During remission, closed ADAMTS13 with all epitopes being cryptic, is predominantly found in patients with restored activity, whereas distinct open ADAMTS13 is present in patients with subclinical disease. Furthermore, IgGs from iTTP patients opened the conformation of ADAMTS13, corroborating the role of pathogenic autoantibodies in opening ADAMTS13 in iTTP. These new cryptic epitope-recognizing mAbs hold the promise to further enhance our understanding of ADAMTS13's compactly closed conformation and may support the prediction of early relapses in the future.

Introduction

Plasma ADAMTS13 circulates as a multi-domain enzyme consisting of an N-terminal active site-bearing metalloprotease (M) domain, a disintegrin-like (D) domain, a first thrombospondin type-1 repeat (T1), a cysteine-rich (C) domain and a spacer (S) domain. The C-terminal tail of ADAMTS13 consists of seven more thrombospondin type-1 repeats (T2-T8) and two CUB (Complement C1r/C1s, Uegf, BMP-1; CUB1-2) domains.[1,2] Over the last decades, crystal structures were resolved for ADAMTS13's DTCS, MDTCS and CUB1-2 domains, and the interdomain interactions between the S and CUB1-2 domains have been extensively investigated.[3–5] Nevertheless, the structure of full-length (FL-) ADAMTS13 remains elusive. Recent AlphaFold predictions suggested FL-ADAMTS13 to compactly fold through additional interdomain contacts besides the well-described S-CUB interaction.[6,7] However, as AlphaFold is a weak predictor of fold-switching proteins[8], structural differences between closed and open ADAMTS13 remain poorly understood.

In immune-mediated thrombotic thrombocytopenic purpura (iTTP), pathogenic IgG autoantibodies induce an open ADAMTS13 conformation.[9,10] As a biomarker for acute and subclinical iTTP, open ADAMTS13 gains clinical interest to assist the diagnosis of patients with borderline ADAMTS13 activity as well as the prediction of relapse risks during remission.[9–13] Upon disruption of the S-CUB interaction, open ADAMTS13 exposes an S domain epitope, which normally remains cryptic in closed ADAMTS13.[9] Similarly, we showed that binding of our anti-CUB1 monoclonal antibody (mAb) 17G2 opens closed ADAMTS13 from healthy donor (HD) plasma, leading to exposure of the cryptic S domain epitope recognized by our mAb 1C4.[9,14,15] Besides conformationally opening ADAMTS13, 17G2 binding allosterically activates the M domain, resulting in the exposure of a cryptic epitope recognized by our anti-M mAb 6A6.[14–16]

With growing interest in open ADAMTS13 as a diagnostic marker, a thorough understanding of its conformation is essential. Therefore, the aim of this study was to characterize the uniformity of open ADAMTS13 conformation across iTTP patients. Through screening of novel cryptic epitopes, our main finding is that one distinct open ADAMTS13 conformation with exposure of multiple cryptic epitopes distributed across several domains characterizes acute iTTP. In addition, IgGs isolated from acute iTTP plasma samples induce this distinct open ADAMTS13 conformation.

Methods

Patient samples

Citrated plasma samples from 53 acute iTTP, 30 remission iTTP, and 25 HDs were available for ADAMTS13 analysis. By pooling plasma from >20 HDs, normal human plasma pool (NHP) was generated. All plasma samples were obtained according to the Declaration of Helsinki, and their use was approved by local ethic committees (N°#2007/23, Marseille, France or S62889 and S66725, UZ/KU Leuven, Belgium).

Monoclonal and total IgG antibodies

To identify novel cryptic epitope-recognizing mAbs, our antibody screen included a total of 60 different mAbs of which some were newly produced as described before, whereas most were previously already described.[9,14,16–18] In various ELISA setups, the mAbs 3H9, 6A6, 1D5, 1C4, 9C12, 19H4 and 10D2 were used as coating antibodies, whereas mAb 17G2 conformationally opens ADAMTS13 enabling detection of captured ADAMTS13 using biotinylated mAbs 15D1, 19H4 or 17G2.[9,14,16–18] These murine anti-human ADAMTS13 mAbs were produced and purified as previously described.[9,14,16–18] Total IgG (auto)antibody fractions were purified from acute iTTP patient (Samples 2, 5, 9, 12 and 20 of the MF-KB-TTPxx-A cohort) and HD plasmas as previously described.[10,19]

Screening of mAbs that recognize conformation sensitive epitopes

Based on our previously described Open/Closed ELISA, 60 anti-ADAMTS13 mAbs were screened for their potential to recognize cryptic epitopes that become exposed upon opening of closed plasma ADAMTS13.[9,14,16–18] Epitopes were considered cryptic when mAbs could not capture closed plasma ADAMTS13 but could capture plasma ADAMTS13 upon opening using mAb 17G2. In brief, candidate mAbs were coated (5 µg/mL in 0.05 M carbonate/bicarbonate buffer at pH 9.6), after which ¼-diluted NHP was added to capture plasma ADAMTS13 that was preincubated in absence (closed ADAMTS13) or presence (open ADAMTS13) of 17G2 (2.5 µg/mL in 0.3% milk powder in phosphate buffered saline (PBS)). Captured, open ADAMTS13 was detected using the biotinylated anti-S mAb 15D1 (1.5 µg/mL in 0.3% milk powder in PBS) and HRP-labelled high-sensitivity streptavidin (1/10 000 in 0.3% milk powder in PBS; Pierce™, Waltham, USA). As a positive control, mAb 1C4 was used, which is known to recognize a cryptic S domain epitope in ADAMTS13. In this case, the biotinylated anti-M mAb 3H9 was used for detection as previously described.[9,10]

ADAMTS13 antigen ELISA

To quantify ADAMTS13 antigen levels in patient or HD plasma, an in-house ELISA assay was used.[20] The anti-M mAb 3H9 (5 µg/mL in 0.05 M carbonate/bicarbonate buffer at pH 9.6) was coated onto a 96-well plate to capture plasma ADAMTS13. Starting at a 1/12.5 or 1/100 dilution for patients or HDs respectively, plasma samples were serially diluted in a 1.5/2.5

ratio. Captured plasma ADAMTS13 was detected using the biotinylated anti-T8 19H4 and anti-CUB1 17G2 mAbs (each at 1.5 µg/mL in 0.3% milk powder in PBS) and HRP-labeled streptavidin (1/10 000 in 0.3% milk powder in PBS; Roche, Basel, Switzerland). To calculate ADAMTS13 antigen present in patient or HD samples, antigen from NHP served as a 1 µg/mL reference.[20]

Evaluation of ADAMTS13 conformation

Cryptic epitope exposure was assessed in patient or HD samples based on our Open/Closed ELISA.[9,10] In brief, cryptic epitope-recognizing mAbs were individually coated (5 µg/mL in carbonate/bicarbonate buffer at pH 9.6), after which a plasma dilution series was added. Plasma samples were first preincubated either in absence or presence of the opening mAb 17G2 (2.5 µg/mL in 0.3% milk powder in PBS). NHP preincubated with the opening mAb 17G2 was used as an intra-assay reference. To verify the trigger for ADAMTS13 opening, a 1/8 dilution of NHP (containing closed plasma ADAMTS13) was preincubated with the total IgG fraction purified from acute iTTP patients (Samples 2, 5, 9, 12 and 20 of the MF-KB-TTPxx-A cohort) or HDs.[10,19] Detection of captured, open ADAMTS13 occurred as described above. When mAb 1C4 was coated, the biotinylated anti-M mAb 3H9 was used, whereas the biotinylated anti-S mAb 15D1 was used for detection when all other cryptic epitope-recognizing mAbs were coated. Cryptic epitope exposure was determined from ADAMTS13's conformation index (CI), which is calculated by correcting OD values for plasma antigen and normalizing for the intra-assay NHP control.[21] As previously described, a CI above 0.50 indicates accessible cryptic epitopes (open ADAMTS13), whereas a CI equal or below 0.50 represents inaccessible cryptic epitopes (closed ADAMTS13).[9,10]

Results

Anti-ADAMTS13 antibodies reveal novel cryptic epitopes in plasma ADAMTS13

To extend our understanding of open ADAMTS13, we first aimed to identify specific epitopes within different ADAMTS13 domains that only become accessible upon transition from its closed to its open conformation. Hereto, 60 mAbs from our anti-ADAMTS13 mAb library were screened for their capacity to only capture mAb 17G2-induced open ADAMTS13 but not closed ADAMTS13. Next to the mAbs 1C4 (anti-S)[9] and 6A6 (anti-M)[16], we identified four mAbs that specifically recognized cryptic epitopes exposed in open ADAMTS13. These included mAbs 1D5, 9C12, 19H4 and 10D2, respectively targeting the D, T7, T8 and CUB1 domains of ADAMTS13.[14,16,17,22] Indeed, all six antibodies showed residual to no binding to closed ADAMTS13 (Figure 1, filled bars). Only upon 17G2-induced opening, all six antibodies showed distinct binding to open ADAMTS13 (Figure 1, open bars), suggesting a significant role for the M, D, S, T7, T8 and CUB1 domains in maintaining the ADAMTS13

closed. Importantly, about 90% of our 60 anti-ADAMTS13 mAbs were found to distinctly capture closed plasma ADAMTS13, suggesting most antibody epitopes to be only partially cryptic or non-cryptic (Figure S1-5).

Closed ADAMTS13 in healthy donors

After identifying this new set of cryptic epitope-recognizing mAbs, we screened a cohort of 25 HDs to verify the CI cut-off that distinguishes between open ADAMTS13 with accessible epitopes or closed ADAMTS13 with cryptic epitopes. Previously, we identified a CI cut-off of 0.50 in our Open/Closed ELISA to evaluate the accessibility of the cryptic epitope recognized by the anti-S mAb 1C4.[9,10] Values above this threshold indicated open ADAMTS13 with accessible 1C4 epitope, while those equal or below the CI cut-off indicated closed ADAMTS13 with a cryptic 1C4 epitope. Similarly, we found that this same CI cut-off of 0.50 can be used to determine whether the cryptic epitopes recognized by mAbs 6A6, 1D5, 9C12, 19H4 and 10D2 are accessible (>0.50) or cryptic (≤0.50). Indeed, the CI values for closed ADAMTS13 (absence of 17G2) were below 0.50 in 22 out of 25 HD plasma samples (range CI_{6A6}=0.06-0.35, range CI_{1D5}=0.05-0.46, range CI_{9C12}=0.04-0.38, range CI_{19H4}=0.07-0.35 and range CI_{10D2}=0.02-0.15), whereas those for open ADAMTS13 (presence of 17G2) were all above 0.50 (range CI_{6A6}=0.83–2.03, range CI_{1D5}=0.66–1.54, range CI_{9C12}=0.58–1.33, range CI_{19H4}=0.67–1.79 and range CI_{10D2}=0.58–1.39) (Figure 2). Of note, three HDs had a $CI_{6A6,}$ and CI_{9C12} above 0.50 indicating that both M and T7 epitopes were accessible in these three HDs while the 1D5, 1C4, 19H4 and 10D2 epitopes within the D, S, T8 and CUB1 domains remained cryptic.

Distinct open ADAMTS13 in acute phase iTTP

Previously, we showed that acute phase iTTP patients presented open ADAMTS13 with an accessible 1C4 S domain epitope. Using our novel cryptic epitope-recognizing mAbs, we now wanted to evaluate whether acute phase iTTP patients present one distinct or multiple variable open ADAMTS13 conformations. Hereto, we screened the accessibility of each of the novel cryptic epitopes in plasma from 53 acute phase iTTP patients. For 14 patients, CI values could not be calculated as ADAMTS13 antigen was undetectable (i.e. ≤0.03 µg/mL).[10,20] Interestingly, for all other acute phase iTTP patients, we found that all cryptic epitopes in the M, D, S, T7, T8 and CUB1 domains are uniformly exposed, regardless of the presence of 17G2 (Figure 3). Indeed, all acute iTTP patients presented CI values above 0.50 (range CI_{6A6}=0.91-18.27, range CI_{1D5}=0.58-9.47, range CI_{9C12}=0.63-25.50, range CI_{19H4}=2.50-

14.59, range CI_{10D2}=0.51-9.42). As expected, the conformation remained open in all iTTP samples when preincubated in presence of 17G2 (range CI_{6A6}=1.33-16.74, range CI_{1D5}=0.73-9.52, range CI_{9C12}=0.58-20.58, range CI_{19H4}=3.07-18.65, range CI_{10D2}=0.62-9.39). Of note, one acute phase iTTP patient indicated a cryptic CUB1 epitope in presence of 17G2 (CI_{10D2}=0.48). Overall, these results indicate that regardless of mAb 17G2 addition, acute phase iTTP patients consistently present one distinct open ADAMTS13 conformation in which all newly identified mAb epitopes are exposed.

Distinct open ADAMTS13 in subclinical iTTP

Despite treatment response and clinical recovery, remission phase iTTP patients remain at risk for clinical and/or ADAMTS13 relapses.[11,23] Reappearance of anti-ADAMTS13 autoantibodies and a drop in ADAMTS13 activity represents ongoing subclinical iTTP, which is linked to open ADAMTS13 with accessible 1C4 S domain epitope.[10] Here, we evaluated the accessibility of the novel cryptic epitopes in remission phase iTTP patients with ADAMTS13 activity above (n=15) or below (n=15) 50% of its normal activity (Figure 4). All remission patient samples showed detectable ADAMTS13 antigen enabling CI calculation for each cryptic epitope recognizing antibody. In remission patients with ADAMTS13 activity restored >50%, about half of the plasma samples (53%, 8/15) indicated a closed ADAMTS13 with cryptic D, S, T7, T8 epitopes. Interestingly, the M domain epitope recognized by mAb 6A6 was found to remain cryptic less frequently (27%, 4/15), whereas the CUB1 domain epitope recognized by mAb 10D2 remained cryptic more frequently (80%, 12/15) in these patients with ADAMTS13 activity restored >50%. Upon addition of the mAb 17G2, closed ADAMTS13 could be opened as reflected by the exposure of all six domain epitopes. In remission patients with ADAMTS13 activity <50%, almost all patients (93%, 14/15) presented the same distinct open ADAMTS13 conformation with all M, D, S, T7, T8 and CUB1 domain epitopes being accessible, regardless of mAb 17G2 addition. Of note, in one patient presenting closed ADAMTS13 with inaccessible D, S, T7, T8 and CUB1 domain epitopes, its M domain epitope was accessible for 6A6 recognition. On the other hand, in another patient presenting open ADAMTS13 with accessible M, D, S, T7 and T8 domain epitopes, its CUB1 domain epitope remained cryptic and inaccessible for 10D2 recognition.

Pathogenic IgGs trigger distinct open ADAMTS13

As pathogenic IgG autoantibodies from iTTP patients were previously found to expose the S domain epitope for 1C4 recognition[10,19], we investigated whether such patient autoantibodies

also trigger the exposure of the M, D, T7, T8 and CUB1 domain epitopes. Hereto, closed plasma ADAMTS13 was incubated with either iTTP patient-purified total IgGs or HD-purified total IgGs (Figure 5). Incubation of a buffer condition (i.e. 'No IgGs), verified that plasma presented closed ADAMTS13 with all six epitopes being cryptic. Also, ADAMTS13's conformation remained closed, with all six epitopes being cryptic, following incubation of the total IgG fraction purified from two different HDs. Only upon incubation of the total IgG fraction purified from five different iTTP patients, ADAMTS13 adopted its distinct open conformation in which all M (range CI_{6A6}: 2.63-3.75), D (range CI_{1D5}: 0.95-1.65), S (range CI_{1C4}: 0.89-1.85), T7 (range CI_{9C12}: 1.68-3.75), T8 (range CI_{19H4}: 1.20-2.06) and CUB1 (range CI_{10D2}: 0.74-1.80) domain epitopes became accessible for mAb recognition. As anti-ADAMTS13 autoantibodies trigger the exposure of cryptic mAb epitopes in multiple ADAMTS13 domains, we studied whether presence of anti-ADAMTS13 autoantibodies in the three healthy donors could explain the presence of variably open ADAMTS13 (with accessible M and T7 epitopes, but inaccessible D, S, T8 and CUB1 epitopes) in these individuals. When evaluating all three HD plasmas using an in-house autoantibody ELISA assay[24], no positivity for anti-ADAMTS13 IgGs (data not shown) was found, suggesting a different trigger to cause this M and T7 domain epitope exposure.

Discussion

In this study, we performed an antibody screen to identify mAbs that selectively recognize previously unidentified cryptic epitopes across different domains in HD plasma ADAMTS13. Besides the previously described mAbs 6A6 (anti-M) and 1C4 (anti-S)[9,16], we here report four mAbs (1D5, 9C12, 19H4 and 10D2) to specifically bind epitopes within the D, T7, T8 and CUB1 domains of open ADAMTS13, but not to bind to closed ADAMTS13.[14,16,17,22] We previously demonstrated that ADAMTS13 adopts an open conformation in acute iTTP, characterized by the exposure of a cryptic epitope within the spacer domain, recognized by monoclonal antibody 1C4.[9] In the present study, we showed that this open conformation also revealed cryptic epitopes within the D, T7, T8, and CUB1 domains. These findings indicate the presence of a distinct and consistent open ADAMTS13 conformation across all patients with acute iTTP. In addition, when ADAMTS13 activity restores above 50% during remission, the enzyme predominantly adopts a closed conformation in most patients, rendering all epitopes cryptic. Interestingly, the same distinct open conformation of ADAMTS13 observed during the acute phase was also present in a subset of remission patients, in whom subclinical disease maintained ADAMTS13 activity below 50%. Finally, pathogenic IgG autoantibodies, isolated from acute iTTP plasmas, were capable to shift the closed ADAMTS13 conformation from healthy donors to a distinct open ADAMTS13 with all six epitopes being exposed. Thereby, we provide novel insight into the extent to which

ADAMTS13 opens its conformation, and thus enhance our understanding of the role for ADAMTS13's conformation in the pathophysiology of iTTP. The mAbs identified herein provide an interesting tool to further characterize the iTTP disease progression. With open ADAMTS13 being a predictive marker for earlier relapse risks, our mAbs might provide future value for the prediction and prevention of iTTP relapses.[12,13]

In absence of pathological IgGs, nearly all HDs adopted closed ADAMTS13 with cryptic M, D, S, T7, T8 and CUB1 domain epitopes. Hence, each of these domains appear to conformationally vary between closed and open ADAMTS13. Although it remains unclear whether these novel cryptic epitopes expose through inter- or intradomain changes, our findings support the hypothesis that closed ADAMTS13 could be compactly folded through multiple interdomain contacts extending beyond the well-described S-CUB interaction.[6,7] With cryptic epitopes in both proximal MDTCS and distal T2C2 domains, our findings for closed ADAMTS13 align with the condensed form of ADAMTS13 observed via electron microscopy, and with the more compact envelope found via small angle X-ray scattering (SAXS) and AlphaFold for FL-ADAMTS13 compared to truncated variants.[16,17,25] Intricately, the cryptic epitopes identified within the T7 and T8 domains corroborate their involvement in the minimal structure for allosterically regulated ADAMTS13 as proposed by SAXS, phylogenetic and functional analysis.[26,27] Curiously, no cryptic epitopes were found within the T3 to T6 domains that are dispensable in ADAMTS13's allosteric regulation.[26,27] As we also found cryptic epitopes within the M and D domains, it is tempting to speculate that these might be shielded by distal domain interactions as predicted by AlphaFold simulations and thus might be involved in ADAMTS13's allosteric regulation by occluding the active or substrate binding sites. Future resolving of the exact residues that shape each of these cryptic epitopes might provide valuable insight into how these domains likely engage in interdomain contacts to close ADAMTS13.

Surprisingly, 3 out of 25 HDs exhibited closed ADAMTS13 with cryptic D, S, T8 and CUB1 epitopes, yet with accessible M and T7 epitopes. As all plasma samples tested negative for anti-ADAMTS13 IgGs, the molecular basis for this variably closed ADAMTS13 remains unknown. Crystallization of the M domain has previously required structural stabilization by the mAb 3H9[4], and hydrogen-deuterium exchange mass spectrometry has shown that mAb binding induces flexibility in the M domain.[14,28] Thus, the accessibility of the 6A6 epitope might be linked to the inherent molecular flexibility of the M domain. Nevertheless, it remains unclear whether the accessibility of both M and T7 epitopes occurred by chance or resulted from the disruption of an interdomain contact. Hence, this finding raises the possibility that the conformation of closed ADAMTS13 may be more variable than previously thought. As

differences in closed ADAMTS13 are poorly explored, its incidence in broader population, and significance for health or disease in circulation remains currently unknown.

In conclusion, our study provides new insights into open and closed ADAMTS13 in two ways. First, we demonstrate that open ADAMTS13 exposes multiple cryptic epitopes across various domains, extending beyond those of the S-CUB interaction. We here reveal that once opened, ADAMTS13 adopts one distinct open conformation that is consistently observed in patients with acute and subclinical iTTP. Secondly, we found that closed ADAMTS13 likely adopts a compact, folded structure stabilized by more interdomain interactions than previously recognized.

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Figure Legends

Figure 1. Open ADAMTS13 reveals cryptic epitopes across various domains. Closed plasma ADAMTS13 was incubated without (black bars) or with (white bars) the anti-CUB1 mAb 17G2, which conformationally opens ADAMTS13 before loading on a 96-well plate coated with the previously described mAbs 1C4 and 6A6,[9,14,16] and the new mAbs 1D5, 9C12, 19H4 and 10D2, directed against epitopes in the S, M, D, T7, T8 and CUB1 domains, respectively. All coated mAbs could capture open ADAMTS13 (white bars) but not closed ADAMTS13 (black bars), indicating that these mAbs recognize cryptic epitopes that become exposed upon mAb 17G2-induced conformational opening of ADAMTS13. For each mAb, data are expressed as relative binding to open ADAMTS13 (mean ± standard deviation, n=3 independent experiments).

Figure 2. ADAMTS13 is closed in healthy donors. Microtiter plates were coated with mAbs to screen cryptic ADAMTS13 epitope exposure in plasma from healthy donors (HDs) in absence or presence of the anti-CUB1 mAb 17G2. Nearly all HD plasma samples (22/25) showed natively closed ADAMTS13 in which all cryptic epitopes were inaccessible (filled circles) but could become exposed upon mAb 17G2 incubation (open circles). Three HDs revealed accessible 6A6 and 9C12-recognized epitopes in the M and T7 domains respectively (open circles), whereas their 1C4, 1D5, 19H4 and 10D2-recognized epitopes in the S, D, T8 and CUB1 domains remained cryptic (filled circles). For each HD, data are expressed as a conformation index by correcting ELISA absorbance values for plasma antigen levels and normalizing for the intra-assay NHP control.[21] The dotted line represents the cut-off that differentiates accessible (>0.50) from inaccessible (≤0.50) ADAMTS13 epitopes, representing open and closed ADAMTS13 respectively.

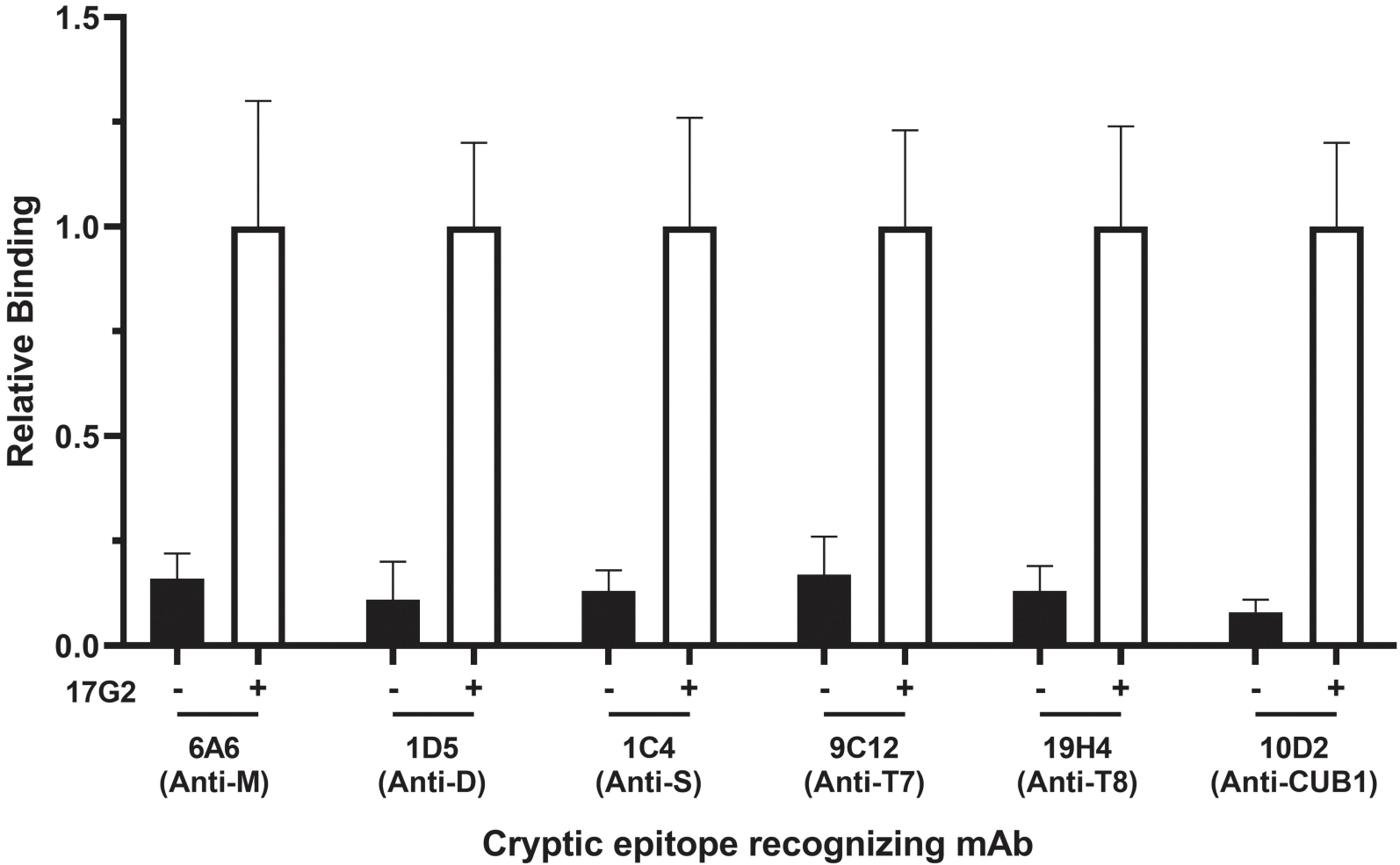
Figure 3. One distinct open ADAMTS13 conformation in acute iTTP. Microtiter plates were coated with mAbs to screen cryptic ADAMTS13 epitope exposure in plasma from acute iTTP patients in absence or presence of the anti-CUB1 mAb 17G2. Plasma samples from 39 acute iTTP patients were eligible for screening their exposure of novel cryptic ADAMTS13 epitopes in ELISA. Regardless of mAb 17G2 addition, all patients presented one distinct open ADAMTS13 conformation (open circles) with exposure of each cryptic epitope. The dotted line represents the cut-off that differentiates accessible (>0.50) from inaccessible (≤0.50) ADAMTS13 epitopes respectively.

Figure 4. One distinct open ADAMTS13 conformation in subclinical iTTP. Microtiter plates were coated with mAbs to screen cryptic ADAMTS13 epitope exposure in plasma from remission iTTP patients in absence or presence of the anti-CUB1 mAb 17G2. Plasma samples from 30 remission iTTP patients were available for cryptic epitope screening in ELISA. In patients with ADAMTS13 activity >50%, over half of the patients (8/15) indicated closed ADAMTS13 with cryptic D, S, T7, T8 epitopes (closed circles). The M domain epitope recognized by mAb 6A6 remained less frequently cryptic (4/15), whereas the CUB1 domain epitope recognized by mAb 10D2 remained more frequently cryptic (12/15) in these patients. In patients with ADAMTS13 activity <50%, almost all patients (13/15) presented one distinct open ADAMTS13 conformation with all M, D, S, T7, T8 and CUB1 domain epitopes being accessible (open circles). Addition of mAb 17G2 opened ADAMTS13 in all patients (30/30) reflected by exposure of all six domain epitopes. The dotted line represents the cut-off that differentiates accessible (>0.50) from inaccessible (≤0.50) ADAMTS13 epitopes.

Figure 5. Pathogenic IgGs trigger distinct open ADAMTS13. Microtiter plates were coated with mAbs to screen cryptic ADAMTS13 epitope exposure in plasma from healthy donors in absence or presence of the total IgG fraction purified from acute iTTP patients or healthy donors (HDs). In absence of IgGs, plasma ADAMTS13 was verified to be closed (closed circles). Following incubation of the total IgG fraction purified from healthy donors (HD IgGs), closed ADAMTS13 was indicated by inaccessible cryptic epitopes. Incubation with iTTP-purified IgGs induced distinct open ADAMTS13 in which all epitopes became accessible. The dotted line represents the cut-off that differentiates accessible (>0.50) from inaccessible (≤0.50) ADAMTS13 epitopes respectively.

Figures

All figures are available as separate .JPG file.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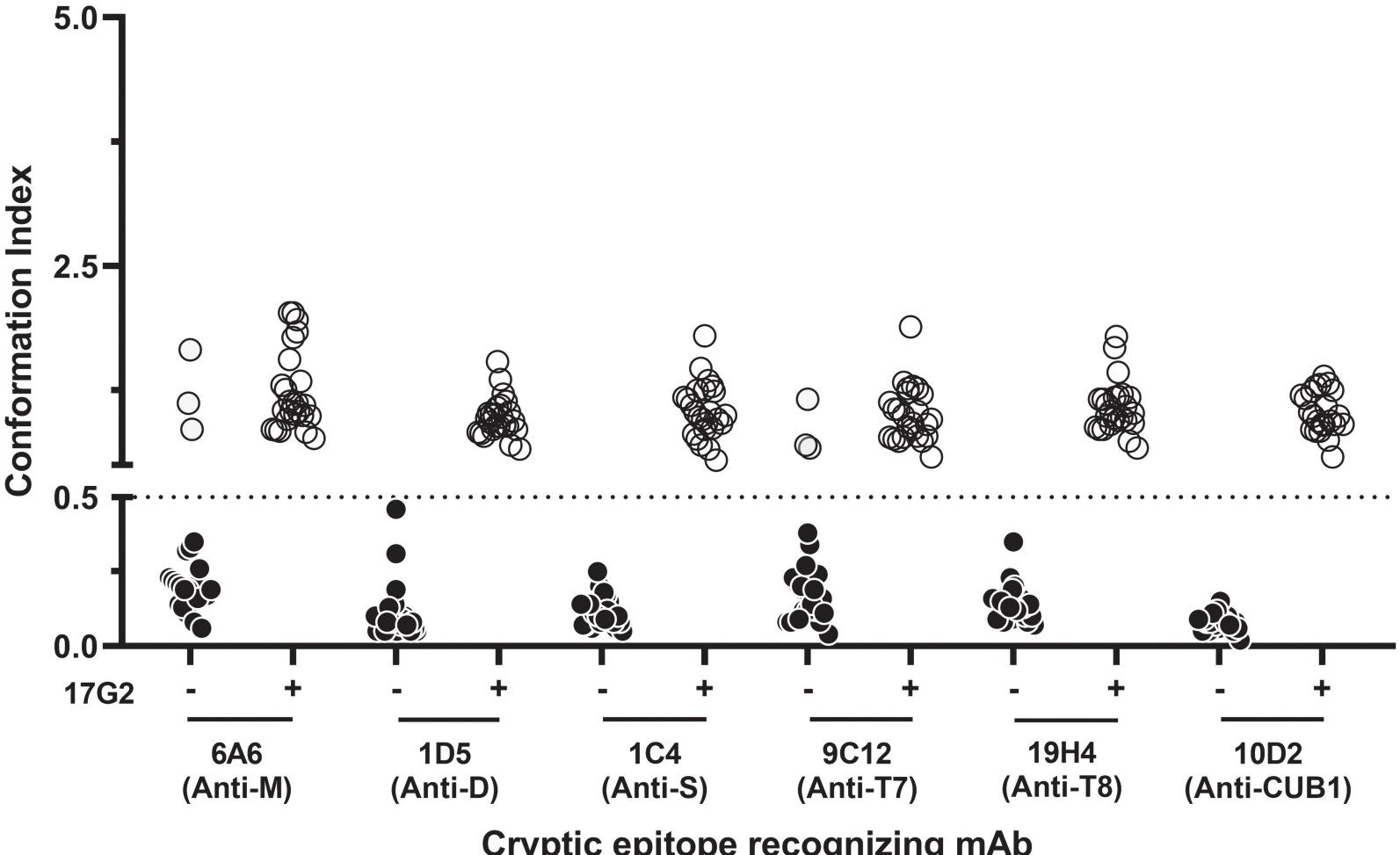
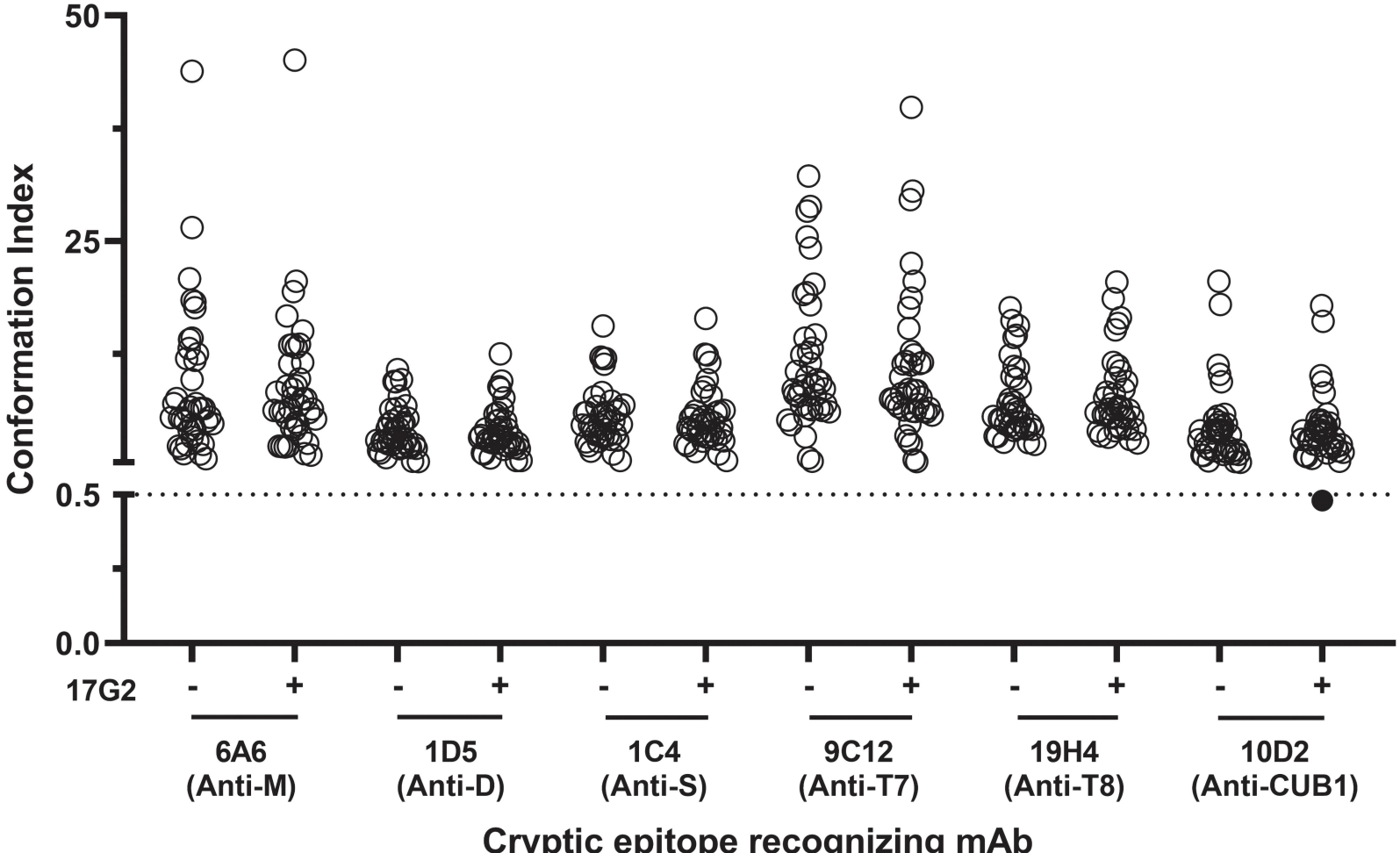
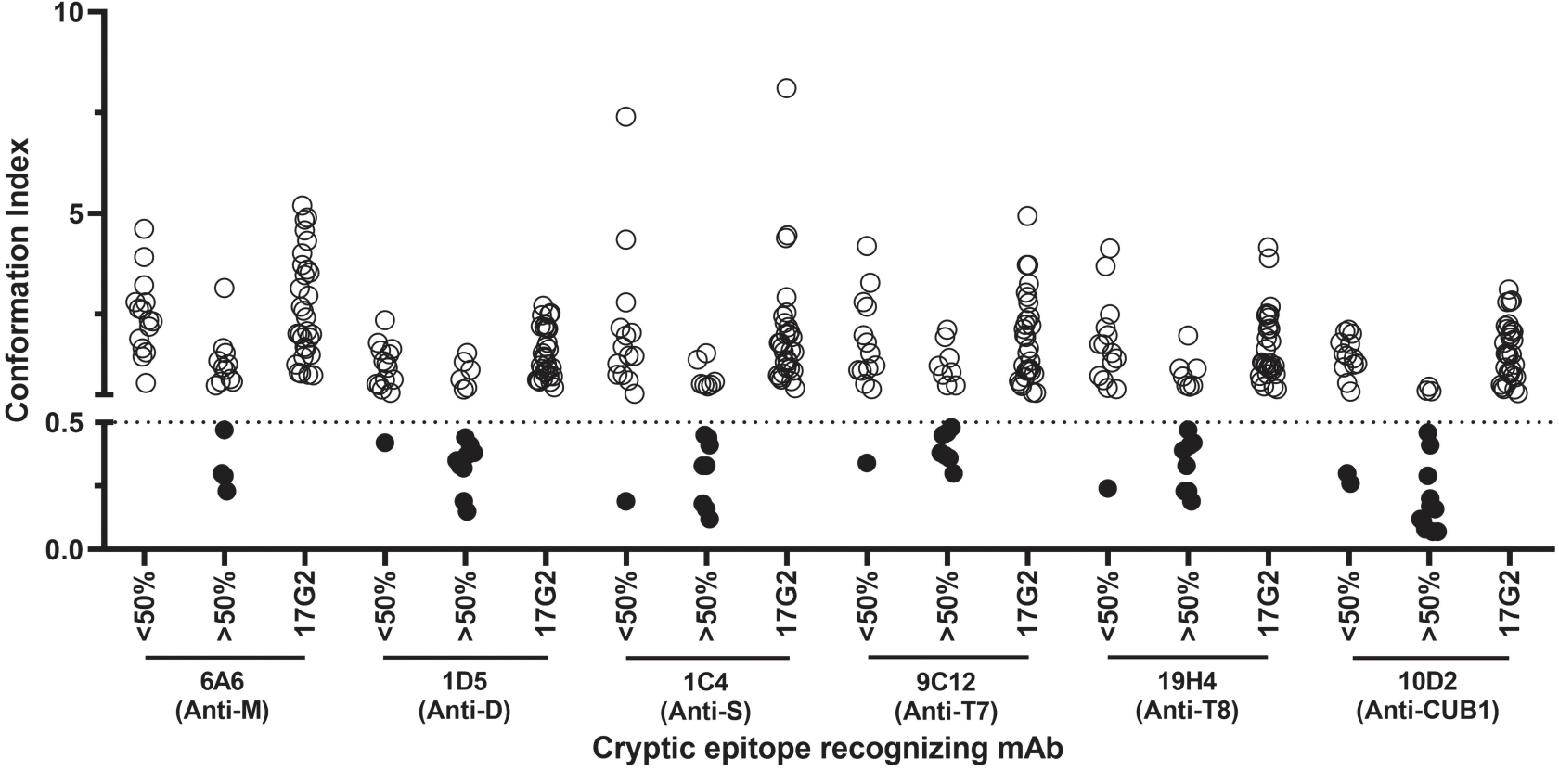
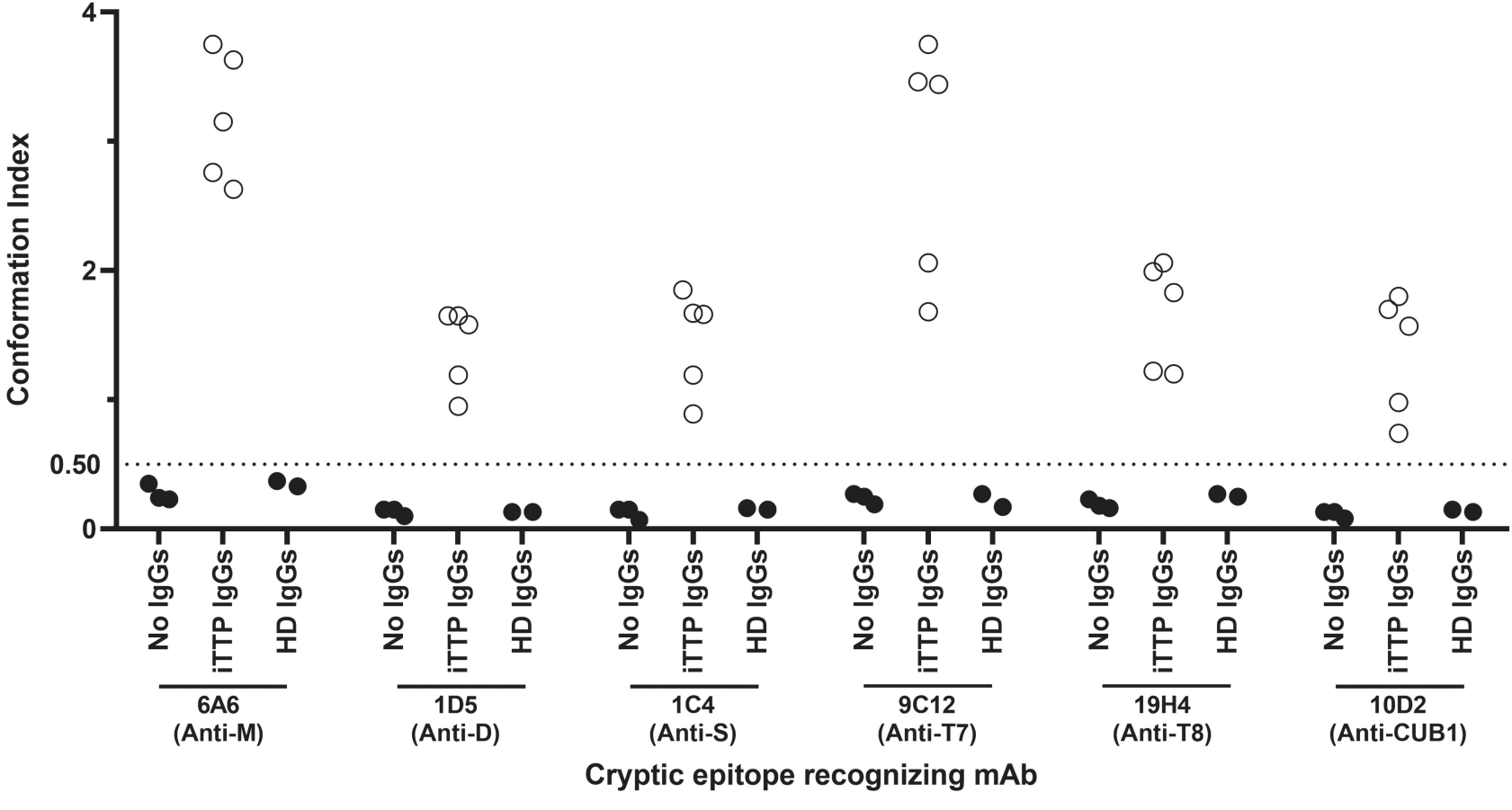
Relative Binding
17G2
- + - + - + - + - + - +
6A6 (Anti-M)
1D5 (Anti-D)
1C4 (Anti-S)
9C12 (Anti-T7)
19H4 (Anti-T8)
10D2 (Anti-CUB1)
Cryptic epitope recognizing mAb


5.0
2.5
0.5
0.0
Conformation Index
17G2
- + - + - + - + - + - +
6A6 (Anti-M)
1D5 (Anti-D)
1C4 (Anti-S)
9C12 (Anti-T7)
19H4 (Anti-T8)
10D2 (Anti-CUB1)
Cryptic epitope recognizing mAb


50
25
0.5
0.0
Conformation Index
17G2
- + - + - + - + - + - +
6A6 (Anti-M)
1D5 (Anti-D)
1C4 (Anti-S)
9C12 (Anti-T7)
19H4 (Anti-T8)
10D2 (Anti-CUB1)
Cryptic epitope recognizing mAb


Conformation Index
10
5
0.5
0.0
<50%
>50%
17G2
6A6
(Anti-M)
1D5
(Anti-D)
1C4
(Anti-S)
9C12
(Anti-T7)
19H4
(Anti-T8)
10D2
(Anti-CUB1)
Cryptic epitope recognizing mAb


Conformation Index
4
2
0.50
0
No IgGs
iTTP IgGs
HD IgGs
6A6
(Anti-M)
1D5
(Anti-D)
1C4
(Anti-S)
9C12
(Anti-T7)
19H4
(Anti-T8)
10D2
(Anti-CUB1)
Cryptic epitope recognizing mAb

Title

Novel cryptic ADAMTS13 epitopes uncover one distinct open ADAMTS13 in iTTP

Authors

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Supplementary data

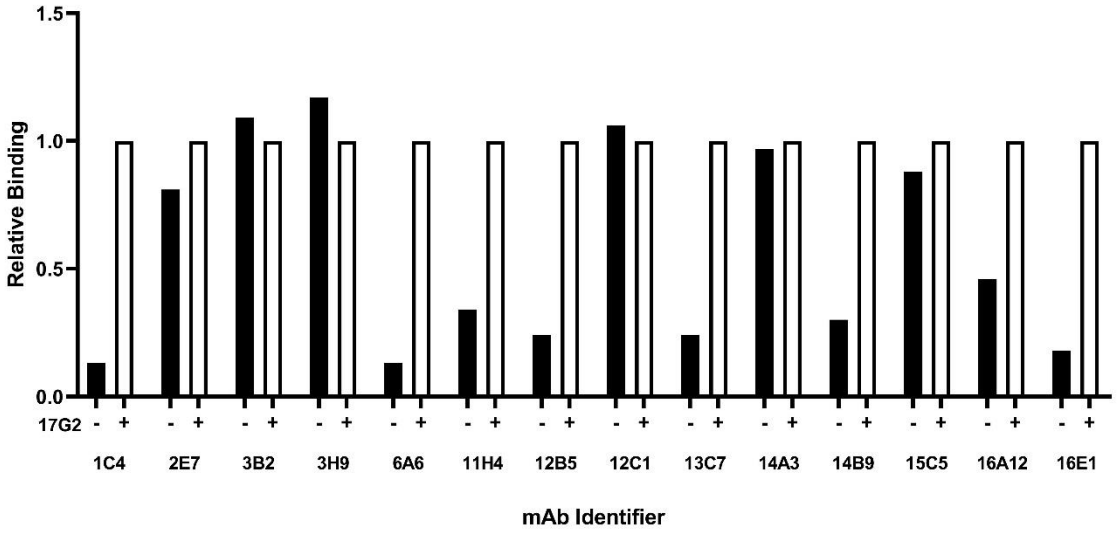

Figure S1. Antibody screen of anti-M domain monoclonal antibodies. A total of 13 anti-M mAbs were screened for their potential to recognize cryptic epitopes in ADAMTS13. Epitopes were considered cryptic when mAbs could not capture closed plasma ADAMTS13 (black bars) but could capture plasma ADAMTS13 upon opening using mAb 17G2 (white bars). As a positive control, mAb 1C4 was used as this mAb is known to recognize a cryptic ADAMTS13 epitope.[1]

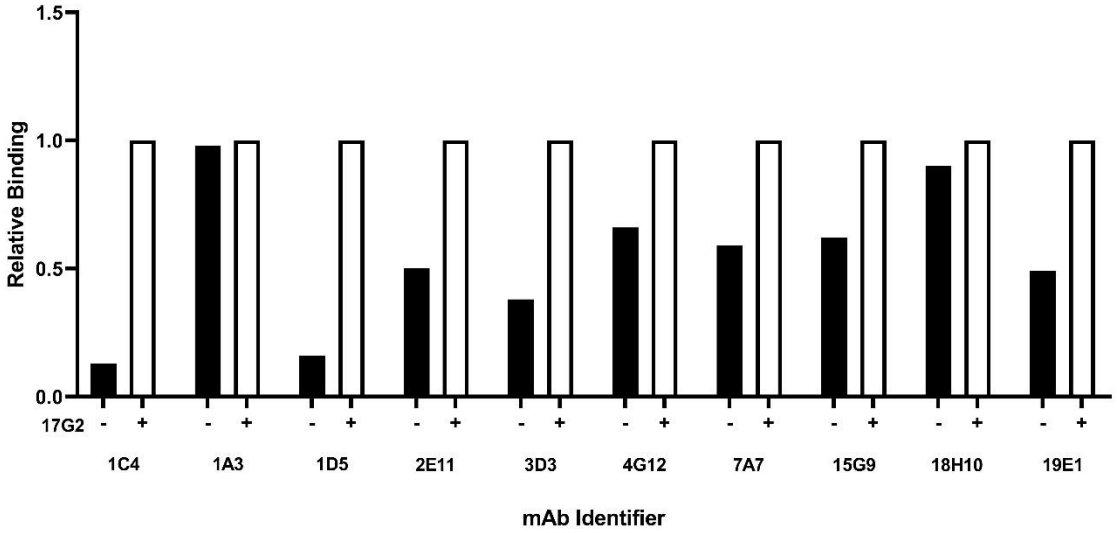

Figure S2. Antibody screen of anti-DT domain monoclonal antibodies. A total of 9 anti-DT mAbs were screened for their potential to recognize cryptic epitopes in ADAMTS13. Epitopes were considered cryptic when mAbs could not capture closed plasma ADAMTS13 (black bars) but could capture plasma ADAMTS13 upon opening using mAb 17G2 (white bars). As a positive control, mAb 1C4 was used as this mAb is known to recognize a cryptic ADAMTS13 epitope.[1]

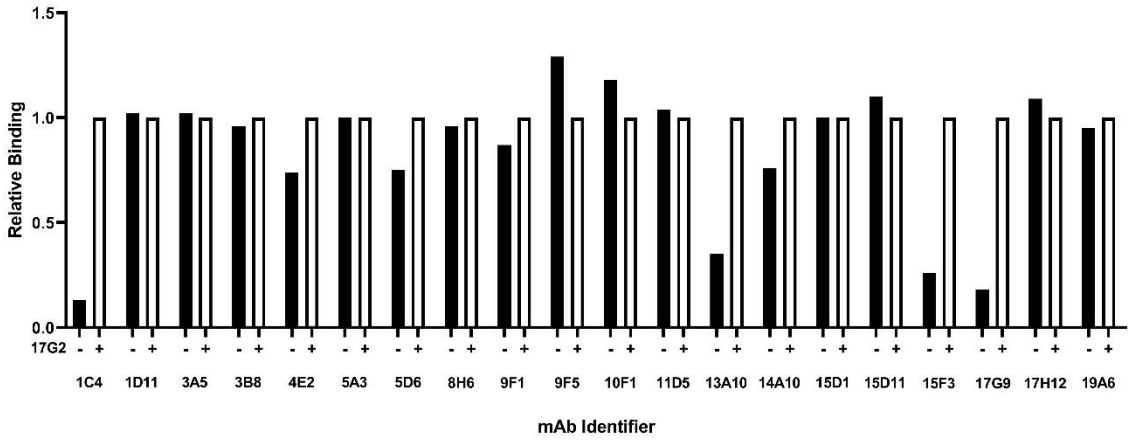

Figure S3. Antibody screen of anti-CS domain monoclonal antibodies. A total of 19 anti-CS mAbs were screened for their potential to recognize cryptic epitopes in ADAMTS13. Epitopes were considered cryptic when mAbs could not capture closed plasma ADAMTS13 (black bars) but could capture plasma ADAMTS13 upon opening using mAb 17G2 (white bars). As a positive control, mAb 1C4 was used as this mAb is known to recognize a cryptic ADAMTS13 epitope.[1]

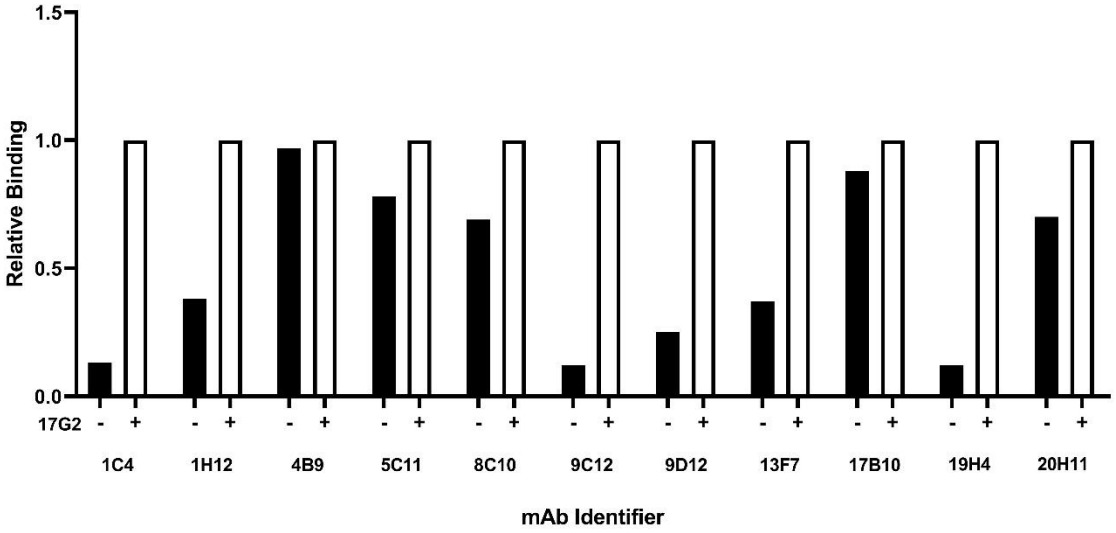

Figure S4. Antibody screen of anti-T2T8 domain monoclonal antibodies. A total of 10 anti-T2T8 mAbs were screened for their potential to recognize cryptic epitopes in ADAMTS13. Epitopes were considered cryptic when mAbs could not capture closed plasma ADAMTS13 (black bars) but could capture plasma ADAMTS13 upon opening using mAb 17G2 (white bars). As a positive control, mAb 1C4 was used as this mAb is known to recognize a cryptic ADAMTS13 epitope.[1]

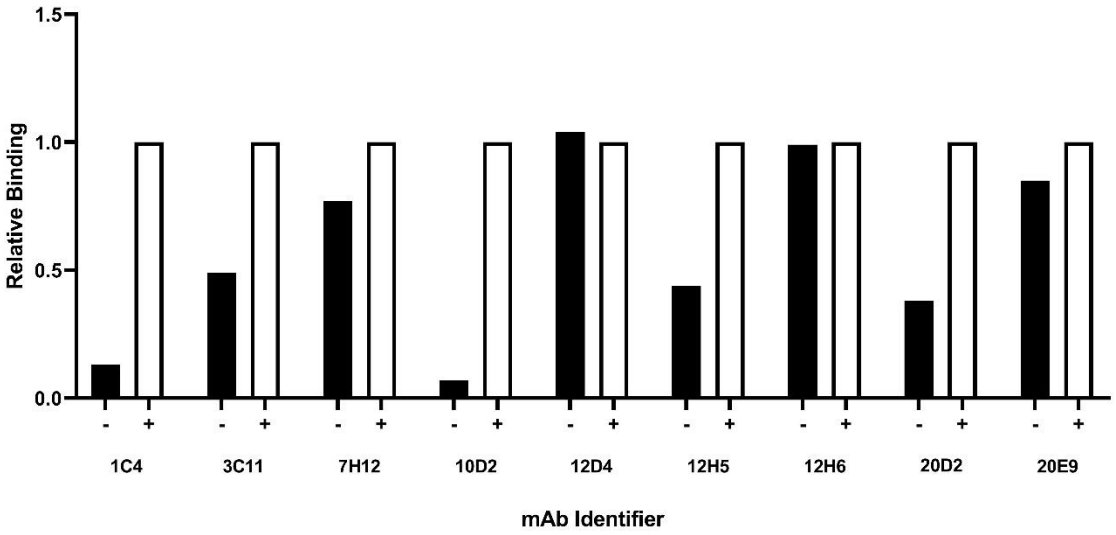

Figure S5. Antibody screen of anti-CUB domain monoclonal antibodies. A total of 8 anti-CUB mAbs were screened for their potential to recognize cryptic epitopes in ADAMTS13. Epitopes were considered cryptic when mAbs could not capture closed plasma ADAMTS13 (black bars) but could capture plasma ADAMTS13 upon opening using mAb 17G2 (white bars). As a positive control, mAb 1C4 was used as this mAb is known to recognize a cryptic ADAMTS13 epitope.[1]

References

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