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# Screening for pulmonary veno-occlusive disease in heterozygous *EIF2AK4* variant carriers

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**Summary (246)**: Comprehensive evaluation as well as longitudinal follow-up of a cohort of *EIF2AK4* heterozygous variant carriers did not raise any suspicion of pulmonary veno-occlusive disease, confirming the recessive inheritance of *EIF2AK4*-linked PVOD.

Pulmonary veno-occlusive disease (PVOD) is a rare form of pulmonary arterial hypertension (PAH), characterised by a specific phenotype and associated with a poor prognosis, often justifying an early referral for lung transplantation [1–4]. Pathological hallmark of PVOD is a preferential involvement of the pulmonary venous system with obliteration of small pulmonary veins by fibrous intimal thickening and patchy capillary proliferation [5].

Heritable forms of PVOD are caused by bi-allelic pathogenic variants in the gene *EIF2AK4* (*eukaryotic translation initiation factor 2 alpha kinase 4*), with an autosomal recessive transmission [2, 4, 6]. Biallelic *EIF2AK4* pathogenic variants are found in nearly all PVOD patients with a family history but are also identified in 8-25% of sporadic cases [2, 7, 8]. Contrarily to idiopathic PVOD, pathogenic variants carriers are characterised by a young age and the absence of other risk factors for PVOD, such as exposure to alkylating agents or organic solvents [1]. The *EIF2AK4* gene encodes a serine-threonine kinase known as general control nonderepressible 2 (GCN2) that phosphorylates the α-subunit of eukaryotic translation initiation factor (eIF2α) under amino acid deprivation, leading to preferential synthesis of stress proteins [7, 9].

Genetic counselling and testing are now an integral part of the management of PAH and have been offered to all patients with PAH and PVOD in the French PH referral centre [10, 11]. Due to its recessive autosomal transmission, detection of first-degree relatives in heritable PVOD allows to identify healthy subjects carrying biallelic pathogenic variants of *EIF2AK4* but also carriers of a single heterozygous variant. While the risk of developing PVOD is well established in carriers with bi-allelic pathogenic variants (penetrance probably nearly complete), the phenotype of heterozygous carriers and their risk of developing PVOD is unknown to date. One can hypothesize that the presence of a heterozygous pathogenic variant results in a decrease in the expression of GCN2 which could be the cause of pulmonary vascular abnormalities. The objective of this study was to determine the phenotype of healthy relatives carrying a heterozygous pathogenic variant of the *EIF2AK4* gene.

In the DELPHI-4 study (ClinicalTrials.gov identifier: NCT03902353), we screened for signs and symptoms of PVOD in a cohort of relatives carrying heterozygous *EIF2AK4* pathogenic variant. Genetic screening and testing were performed as previously described [10, 12]. All variants are classified as pathogenic class 5 variants according to ACMG classification criteria (**Table 1**). Clinical evaluation comprised dyspnoea assessed by the modified New York Heart Association functional class (NYHA FC), 6-minute walk distance (6MWD), pulmonary function tests (PFTs) including the diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (DLCO), electrocardiogram, echocardiography, cardio-pulmonary exercise testing (CPET), high-resolution computed tomography (HRCT) of the chest, abdominal ultrasound searching for hepatic abnormalities, standard biological assessment and N-terminal brain natriuretic peptide (NT pro-BNP) level. In agreement with French bioethics laws (Commission de Protection des Personnes 2017-A02448-45), all participants provided informed consent to undergo a non-invasive clinical assessment at inclusion and for a yearly follow-up by phone consultation.

Fifteen subjects were included (eight females, 53%) with a median [min-max] age of 50 [26-86] years (**Table 1**). At inclusion, all subjects were in NYHA FC I except for the two eldest individuals (85 and 86 years) who were in class II. Three subjects were smokers and 4 were former smokers. A significant exposure to solvents was reported in one subject. No clinical signs of right heart failure were noticed. Four subjects had an incomplete right bundle branch block on electrocardiogram. No signs of PH were found on echocardiography with an absence of elevated tricuspid regurgitation velocity and a normal tricuspid annular plane systolic excursion (TAPSE) 21 [17-28] mm. PFTs were normal with a median DLCO of 96 [68-118] % of predicted values. NT pro-BNP levels were normal (NT pro-BNP 45 [5-299] ng/l) for all subjects. CPET did not find any signs suggestive of pulmonary hypertension and showed a preserved exercise capacity with a median peak $VO_2$ of 24 [12.7-34.5] ml/min/kg and a normal $VE/VCO_2$ slope of 28 [18-43]. Blood gas analyses were normal except for the eldest subject who had mild hypoxemia at rest and a reduced exercise capacity with a peak $VO_2$ of 12.7 ml/mn/kg. Cardiologic evaluation revealed heart failure with preserved ejection fraction. Two

out of 15 chest CT scans displayed nonspecific pulmonary micronodules, without any signs suggestive of PAH or PVOD. Thirteen abdominal ultrasounds were unremarkable, one hyperechogenic liver was related to another medical condition. An adrenal nodule was found in another subject, which eventually led to the resection of an adrenocortical oncocytoma, treated successfully. Follow-up after inclusion (27 [7-31] months) did not raise any suspicion of PVOD.

We report the first cohort of carriers of heterozygous *EIF2AK4* pathogenic variant, although of limited size, and we did not demonstrate any signs suggesting pulmonary vascular abnormalities. Exhaustive clinical evaluation did not raise PH suspicion in any of the 15 subjects including the two eldest ones. Chest CT scans did not display the classical radiological features of PVOD, such as increased septal lines, centrilobular ground-glass opacities and mediastinal lymph node enlargements [1]. Of note, median age at diagnosis of heritable PVOD is 26 years [4] and we did not find any signs suggestive of PVOD in our cohort of heterozygous variant carriers at a median age of 50 years. These data are reassuring on the absence of a risk of PVOD, even if we cannot fully exclude a very late onset of the disease.

These results support that heterozygous *EIF2AK4* pathogenic variant is not associated with a specific phenotype of mild PVOD [4]. However, Hadinnapola et al. identified an overrepresentation of rare and predicted deleterious *EIF2AK4* variants in idiopathic PAH (1.2%) as compared to control subjects (0.5%) [6]. Indeed, Eichstaedt et al. reported an isolated family with frameshift mutation in the *BMPR2* gene and a splice site mutation in the *EIF2AK4* gene, suggesting that *EIF2AK4* pathogenic variant may act as a “second hit” explaining the variable penetrance in this family [13]. In our cohort, one subject had been exposed to solvent and seven were active or former smokers. Since we cannot fully exclude that *EIF2AK4* pathogenic variant carriers have an increased risk of developing PVOD through toxic exposure, we proposed an educational program for these relatives to limit toxic exposure (tobacco, occupational exposure) and a clinical evaluation in case of unexplained dyspnoea.

Two of the 15 subjects developed rare tumours, including one medulloblastoma and one adrenocortical oncocytoma. As GCN2 is widely expressed in different tissues, we cannot rule out a potential link with *EIF2AK4* pathogenic variant. GCN2 is an eIF2α kinase responsible for entirely rewiring the metabolism of cells in response to amino acid starvation stress and it has been demonstrated that more than 10% of cancer cell lines appear to be dependent on GCN2 [14, 15]. However, GCN2 is considered as a potential regulator of cancer cell metabolism and it is the increased rather than decreased expression, as expected in the presence of a heterozygous variant, which could be associated with a higher cancer risk. Indeed, no increased tumour risk has been reported in patients with heritable PVOD due to *EIF2AK4* pathogenic variants, even under immunosuppressants after lung transplantation.

In conclusion, our study covering a small but unique cohort of relatives carrying heterozygous *EIF2AK4* pathogenic variant does not report any abnormalities suggesting a silent or mild pulmonary vascular disease. Our work is, to our knowledge, the first piece of evidence supporting the common statement that *EIF2AK4* heterozygous variant carriers have no risk of developing PVOD, confirming the recessive inheritance of *EIF2AK4*-linked PVOD. This study provides important clinical evidence for genetic counselling in PH referral centres. Prospective follow-up is now ongoing in our cohort; besides, international large prospective registries are warranted to confirm our findings among heterozygous *EIF2AK4* variant carriers.

**Conflict of interest**: B. Lechartier has nothing to disclose. B. Girerd has nothing to disclose. M. Eyries has nothing to disclose. A. Beurnier has nothing to disclose. M. Humbert reports grants and personal fees from Acceleron, Janssen and Merck, personal fees from Altavant, Morphogen-IX and Bayer, outside the submitted work. D. Montani reports grants and personal fees from Acceleron, Janssen and Merck, personal fees from Bayer, outside the submitted work.

**Table 1. Clinical characteristics of the heterozygous *EIF2AK4* variant carrier cohort**.

| | ***EIF2AK4* variant** | **Protein change** | **Sex** | **Age** *yrs* | **Medical History** | **NYHA FC** | **6MWD** *m* | **TAPSE** *mm* | **DLCO** *%* | **$VO_2$** *ml/min/kg* |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | c.1392del | p.(Arg465Valfs*38) | F | 58 | - | 1 | 596 | 17 | 85 | 22.7 |
| 2 | c.3802C>T | p.(Gln1268*) | M | 59 | Systemic hypertension, hemochromatosis, stroke | 1 | 605 | 19 | 108 | 29.2 |
| 3 | c.354_355del | p.(Cys118Trpfs*7) | M | 85 | Prostate cancer | 2 | 370 | 28 | 74 | 18.5 |
| 4 | c.1554-4C>A | p.(Cys519Aspfs*17) | F | 86 | - | 2 | 330 | 23 | 68 | 12.7 |
| 5 | c.1554-4C>A | p.(Cys519Aspfs*17) | F | 30 | Adrenocortical oncocytoma | 1 | 630 | 21 | 100 | 27.7 |
| 6 | c.354_355del | p.(Cys118Trpfs*7) | M | 26 | - | 1 | 496 | 21 | 91 | 34.5 |
| 7 | c.354_355del | p.(Cys118Trpfs*7) | M | 39 | Allergic asthma | 1 | 573 | 27 | 106 | 24.1 |
| 8 | c.1554-4C>A | p.(Cys519Aspfs*17) | M | 32 | - | 1 | 668 | 21 | 89 | 29.5 |
| 9 | c.354_355del | p.(Cys118Trpfs*7) | F | 37 | - | 1 | 584 | 25 | 89 | 25.9 |
| 10 | c.2136_2139dup | p.(Ser714Hisfs*21) | M | 67 | - | 1 | 550 | 23 | 102 | 23.1 |
| 11 | c.745C>T | p.(Arg249*) | F | 62 | - | 1 | 520 | 21 | 114 | 21 |
| 12 | c.(?_-73)_ (859+1_860-1) | p.([?]) | F | 57 | - | 1 | 572 | 20 | 89 | 28.5 |
| 13 | c.3159G>A | p.(Lys975_Lys1053del) | F | 29 | Allergic asthma | 1 | 610 | 19 | 99 | 18 |
| 14 | c.3159G>A | p.(Lys975_Lys1053del) | F | 50 | - | 1 | 562 | 28 | 118 | 22.3 |
| 15 | c.2319+1G>A | p.([?]) | M | 40 | Medulloblastoma | 1 | 714 | 30 | 96 | 32 |

*Data are presented as absolute or median values. NYHA FC: functional class according to the NYHA scale; 6MWD: 6-minute walking distance (m); TAPSE: tricuspid annular plane systolic excursion (mm); DLCO: diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (% predicted); $VO_2$: peak oxygen consumption (ml/min/kg).*

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