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Factor V Leiden is not common in patients diagnosed with primary pulmonary hypertension

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Factor V Leiden is not common in patients diagnosed with primary pulmonary hypertension. C.G. Elliott, M.F. Leppert, G.J. Alexander, K. Ward, L. Nelson, G.G. Pietra. ©ERS Journals Ltd 1998.

ABSTRACT: Substantial evidence suggests that thrombosis contributes to the pathogenesis of primary pulmonary hypertension (PPH). An abnormal factor V (factor V Leiden) may contribute to thrombosis in the pulmonary microcirculation of PPH patients. A point mutation in which adenine is substituted for guanine at nucleotide 1691 (1691A) alters factor V so that it resists cleavage by activated protein C. Heterozygosity for the 1691A mutation is more common (2–8%) in Caucasian Europeans and Americans than in Africans (1%) and Asians (<1%). The aim of the study was to examine the prevalence of the mutation that codes for factor V Leiden in individuals with PPH.

We identified 42 Caucasians diagnosed with PPH. We extracted deoxyribonucleic acid (DNA) from whole blood and assayed DNA samples for the point mutation (1691A) that codes for factor V Leiden.

One out of 42 (2.4%; 95% confidence interval=0.1–12.6) Caucasians diagnosed with PPH was heterozygous for the normal 1691G and mutant 1691A allele. All 10 individuals with familial PPH were homozygous for the normal 1691G allele. The prevalence of heterozygosity for the 1691A allele and the normal 1691G allele does not differ from that observed in reference (control) populations.

The low prevalence of the 1691A mutation among individuals diagnosed with primary pulmonary hypertension provides evidence that factor V Leiden does not contribute to the pathogenesis of the disease in most patients.

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Primary pulmonary hypertension (PPH) is a rare disorder of uncertain pathogenesis characterized by severe pulmonary hypertension in the absence of an identifiable cause [1–3]. A spectrum of pathological changes involve the muscular pulmonary arteries and arterioles of patients with a clinical diagnosis of PPH [4]. Investigators have hypothesized that pulmonary vasoconstriction [5, 6], disrupted and deranged vascular growth and remodelling of the pulmonary vascular bed [7], and coagulation abnormalities [8, 9], contribute to the development of PPH.

Previous investigations have provided evidence that thrombosis represents either a primary, or an important secondary, pathogenetic mechanism for PPH [9–13]. Thrombotic lesions often are present in the small pulmonary arteries and arterioles of PPH patients [4, 10], and fibrinopeptide A, released when thrombin converts fibrinogen to fibrin, is increased in PPH [12]. Thrombomodulin, which binds thrombin at the endothelial surface and opposes thrombosis by activating protein C, is decreased in PPH [9], as is the expression of von-Willebrand factor [14]. Furthermore, chronic anticoagulation with warfarin appears to improve the survival of individuals with PPH [11, 15].

An abnormal factor V (factor V Leiden) is a newly understood cause for thrombosis and familial thrombophilia [16, 17] that may represent an important mechanism in the pathogenesis of PPH [8]. A mutation of the factor V gene replaces arginine with glutamine in position 506 of the protein. Activated protein C (APC) cleaves activated factor V at position 506, and the mutated factor V resists cleavage by APC, but has normal procoagulant activity. APC resistance has been associated with increased rates of pathological thrombus formation in the venous, but not the arterial circulation [18, 19], and in increased propensity for recurrent pathological venous thrombosis [20]. Venous thrombosis often becomes clinically apparent after the age of 40 yrs [19], a feature which is compatible with the often delayed onset of PPH. Furthermore, factor V Leiden may combine with other procoagulants, *e.g.* oestrogens [21], homocysteine [22], and natural events, *e.g.* pregnancy [18] to cause thrombosis.

Because of evidence that suggests that thrombosis contributes to the pathogenesis of PPH, we hypothesized that the prevalence of factor V Leiden would be significantly higher in patients with PPH than in normal reference populations. To test this hypothesis, we measured the prevalence of the factor V Leiden mutation (nucleotide 1691 guanine (G)→adenine (A)) in Caucasians diagnosed with PPH and compared this result with the prevalence of factor V Leiden in previously reported reference Caucasian populations [19, 23, 24].

Methods

We evaluated the presence of the 1691A mutation in the coagulation factor V gene by studying deoxyribonucleic acid (DNA) sampled from a cohort of Caucasian PPH patients. Participants in this study provided informed written consent for extraction and study of DNA according to a protocol approved by the institutional review board of the University of Utah.

Study subjects

We identified patients through a national patient support group (United Patients Association for Pulmonary Hypertension (UPAPH)). We also identified patients who were referred to the LDS Hospital for the evaluation and treatment of PPH. We used the National Institute of Health registry definition for PPH (pulmonary artery pressure (P_{pa}) >25 mmHg (3.33 kPa) at rest or P_{pa} >30 mmHg (3.99 kPa) during exercise and no identifiable underlying cause) [2]. Lung transplantation or autopsy results were examined when available (n=4) to support the diagnosis of PPH and to characterize the histopathology.

All PPH patients or their family members completed a detailed family history questionnaire. Familial PPH was diagnosed when a first-degree family member (parent, sibling, or child) had PPH confirmed, using the same methods that were used for the diagnosis of PPH in the patient cohort. When no first-degree relatives with PPH were identified, PPH patients were classified as "sporadic" PPH.

Pathology

Specimens were obtained according to local pathology practice. Slides were stained with haematoxylin and eosin. Pulmonary arteries were examined (by G.G. Pietra) and classified for medial hypertrophy, muscularization of arterioles, concentric laminar intimal proliferation and fibrosis, eccentric intimal proliferation and fibrosis, plexiform and dilation lesions, pulmonary arteritis, and fresh and recanalized thrombi as described previously [4]. Occlusive blood clots or eccentric intimal thickening and/or recanalized channels in muscular arteries or arterioles were considered thrombotic in origin.

Blood samples

We collected blood from PPH patients in vacutainer tubes which contained 0.5 mL of 0.12 M sodium citrate. DNA was extracted from whole blood using standard phenol/chloroform methods.

Normal reference populations

We used the allele frequencies for 1691A as determined previously by REES *et al.* [24] and RIDKER *et al.* [19, 23] for comparison with the 1691A allele frequency among the population of patients with PPH.

Factor V gene analysis

Polymerase chain reaction (PCR) was used to amplify exon 10 of the factor V gene that contains the G→A transition at nucleotide 1691. The reaction was performed on a Techne PH-3 thermal cycler (Techne Cambridge Ltd, Duxford, Cambridge, UK) using the primers and amplification conditions of BERTINA *et al.* [17]. Allele-specific digestion was performed using a restriction endonuclease *Mnl*1 (New England Biolabs (NEB), Beverly, MA, USA) in a final volume of 40 µL which included 30 µL of PCR product, 10 units *Mnl*1, and 1×NEB buffer. Incubation was overnight at 37°C. The product was electrophoresed on a 2% SeaKem agarose gel (FMC, Rockland, ME, USA) and visualized with ethidium bromide. A 1691G allele yields restriction fragments of 37, 67, and 163-base pairs (bp), whereas a 1691A allele yields 67, and 200-bp fragments.

Statistics

Data are presented as means±SEM and 95% confidence intervals (CI). The observed 1691A allele frequency in PPH was compared with the observed 1691A allele frequency in European and American Caucasian reference populations by the Chi-squared statistic. Before subjecting the data to analysis, we chose a one-tailed significance level of $p<0.05$ to test the hypothesis that the mutation for factor V Leiden was more prevalent in Caucasian patients diagnosed with PPH than in Caucasian reference populations.

Results

Study subjects

Ten sporadic PPH patients were identified by referral to the LDS hospital; 25 sporadic PPH patients and 10 familial PPH patients were identified through UPAPH. We excluded three patients who were not Caucasian in order to assure comparable ancestry of the PPH and reference Caucasian populations. This patient population had the typical epidemiological and physiological features of PPH (table 1).

Table 1. – Characteristics of 42 Caucasian primary pulmonary hypertension patients

	Sporadic	Familial	Total
Patients n	32	10	42
Age yrs*	35±2	35±4	35±2
Sex F/M	23/9	5/5	28/14
P_{ra} mmHg	8±1	10±2	8±1
P_{pa} mmHg	62±3	53±4	60±2
P_{pcw} mmHg**	8±1	11±2	9±1
CI $L \cdot min^{-1} \cdot m^{-2}$	2.4±0.1	1.7±0.2	2.2±0.1
PVR $mmHg \cdot L^{-1} \cdot min^{-1}$	16±3	11±2	15±2

F: female; M: male; P_{ra}: right atrial pressure; P_{pa} : mean pulmonary artery pressure; P_{pcw}: pulmonary capillary wedge pressure; CI: cardiac index; PVR: pulmonary vascular resistance. All measurements are reported as mean±SEM. *: age at time of diagnostic cardiac catheterization; **: P_{pcw} measurements were available for 32 out of 42 Caucasian primary pulmonary hypertension patients.

Lung perfusion scans

Lung perfusion scan reports were available for 40 out of 42 patients. In addition, two patients had perfusion scans that were described as negative for pulmonary embolism in the medical records, but the reports were not available. Seventeen out of 40 lung perfusion scans were interpreted to be normal. The remaining 23 lung perfusion scans showed a spectrum of patchy perfusion abnormalities and subsegmental defects.

Pathology

Four patients had had a lung transplant, and tissue samples were available from the native (PPH) lungs. Three of the four cases had classical lesions of plexogenic hypertensive pulmonary arteriopathy including marked medial hypertrophy, concentric laminar intimal fibrosis, and plexiform lesions. Occasional fibrin thrombi were observed in one of these specimens. The fourth case demonstrated hypertensive pulmonary arteriopathy of the thrombotic type including medial hypertrophy, eccentric intimal fibrosis, and recanalized thrombi.

Prevalence of factor V Leiden

One out of 42 Caucasians diagnosed with PPH (2.4%, 95% CI=0.1–12.6) was heterozygous for factor V Leiden. This prevalence did not differ from the prevalence of factor V Leiden in European (χ^2=1.87; p=0.17) or the American (χ^2=0.56; p=0.45) Caucasian populations for whom the prevalence of the factor V Leiden mutation is 8.2% (95% CI=6.2–10.7) or 4.9% (95% CI=3.8–6.2), respectively [19, 25, 26]. The 10 Caucasians with familial PPH were homozygous for the normal 1691G allele.

Discussion

The present study demonstrates a low prevalence of factor V Leiden mutation (1691A) among Caucasian patients with PPH. Only one out of 42 patients were heterozygous for both the 1691A allele and the 1691G allele. No homozygotes for the abnormal 1691A allele were found. These observations do not differ from the observed prevalence of factor V Leiden in other Caucasian populations where the prevalence ranged 2–8% [17, 18, 22, 24, 25]. In contrast, the prevalence of factor V Leiden mutation is substantially higher among European populations with venous thromboembolism [26].

The demonstration of a low prevalence of factor V Leiden associated with PPH is important because factor V Leiden predisposes individuals to venous thrombosis, and because evidence suggests that thrombosis is an important mechanism in the pathogenesis of PPH [8, 12] and familial PPH [27]. The finding of a low prevalence of factor V Leiden mutation among PPH patients suggests that mechanisms other than resistance of an abnormal factor V to APC explain the occurrence of thrombotic arteriopathy in PPH and the apparent efficacy of anticoagulation with warfarin for the treatment of PPH. Such mechanisms may reflect a combination of low flow (stasis) and alterations of normal homeostatic mechanisms that oppose *in situ* thrombosis of small pulmonary vessels. The observation that factor V Leiden is not more prevalent among patients with advanced PPH is consistent with previous reports that factor V Leiden is not associated with arterial thrombosis, *i.e. in situ* thrombosis complicating diseased arteries. It also provides evidence suggesting that thrombotic lesions in PPH do not arise in the venous circulation.

The present study provides a confident estimate of the prevalence of the factor V Leiden mutation in PPH because the patient population was sufficiently large and broadly representative of PPH. Our estimate suggests that it is highly unlikely that >13% of all Caucasian PPH patients have the point mutation that codes for factor V Leiden. The population was representative of PPH patients and included a number of individuals who had either histological or lung perfusion scan evidence of thrombotic pulmonary arteriopathy. These observations agree with previous studies of the histopathology of PPH that identified thrombotic lesions in 33–56% of PPH specimens [4, 13, 28].

Whether or not factor V Leiden accelerates the progression of primary pulmonary hypertension remains uncertain. One individual in the present series had primary pulmonary hypertension and was heterozygous for the mutation for factor V Leiden. In this case, factor V Leiden was associated with a younger age of onset (18 yrs old) than 90% of PPH patients [2]. Furthermore, in this individual, nonsegmental perfusion lung scan defects suggested that vascular thrombosis had complicated the primary pulmonary hypertension [13]. Interestingly, this individual had undergone a successful heart and lung transplant 5 yrs earlier. Histological studies of the native hypertensive lungs had demonstrated a marked intimal thickening that may have resulted from organized *in situ* thrombosis [4].

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