ORIGINAL ARTICLE
PULMONARY VASCULAR DISEASE

The ADAMTS13–VWF axis is dysregulated in chronic thromboembolic pulmonary hypertension

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The ADAMTS-13–VWF axis is dysregulated in chronic thromboembolism with and without pulmonary hypertension and is implicated in the pathogenesis http://ow.ly/J9SC30nh5T0

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ABSTRACT Chronic thromboembolic pulmonary hypertension (CTEPH) is an important consequence of pulmonary embolism that is associated with abnormalities in haemostasis. We investigated the ADAMTS13–von Willebrand factor (VWF) axis in CTEPH, including its relationship with disease severity, inflammation, *ABO* groups and *ADAMTS13* genetic variants.

ADAMTS13 and VWF plasma antigen levels were measured in patients with CTEPH (n=208), chronic thromboembolic disease without pulmonary hypertension (CTED) (n=35), resolved pulmonary embolism (n=28), idiopathic pulmonary arterial hypertension (n=30) and healthy controls (n=68). CTEPH genetic *ABO* associations and protein quantitative trait loci were investigated. ADAMTS13–VWF axis abnormalities were assessed in CTEPH and healthy control subsets by measuring ADAMTS13 activity, D-dimers and VWF multimeric size.

Patients with CTEPH had decreased ADAMTS13 (adjusted β −23.4%, 95% CI −30.9– −15.1%, p<0.001) and increased VWF levels (β +75.5%, 95% CI 44.8–113%, p<0.001) compared to healthy controls. ADAMTS13 levels remained low after reversal of pulmonary hypertension by pulmonary endarterectomy surgery and were equally reduced in CTED. We identified a genetic variant near the *ADAMTS13* gene associated with ADAMTS13 protein that accounted for ∼8% of the variation in levels.

The ADAMTS13–VWF axis is dysregulated in CTEPH. This is unrelated to pulmonary hypertension, disease severity or markers of systemic inflammation and implicates the ADAMTS13–VWF axis in CTEPH pathobiology.

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Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) results from failure of thrombus resolution in the pulmonary arteries following acute pulmonary embolism (PE) in 3% of cases [1]. Organisation and fibrosis of thrombotic material leads to the obstruction of proximal pulmonary arteries and the subsequent development of a secondary small vessel vasculopathy, both of which contribute to pulmonary hypertension and subsequent right heart failure [2, 3].

Abnormalities in haemostasis are implicated in CTEPH pathobiology [4, 5]. This includes elevated von Willebrand factor (VWF), a multimeric plasma glycoprotein that is synthesised by vascular endothelial cells and megakaryocytes [6, 7]. VWF plays an important role in platelet recruitment by mediating the adhesion of platelets to the endothelium and is also a carrier protein for the pro-coagulant blood clotting Factor VIII [7]. VWF activity is normally regulated by ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), a plasma protein that specifically cleaves the more active high molecular weight VWF multimers [8]. ADAMTS13 is predominately produced by hepatic stellate cells, in addition to vascular endothelial cells and megakaryocytes [9]. The critical role of ADAMTS13 levels in haemostasis is exemplified by thrombotic thrombocytopenic purpura (TTP), characterised by micro-angiopathic thrombosis, in which plasma levels of ADAMTS13 are severely reduced by autoantibodies or rare *ADAMTS13* mutations [10].

Plasma VWF levels are increased in a range of thrombotic conditions, including coronary artery disease (CAD), ischaemic stroke and venous thromboembolism [11, 12]. Conversely, plasma ADAMTS13 levels are modestly reduced in CAD and ischaemic stroke [11, 13]. There are discordant findings in patients with acute PE, with ADAMTS13 levels reported to be increased, no different and decreased [14–16]. VWF and Factor VIII are known to be elevated in CTEPH and do not change following pulmonary endarterectomy (PEA), suggesting a role in pathogenesis [6]. Whilst VWF cleaving protease has been indirectly assessed in CTEPH, the direct role of ADAMTS13 has not been investigated to date [6].

A large proportion of the variation in VWF levels is genetically determined, with 30% due to *ABO* groups [17]. The *ADAMTS13* gene is situated ∼200 kilobases (kb) downstream of *ABO* and is genetically regulated, with 20% of its variance attributable to common variants at the *ADAMTS13* locus [18]. ADAMTS13 is not known to vary with *ABO* groups in healthy cohorts [19]. Similar to in other thrombotic diseases, the non-O blood groups are over-represented in CTEPH, suggesting a mechanism by which VWF levels are increased [20]. We aimed to investigate the ADAMTS13–VWF axis in CTEPH patients, including its relationship with *ABO* groups and *ADAMTS13* genetic variants.

Methods

Study samples and participants

The study was approved by the regional ethics committee (REC no. 08/H0304/56 and 08/H0802/32) and all study participants provided written informed consent from their respective institutions.

Consecutive CTEPH patients from the national PEA centre (Royal Papworth Hospital, Cambridge, UK) with available plasma samples (August 2013–December 2016) (supplementary figure S1) and genotype data were included in the study (n=208). Healthy volunteers (n=68) were used as a control group (Royal Papworth Hospital and Hammersmith Hospital, London, UK). Additional patient groups were recruited as disease comparators, including patients with chronic thromboembolic disease (CTED) (n=35), idiopathic pulmonary arterial hypertension (IPAH) (n=30) and PE (n=28). CTED was characterised by persistent pulmonary arterial thromboembolic occlusions without pulmonary hypertension (mean pulmonary arterial pressure <25 mmHg) in symptomatic patients, and other diagnoses were made using international criteria [21, 22].

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ADAMTS13 and VWF plasma concentrations

Plasma samples were used to measure ADAMTS13 and VWF antigen (Ag) levels by ELISA. Samples for the CTEPH, CTED and IPAH groups were obtained closest to the time of diagnosis, and pre-operatively for the CTEPH and CTED patients undergoing PEA. Additionally, ADAMTS13 and VWF levels were measured in 22 paired post-PEA samples taken within 1 year of surgery to assess the effect of PEA. The PE group was sampled from a specialist PE follow-up service (Hammersmith Hospital, London) at a median (interquartile range (IQR)) of 220 (218) days following an acute PE.

ADAMTS13 and VWF plasma antigen levels were quantified using polyclonal rabbit anti-ADAMTS13 and anti-VWF antibodies as previously described (supplementary material) [19, 23].

ADAMTS13 activity, D-dimer, anti-ADAMTS13 autoantibodies and VWF multimeric size

Additional experiments were performed on a subset of the CTEPH (n=21–23) and healthy control (n=14) groups to identify potential mechanisms for any dysregulation of the ADAMTS13–VWF axis. Plasma samples were used to measure ADAMTS13 activity (fluorescence resonance energy transfer assay), D-dimer concentrations (ELISA) and anti-ADAMTS13 autoantibodies (CTEPH, n=23), with further details in the supplementary material. An estimate of VWF multimeric size was made by measuring VWF collagen binding (VWF collagen binding assay (CBA)) and comparing this with VWF antigen levels (CTEPH, n=21).

Clinical phenotype data

Phenotype data for the CTEPH, CTED and IPAH groups were recorded closest to the time of diagnosis and pre-operatively for the CTEPH and CTED patients undergoing PEA. This included demographics, haemodynamics, World Health Organization functional class, 6-minute walk distance (6MWD), clinical blood tests, smoking history and anticoagulation therapy usage.

Genotype data

Imputed genotype dosages were available from an ongoing international genome-wide association study (GWAS) in CTEPH that will be published separately on recruitment of a validation cohort. All individuals were genotyped on commercially available Illumina assays (Illumina, Cambridge, UK) and imputed to the Haplotype Reference Consortium build 1.1 [24]. Additional details and quality controls steps are described in the supplementary material.

Genotypes were available for 207 patients (185 CTEPH, 22 CTED) after GWAS quality control exclusions. These patients were included in the genetic *ABO* group and protein quantitative trait loci (pQTL) analyses. Matched genotypes and ADAMTS13/VWF antigen levels were not available for the healthy control, IPAH or PE groups.

Genetic ABO groups

The *ABO* groups A1, A2, B and O were reconstructed using haplotypes from phased data and a described list of tagging *ABO* single nucleotide polymorphisms (SNPs) (supplementary material). This resulted in 10 groups (A1A1, A1A2, A1B, A1O, A2A2, A2B, A2O, BB, BO, OO), from which blood groups A, B, AB and O were inferred.

Protein quantitative trait loci

Associations between genetic variants in the *ADAMTS13* gene ±40 kb (n=396 variants) and ADAMTS13 protein levels were evaluated using multivariable linear regression. The model was adjusted for age, sex and ADAMTS13 plasma antigen experimental batch. Additional models were adjusted for VWF antigen levels and the first five ancestry informative principal components used in the GWAS analysis. The *ADAMTS13* ±40 kb region included the *ADAMTS13* cis-pQTLs that have previously been described [18, 25, 26].

Statistical analysis

Group differences in ADAMTS13 and VWF antigen levels were assessed using multivariable linear regression adjusted for age, sex, experimental batch (batch1 *versus* batch2) and self-reported ethnicity (Caucasian *versus* non-Caucasian). The β coefficients and confidence intervals are presented as percentage change.

Data are presented as median (IQR). Spearman's rank correlation coefficients were used to describe associations between ADAMTS13 or VWF protein levels and clinical phenotypes associated with disease severity and blood markers of inflammation.

Results

Baseline group characteristics are summarised in table 1 and supplementary table S1. Age and sex differed across the groups (p<0.001 and p=0.014), with CTEPH patients being older (64 (19) years) than healthy controls (49 (24) years). Ethnicity also differed (p<0.001) with more non-Caucasians in the PE group. In the CTEPH group, 176 patients (87%) had a proximal distribution of pulmonary arterial obstruction deemed to be surgically accessible and 150 (72%) underwent PEA.

ADAMTS13 plasma concentrations

ADAMTS13 antigen levels were decreased in CTEPH patients (0.889 (0.397) $\mu g \cdot mL^{-1}$, p<0.001) compared to healthy controls (1.15 (0.300) $\mu g \cdot mL^{-1}$) (figure 1a). ADAMTS13 was also reduced in CTED (0.831 (0.224) $\mu g \cdot mL^{-1}$, p<0.001) but levels were similar to those in CTEPH (p=0.205) (supplementary table S2). There was no difference in ADAMTS13 levels between IPAH (1.12 (0.413) $\mu g \cdot mL^{-1}$, p=0.373) and healthy controls, though the PE group did exhibit slightly lower levels (0.969 (0.704) $\mu g \cdot mL^{-1}$, p=0.049).

Multivariable linear regression confirmed that ADAMTS13 was lowest in the CTEPH (β 23.4%, 95% CI −30.9– −15.1%, p<0.001) and CTED (β −25.9%, 95% CI −35.1– −15.4%, p<0.001) groups (supplementary table S3). These observations should be interpreted with the additional models utilising interaction terms presented in the supplementary materials. Increasing age was also associated with lower ADAMTS13 (β −5.06%, 95% CI −2.99– −7.08% per 10 years, p<0.001). ADAMTS13 antigen levels were not significantly associated with the PE group (β −12.0%, 95% CI −24.0–1.97%, p=0.089) nor IPAH, sex or ethnicity.

TABLE 1 Baseline group characteristics

	Healthy control	CTEPH	CTED	IPAH	PE
Subjects n	68	208	35	30	28
Age years	49 (24)	64 (19)	58 (27)	64 (27)	52 (26)
Female sex	32 (47%)	90 (43%)	9 (26%)	21 (70%)	15 (54%)
Caucasian ethnicity	53 (78%)	180 (95%)	28 (88%)	26 (90%)	13 (54%)
WHO functional class					
1		4 (2%)	6 (18%)	5 (17%)	
2		42 (21%)	17 (50%)	4 (13%)	
3		151 (74%)	11 (32%)	21 (70%)	
4		7 (3%)	0 (0%)	0 (0%)	
6MWD m		318 (176)	366 (180)	342 (244)	
Pulmonary haemodynamics					
mPAP mmHg		42 (18)	21 (4)	42 (17)	
Cardiac index $L \cdot min^{-1} \cdot m^{-2}$		2 (0.6)	2.4 (0.6)	1.7 (0.8)	
PVR $dynes \cdot s \cdot cm^{-5}$		639 (476)	151 (71)	808 (642)	
Clinical blood tests					
Haemoglobin $g \cdot L^{-1}$		140 (27)	138 (16)	142 (22)	
Platelet count $\times 10^9$		246 (82)	200 (56)	222 (77)	
WCC $\times 10^9$		7 (3)	6.6 (2.1)	6.9 (2.4)	
Lymphocyte %		25 (10)	28 (13)	18 (13)	
Neutrophil %		64 (14)	59 (14)	72 (14)	
CRP $mg \cdot L^{-1}$		5 (10)	3 (3)	3 (4)	
NT-proBNP $pg \cdot mL^{-1}$		592 (1576)	113 (194)	334 (695)	
Smoking status					
Never		91 (47%)	16 (50%)	15 (52%)	
Ex-smoker		87 (45%)	13 (41%)	11 (38%)	
Current smoker		15 (8%)	3 (9%)	3 (10%)	
Anticoagulation medication		137 (94%)	15 (94%)	30 (100%)	

Data are presented as median (interquartile range) or n (%). Percentages were calculated using the number of patients for whom data were available as the denominator. CTEPH: chronic thromboembolic pulmonary hypertension; CTED: chronic thromboembolic disease; IPAH: idiopathic pulmonary arterial hypertension; PE: pulmonary embolism; WHO: World Health Organization; 6MWD: 6-minute walk distance; mPAP: mean pulmonary arterial pressure; PVR: pulmonary vascular resistance; WCC: white cell count; CRP: C-reactive protein; NT-proBNP: N-terminal pro-brain natriuretic peptide.

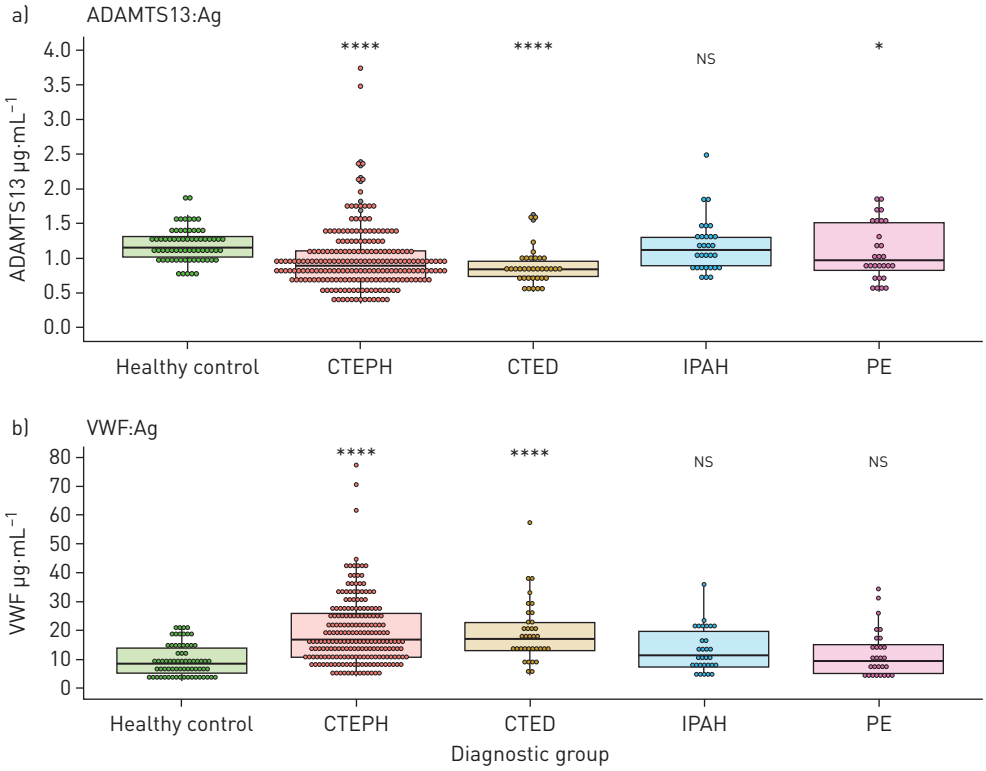

FIGURE 1 a) ADAMTS13 and b) von Willebrand factor (VWF) antigen (Ag) levels by diagnostic group. Dunn's test with false discovery rate adjustment was used to calculate p-values. Healthy control, n=68; chronic thromboembolic pulmonary hypertension (CTEPH), n=208; chronic thromboembolic disease (CTED), n=35; idiopathic pulmonary arterial hypertension (IPAH), n=28; pulmonary embolism (PE), n=28. *: p≤0.05; **: p≤0.01; ***: p≤0.001; ****: p≤0.0001; NS: nonsignificant.

VWF plasma concentrations

We confirmed that VWF antigen levels are increased in CTEPH (16.7 (15.2) µg·mL^{-1}, p<0.001) compared to healthy controls (8.45 (8.77) µg·mL^{-1}) (figure 1b). Furthermore, VWF levels were increased in CTED (17.0 (10.1) µg·mL^{-1}, p<0.001) compared to healthy controls, but were no different to those in CTEPH (p=0.834) (supplementary table S2). There was no difference in VWF antigen levels between healthy controls and patients with IPAH (11.6 (12.3) µg·mL^{-1}, p=0.071) or PE (9.23 (9.82) µg·mL^{-1}, p=0.433).

Multivariable linear regression was also used for VWF plasma concentrations as described for ADAMTS13. This confirmed that VWF was significantly increased in the CTEPH (β +75.5%, 95% CI 44.8–113%, p<0.001) and CTED (β +89.5%, 95% CI 48.0–143%, p<0.001) groups (supplementary table S4). VWF plasma concentrations were not significantly associated with IPAH, PE, sex or ethnicity.

The combination of low ADAMTS13 and high VWF antigen levels had a synergistic effect on the odds of CTEPH (OR 14.5, 95% CI 5.33–47.4, p<0.001) compared with healthy controls (supplementary figure S2 and table S5).

ADAMTS13 and VWF: pre- and post-pulmonary endarterectomy

Matched samples were taken post-PEA from 22 CTEPH patients after a median of 343 (216) days. There were no differences in levels of ADAMTS13 (median of differences (IQR) −0.0328 (0.250) µg·mL^{-1}, p=0.777) or VWF protein (−3.05 (10.7) µg·mL^{-1}, p=0.777) following removal of proximal organised thrombus material by PEA (figure 2).

ADAMTS13 activity, D-dimer, anti-ADAMTS13 autoantibodies and VWF multimers

Specific ADAMTS13 activity (activity:antigen (Act:Ag) ratio) was increased in CTEPH (Act:Ag 1.57 (0.32)) compared with healthy controls (1.05 (0.190), p<0.001) (figure 3a).

Plasmin and thrombin are able to inactivate ADAMTS13 proteolytically *in vitro* and plasmin-mediated ADAMTS13 cleavage has been observed in TTP [27, 28]. Furthermore, abnormalities in the fibrinolysis pathway have been implicated in CTEPH [4]. Therefore, we used fibrinogen degradation products

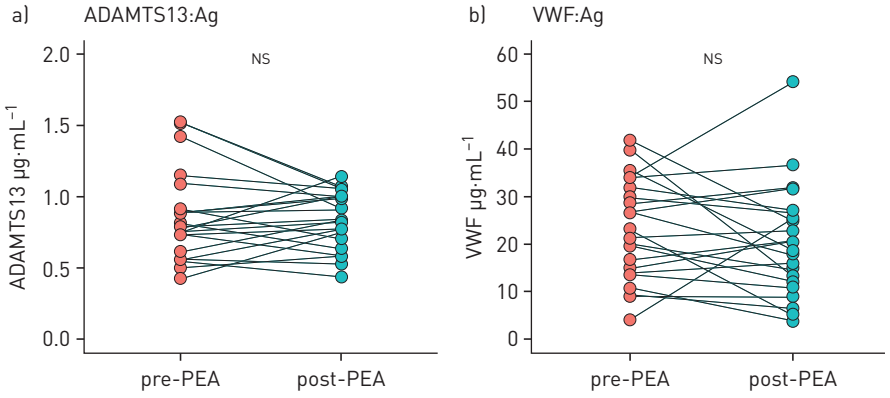

FIGURE 2 a) ADAMTS13 and b) von Willebrand factor (VWF) antigen (Ag) levels pre- and post-pulmonary endarterectomy (PEA) (n=22). Wilcoxon signed-rank test was used to calculate p-values. NS: nonsignificant.

measured by D-dimer as a potential surrogate marker of plasmin and thrombin activity. D-dimer was increased in CTEPH (1.24 (1.25) µg·mL^{-1}) compared to healthy controls (0.538 (0.344) µg·mL^{-1}, p=0.030) (figure 3b). Specific ADAMTS13 activity was not correlated with D-dimer in the CTEPH (rho=0.0938, p=0.761) or healthy control (rho= −0.220, p=0.313) groups (figure 3c).

Because the ADAMT13 reduction in TTP has an autoimmune mechanism, we investigated whether anti-ADAMTS13 autoantibodies are increased in CTEPH. There was no significant difference in

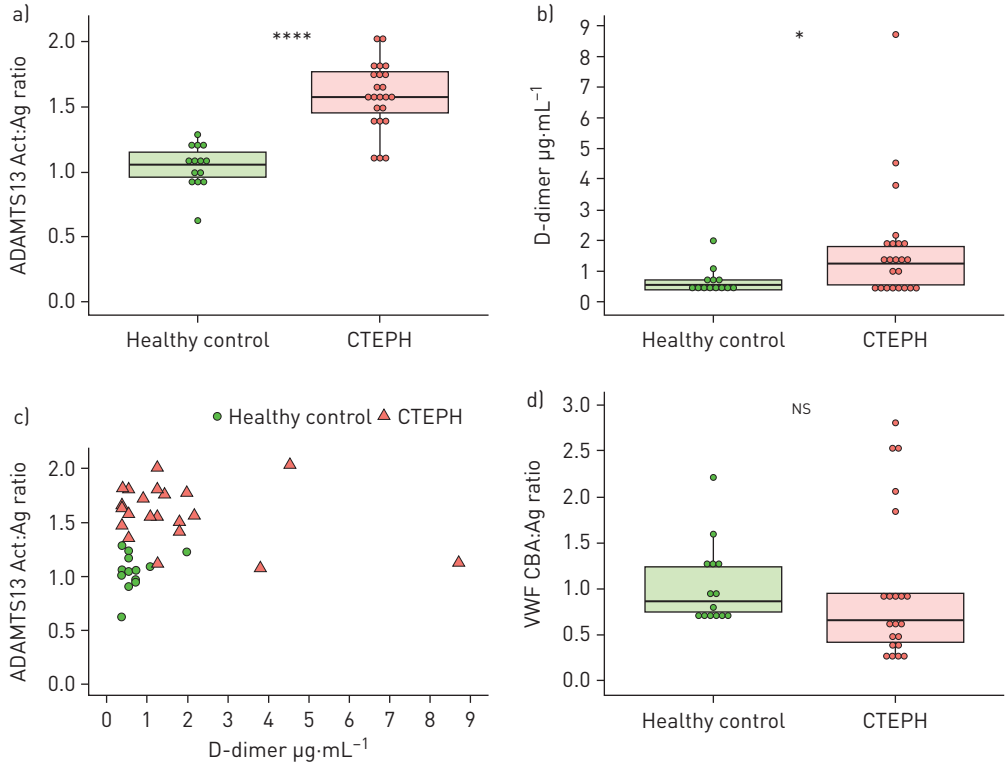

FIGURE 3 ADAMTS13 activity, D-dimer and von Willebrand factor (VWF) multimeric size in chronic thromboembolic pulmonary hypertension (CTEPH) and healthy controls. a) Specific ADAMTS13 activity (activity:antigen (Act:Ag) ratio). b) D-dimer antigen levels. c) Specific ADAMTS13 activity and D-dimer antigen correlation. Healthy control correlation: rho=0.0938, p=0.761; CTEPH correlation: rho= −0.220, p=0.313. d) VWF multimeric size (VWF collagen binding assay (CBA):Ag ratio). A subset of CTEPH patients (n=23) with the lowest ADAMTS13 antigen levels (below the first quartile of the CTEPH group) and healthy controls (n=14) were used for a–c. VWF multimeric size was measured in CTEPH (n=21) samples with the highest VWF antigen concentrations (above the third quartile of the CTEPH group) using the same healthy control subset and displayed in d. The Mann–Whitney U test was used to calculate group differences (a, b, d) and correlation was assessed using Spearman's rank correlation coefficients (c).

anti-ADAMTS13 autoantibodies between CTEPH (92.3% (38.9%)) and healthy controls (76.0% (16.5%), p=0.180) (supplementary figure S3).

We hypothesised that a decrease in ADAMTS13 antigen levels would result in reduced VWF cleavage and an increase in high multimeric VWF, as occurs in TTP [29]. There was no difference in VWF multimeric size between CTEPH (VWF CBA:Ag ratio, 0.659 (0.537)) and healthy controls (0.866 (0.494), p=0.160) (figure 3d).

Clinical phenotype associations with ADAMTS13 and VWF

In CTEPH, ADAMTS13 and VWF did not significantly correlate with markers of disease severity (6MWD, pulmonary vascular resistance or N-terminal pro-brain natriuretic peptide) (supplementary figure S4). Given that inflammation has been associated with both CTEPH and abnormalities in the ADAMTS13–VWF axis, we investigated if they were correlated [30, 31]. There were no correlations with blood markers of inflammation (C-reactive protein, white cell count, neutrophil and lymphocyte percentages) (supplementary figure S5).

ABO groups and ADAMTS13–VWF

There was no difference in ADAMTS13 antigen levels when stratified by simple genetic *ABO* groups (O, A, B, AB; p=0.443) (figure 4a) or more comprehensive genetic *ABO* groups (p=0.616) (supplementary figure S6a).

VWF levels did not vary by *ABO* group (figure 4b and supplementary figure S6b); however, when accounting for covariates (supplementary table S6), *ABO* group B had a higher VWF level (β +51.3%, 95% CI 5.30–117%, p<0.001) than group O. *ABO* group A also had a higher VWF level, although this was not statistically significant (β +19.8%, 95% CI −1.75–46.1%, p=0.073). Patients with *ABO* group O had the lowest VWF levels within the CTEPH group (14.5 (13.0) $\mu g \cdot mL^{-1}$), which were still significantly higher than those of healthy controls (8.45 (8.77) $\mu g \cdot mL^{-1}$, p<0.001).

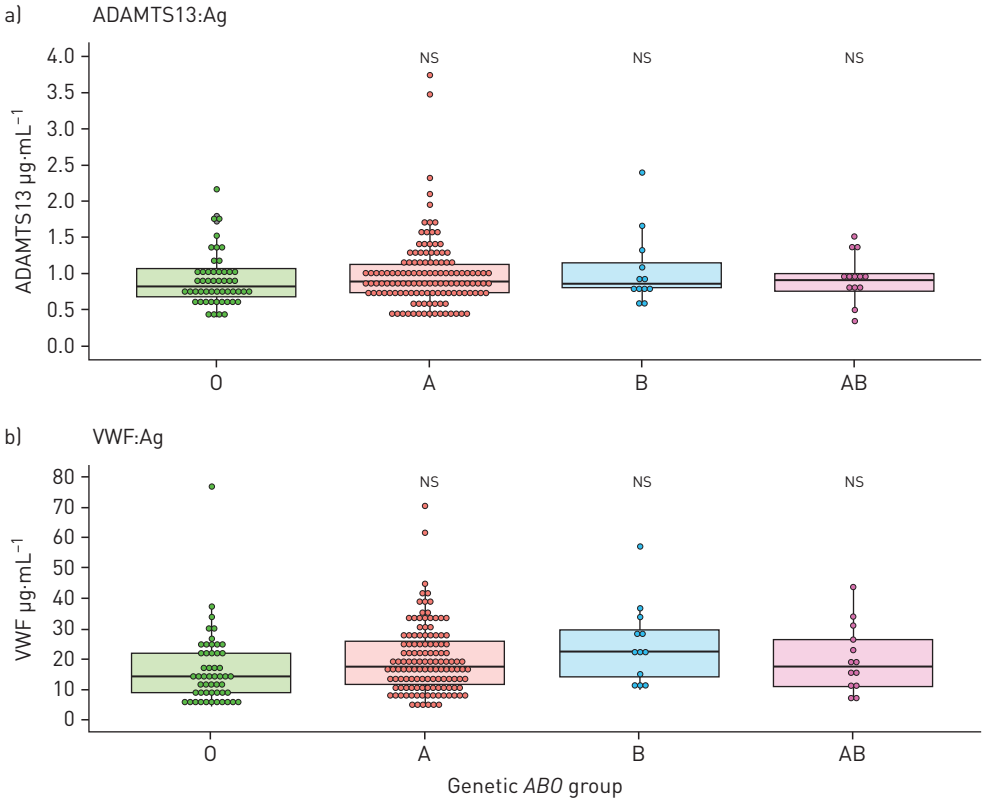

FIGURE 4 ADAMTS13 and von Willebrand factor (VWF) antigen (Ag) levels by *ABO* genetic groups. Chronic thromboembolic pulmonary hypertension (CTEPH) (n=182) and chronic thromboembolic disease (CTED) (n=22) patients with genotypes and protein levels available (in n=3, a genetic *ABO* group could not be inferred) were included. Dunn's test with false discovery rate adjustment was used to calculate p-values. O group, n=51; A group, n=128; B group, n=12; AB group, n=13.

There was no difference in ADAMTS13 antigen levels between *ABO* groups when accounting for covariates with multivariable linear regression.

Protein quantitative trait loci for ADAMTS13

There were five SNPs in the *ADAMTS13* ±40 kb region that were significantly associated with ADAMTS13 protein in a multivariable linear regression model (supplementary table S7). The most significant SNP (rs3739893, risk allele C, β −37.1%, 95% CI −48.1– −23.8%, $p=3.78\times10^{-06}$) is a 5′ untranslated region variant in the *C9orf96* gene, which is ∼8 kb 5′ of the *ADAMTS13* gene. In a model adjusted for age, sex and batch, the lead SNP (rs3739893) explained 7.7% of the variance in ADAMTS13 levels within the CTEPH group (supplementary table S8). In the whole CTEPH GWAS, the effect allele frequency for rs3739893 in CTEPH cases (0.0128) and healthy controls (0.0158) was not significantly different, which suggests that it is not associated with CTEPH disease risk.

Discussion

This is the first study demonstrating a marked reduction in plasma levels of ADAMTS13 in CTEPH. This is independent of pulmonary hypertension, disease severity or systemic inflammation. We confirm that VWF is increased in CTEPH and implicate dysregulation of the ADAMTS13–VWF axis in CTEPH pathobiology.

The magnitude of ADAMTS13 reduction and VWF increase in CTEPH is greater than observed in studies of ischaemic stroke using the same methodology [23]. Furthermore, levels are lower in CTEPH than in CAD when considering the proportion of patients in the lowest ADAMTS13 quartile (65% *versus* 28%, respectively) [13]. Additionally, the combination of decreased ADAMTS13 and increased VWF has a synergistic effect on the odds of CTEPH that is greater than observed in CAD or ischaemic stroke [23]. The more pronounced ADAMTS13–VWF dysregulation in CTEPH may reflect the larger surface area of the vascular endothelium involved or, alternatively, that ADAMTS13–VWF dysregulation is more important in CTEPH pathobiology. Although ADAMTS13 is predominately produced by the liver, the contribution to plasma levels from vascular endothelial cells could be substantial given the large surface area of the lung vasculature [9]. A reciprocal relationship has previously been described between ADAMTS13 and VWF [32, 33]. The reduction in ADAMTS13 remained in our study when VWF levels were adjusted for, which is consistent with low ADAMTS13 being an independent risk factor in other thrombotic diseases [11].

Following PEA and removal of proximal thromboembolic material, the ADAMTS13–VWF axis remained dysregulated despite normalisation of haemodynamic parameters. Additionally, there was equal perturbation of the axis in CTED, and no correlation with CTEPH disease severity, confirming that the changes are not due to the presence of pulmonary hypertension or organised thrombus *per se*. Interestingly, there was no abnormality in ADAMTS13 levels in IPAH despite this group having higher pulmonary vascular resistance, implying that distal pulmonary artery endothelial dysfunction and small vessel vasculopathy are not responsible [34]. Taken together, these observations demonstrate dysregulation of the ADAMTS13–VWF axis in CTEPH pathogenesis.

Low ADAMTS13 levels could be driven by the activation of fibrinolytic pathways and an increase in thrombin and/or plasmin, which have the potential to proteolytically inactivate ADAMTS13 [27]. D-dimer was raised in CTEPH, though there was no correlation with ADAMTS13. High multimeric forms of VWF do not appear to be increased in CTEPH. This is surprising, because increased high multimeric VWF occurs when ADAMTS13 is reduced in TTP and has been suggested to occur in ischaemic stroke and CAD [23, 29]. Measuring VWF multimeric size systemically may not reflect the local disease microenvironment in the pulmonary vascular endothelium. Additionally, the localised flow conditions that may be altered in CTEPH are important in VWF structure, cleavage by ADAMTS13 and thrombus resolution [35]. The increase in specific ADAMTS13 activity in CTEPH may reflect an increased conformational activation of ADAMTS13 by its substrate VWF, due to the altered ADAMTS13:VWF ratio [36].

The *ABO* gene is located in close proximity to and modest linkage disequilibrium with the *ADAMTS13* gene, raising the possibility that *ABO* may influence the ADAMTS13–VWF axis. ABO blood groups are associated with CTEPH, with an over-representation of the non-O groups [20]. Genetic variation in *ABO* has also been associated with ischaemic stroke, CAD and venous thromboembolism [37, 38]. The proposed mechanism of this association has been *via* VWF plasma levels, which are 25% higher in non-O individuals [39]. We found that VWF was increased in some non-O groups within CTEPH; however, VWF was still significantly higher in the CTEPH O group than in healthy controls. This implies that there are additional contributing causes for the increased VWF in CTEPH. Conversely, *ABO* is a pleiotropic

locus and may have alternative functional effects in CTEPH, including mediating pathways involved in inflammation and angiogenesis [26].

We identified a pQTL (rs3739893) in the *C9orf96* gene (~8 kb 5′ of the *ADAMTS13* gene) that is associated with ADAMTS13 protein levels and has been described in two previous studies [18, 25]. In a GWAS of ADAMTS13 antigen levels in a healthy cohort, this SNP was significantly associated with a similar effect size (β −22.3%). Whilst this confirms that ADAMTS13 protein is genetically regulated, this SNP only accounts for a modest variance of ~8% in ADAMTS13 protein levels and is not primarily associated with CTEPH disease risk.

A strength of this study is that we investigated the ADAMTS13–VWF axis in a spectrum of thromboembolic disease from acute PE to chronic thromboembolism with and without pulmonary hypertension. Our study contains a large sample of well-characterised CTEPH patients who have been extensively phenotyped in an experienced national CTEPH centre. ADAMTS13–VWF imbalance does not occur in PE when assessed by multivariable regression, although we were underpowered to detect smaller effect sizes. This raises an intriguing possibility, that there are differences in the ADAMTS13–VWF axis in the spectrum of thromboembolic disease. Future studies using robustly phenotyped PE cohorts to ascertain the presence and extent of residual perfusion defects should investigate if the ADAMTS13–VWF axis varies in post-PE syndrome. Clinical prediction scores for CTEPH following acute PE do not currently incorporate biomarkers [40]. Determining if dysregulation of the ADAMTS13–VWF axis precedes the development of chronic thromboembolic pathology could inform CTEPH risk stratification.

In summary, we report that the ADAMTS13–VWF axis is dysregulated in CTEPH and this is unrelated to pulmonary hypertension, disease severity or systemic inflammation. This implicates the ADAMTS13–VWF axis in CTEPH pathogenesis.

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