The Factor V Leiden variant and risk of chronic thromboembolic pulmonary hypertension

To the Editor:

Chronic thromboembolic pulmonary hypertension (CTEPH) is a devastating complication that occurs in about 3% of survivors of acute pulmonary embolism (PE) [1]. Genetic risk factors may differentiate patients with acute PE who develop CTEPH from those who do not develop CTEPH. The Factor V Leiden (FVL) and Prothrombin G20210A (PT) variants are the most common genetic risk factors for venous thromboembolism (VTE) [2, 3]. In European CTEPH patients, the frequencies of these variants are not increased compared with either patients with non-CTEPH pulmonary hypertension or healthy controls [4–7]. While these data suggest that genetic risk factors for CTEPH and PE may be distinct [8], no studies have directly compared the frequencies of these variants in patients with CTEPH and patients with acute PE who did not develop CTEPH.

We enrolled 91 consecutive CTEPH patients evaluated at the Intermountain Medical Center pulmonary hypertension clinic, and 157 consecutive control patients with a history of acute PE in whom CTEPH was not suspected (based on symptoms and physical examination) who were seen in the Intermountain Medical Center thrombosis clinic. Each CTEPH subject was matched to one or two PE subjects based on clinical factors known to influence the likelihood of inherited thrombophilia carriage: age at first diagnosed VTE (±5 years) [9], history of VTE without an environmental risk factor [9, 10], and history of DVT (as opposed to isolated PE) [11]. Enrolled patients were tested for FVL and PT using polymerase chain reaction amplification with fluorescence monitoring for *F5* c.1601G>A and *F2* c.*97G>A. Clinical tests for either FVL or PT were available in 21.7% of PE subjects and 25.2% of CTEPH subjects (before the diagnosis of CTEPH), and in these cases the testing was not repeated.

CTEPH patients had a median age at CTEPH diagnosis of 63 years (interquartile range (IQR) 52–71 years). Almost half were female (47.3%), and 91.2% were Caucasian. A history of acute PE prior to CTEPH diagnosis was reported in 78.0%, and prior DVT in 53.8%. Median age at first VTE diagnosis was 59 years (IQR 47–68 years), and the median duration from first VTE to CTEPH diagnosis was 3.0 years (IQR 1.3–8.7 years). Pulmonary thromboendarterectomy (PTE) had been performed in 60.4% of CTEPH subjects. The demographics of the PE cohort closely matched those of the CTEPH cohort. Among PE subjects, the median age at first VTE diagnosis was 55 years (IQR 42–65 years), and median follow up from first VTE to study enrolment was 2.7 years (IQR 0.9–7.5 years).

There were no significant differences in the frequencies of FVL or PT carriage among subjects in the Intermountain CTEPH and PE cohorts (figure 1), although there was a trend towards a lower frequency of FVL and a higher frequency of PT among CTEPH patients. There was no difference in the frequencies of FVL or PT between CTEPH patients who had acute PE prior to CTEPH diagnosis (n=71; FVL: 8.5%, PT: 8.5%) *versus* those who did not (n=20; FVL 10.0%, PT 5.0%).

Several clinical factors are known to associate with increased frequency of carriage of thrombophilic genetic variants in patients with VTE, including younger age at VTE diagnosis [9], VTE without an environmental risk factor [9, 10], and a history of DVT (as opposed to isolated PE) [11]. We performed an exploratory subgroup analysis to determine whether these factors have the same effect on the frequency of FVL and PT carriage in patients with CTEPH. We observed the same expected trend towards increased

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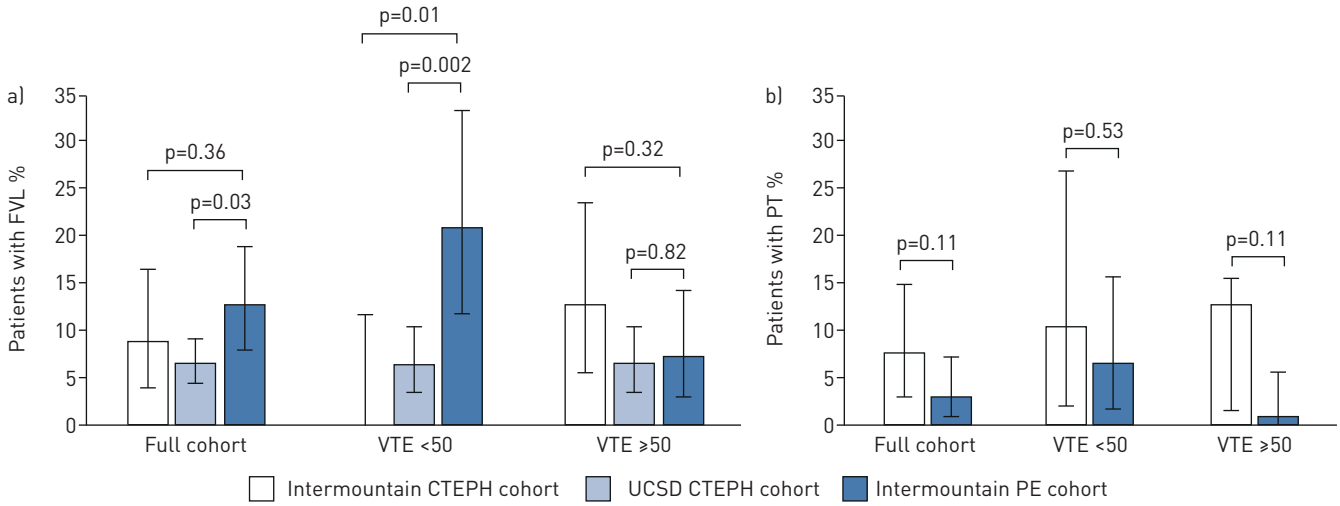

FIGURE 1 Frequency of Factor V Leiden (FVL) and Prothrombin G20210A (PT) variant carriage among the chronic thromboembolic pulmonary hypertension (CTEPH) and pulmonary embolism (PE) cohorts. There was a reduced frequency of activated protein C (APC) resistance in the full University of California San Diego (UCSD) CTEPH cohort (n=447, FVL 6.5%) compared with the frequency of FVL carriage in the full Intermountain PE cohort (n=157, FVL 12.7%). There was a trend towards reduced frequency of FVL in the full Intermountain CTEPH cohort (n=91, FVL 8.8%) relative to the Intermountain PE cohort. When considering only subjects who had their first venous thromboembolism (VTE) diagnosed prior to age 50 years, FVL frequency was significantly lower in both the Intermountain CTEPH cohort (n=29, FVL 0%) and the UCSD CTEPH cohort (median n=220 from 50 independent datasets of imputed age at first VTE, interquartile range (IQR) 224–230; median APC resistance frequency 6.3%, IQR 5.9–6.8%) than in the Intermountain PE cohort (n=61, FVL 21.3%). PE subjects were significantly more likely to carry FVL if they had their first VTE diagnosed prior to age 50 years compared with those who had their first VTE diagnosed at age 50 years or greater (21.3% *versus* 7.3%; p=0.03). In contrast, there was no significant difference in the frequency of FVL or APC resistance in the Intermountain and UCSD CTEPH cohorts among patients with age at first VTE of less than 50 years *versus* 50 years or greater (Intermountain CTEPH cohort: 0% *versus* 12.9%; p=0.06; UCSD CTEPH cohort: 6.3% (IQR 5.9–6.8%) *versus* 6.6% (IQR 6.2–7.0%); p=0.92). There was a trend towards increased frequency of PT carriage among Intermountain CTEPH patients (n=91, PT 7.7%) compared with Intermountain PE patients (n=157, PT 3.2%); however, this did not reach statistical significance. Unlike with FVL, both the Intermountain CTEPH and PE cohorts showed the same general trend towards increased PT frequency among those with VTE diagnosed prior to age 50 years (CTEPH: 10.3%, n=29; PE: 6.5%, n=61) compared with those diagnosed with their first VTE at age 50 years or greater (CTEPH: 6.5%, n=62; PE: 1.0%, n=96). Reported p-values were calculated using the Chi-square test for proportions and were corrected for multiple hypothesis testing. Error bars represent 95% exact binomial confidence intervals.

carriage of both FVL and PT amongst CTEPH and PE patients with a history of DVT and VTE without an environmental risk factor (data not shown). However, there was a significantly lower frequency of FVL observed in the Intermountain CTEPH cohort relative to the Intermountain PE cohort among subjects with first VTE diagnosed prior to age 50 years (figure 1). The same was not observed with PT, in which both Intermountain CTEPH and PE subjects showed the same expected trend towards increased PT carriage among those who were diagnosed with VTE prior to age 50 years (figure 1).

We sought to validate this exploratory observation in an independent population. The University of California San Diego (UCSD) is a high-volume PTE centre and routinely tests for activated protein C (APC) resistance on patients undergoing PTE using the Pefakit assay (Pentapharm Ltd., Switzerland), which has high sensitivity and specificity compared with genetic testing for FVL [12]. We retrospectively analysed the association between age and APC resistance in 447 consecutive patients who underwent PTE at UCSD. The UCSD CTEPH cohort was younger (median age at PTE of 58 years, IQR 42–66 years) and more racially diverse (70.5% Caucasian) than the Intermountain CTEPH cohort. The frequency of APC resistance was 6.5% among the UCSD CTEPH cohort (figure 1), slightly lower than the FVL frequency in the Intermountain CTEPH cohort. This likely in part reflects the differing racial compositions of the two cohorts, as FVL is most common in Caucasians [13]. Indeed, among Caucasians in the UCSD CTEPH cohort, the frequency of APC resistance was 7.3%.

As data regarding age at first VTE diagnosis was not available in the UCSD dataset, we imputed this by using a geometric distribution to characterise the time elapsed between first VTE and PTE among Intermountain CTEPH subjects who had undergone PTE. We then generated 50 independent datasets of imputed age at first VTE among UCSD CTEPH subjects by randomly applying this distribution to the UCSD cohort and then determined the frequency of APC resistance by imputed age at first VTE for each of these 50 datasets. We report the median values from this analysis. Similar to what was observed with FVL in the Intermountain CTEPH cohort, APC resistance was significantly less frequent in UCSD CTEPH subjects with imputed age at first VTE of less than 50 years than in Intermountain PE subjects who were diagnosed with VTE prior to age 50 (figure 1). These data were not significantly changed when we restricted the analysis to only Caucasians within the UCSD dataset (data not shown).

Overall, we identified FVL or APC resistance in 37 of 538 CTEPH patients (6.9%), *versus* 20 of 157 (12.7%) PE controls, suggesting that FVL is less frequent in patients with CTEPH than in similar patients with PE who did not develop CTEPH (p=0.02). The key finding of this analysis, however, is that the lower frequency of FVL in CTEPH patients is entirely driven by the low frequency of FVL among patients with CTEPH who had their first VTE at a young age. We propose that the presence of FVL in younger patients with PE is associated with a reduced risk of developing CTEPH. This may be due to the presence of other risk factors (either genetic or environmental) that exert a particularly strong effect on CTEPH risk in younger patients. Alternatively, FVL could alter thrombus stability in an age-dependent manner through effects on fibrinolysis [14, 15]. A major strength of our study is confirmation of the key finding in an independent population. However, because this observation was made in a *post hoc* analysis of the primary dataset, and because age at first VTE had to be imputed in the validation cohort, our results should be considered hypothesis generating.

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