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CASE REPORT

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An adult patient with pulmonary arterial hypertension, a *NOTCH3* mutation, and leflunomide exposure

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Abstract

Pulmonary arterial hypertension (PAH) is a poorly understood disease of the small pulmonary arteries. Pulmonary vascular remodeling and progressively rising pulmonary vascular resistance are hallmarks of the disease that ultimately result in right heart failure. Several genetic mutations, most notably in bone morphogenetic protein receptor type 2, have a causal association with heritable forms of PAH. Mutations in *neurogenic locus notch homolog protein 3 (NOTCH3)* have been reported in adults and children with PAH, but whether *NOTCH3* is causally associated with PAH is debated. With this case report, we describe the clinical characteristics, comorbidities, and exposure history of an adult patient with PAH and multiple sclerosis who was found to have a *NOTCH3* missense mutation and exposure to leflunomide.

KEYWORDS

case report, genetic counseling, multiple sclerosis, NOTCH3, Pulmonary arterial hypertension

A 39-year-old white woman with relapsing-remitting multiple sclerosis (MS) and ulcerative colitis was referred to our pulmonary hypertension (PH) clinic for evaluation of progressive dyspnea on exertion. Before her referral, her chest X-ray was unremarkable. An outside transthoracic echocardiogram demonstrated normal biventricular structure/function and a right ventricular systolic pressure estimate of 17 mmHg. Pulmonary function testing showed normal spirometry, but a diffusing capacity of the lung for carbon monoxide (DLCO) of 40% predicted. Chest computed tomography (CT) with pulmonary angiography demonstrated no parenchymal abnormalities and no pulmonary embolus. Her isolated low DLCO and normal chest CT raised concern for pulmonary vascular disease and prompted referral to our PH clinic.

During the visit, she reported taking teriflunomide for MS and mesalamine for ulcerative colitis and was on no other medications. She denied the use of tobacco cigarettes, recreational drugs, and anorexigens. She worked from home and denied occupational/recreational exposures. There was no family history of PH, autoimmunity, or hypercoagulable disease. Physical examination was notable for a SpO2 of 94% on ambient air, scattered telangiectasias across the face, and a prominent S2 with expiratory splitting.

We obtained a 6-min walk test, during which she walked 274 meters and desaturated to 86%. A TTE with agitated saline was negative for shunt. V/Q scan did not reveal unmatched perfusion defects. A sleep test demonstrated nocturnal hypoxemia without obstructive sleep apnea. Tests for autoimmune or inflammatory conditions, including anti-nuclear antibody, anti-centromere antibody, anti-Scl-70, anti-RNA polymerase III, anti-dsDNA, anti-Smith, anti-ribonucleoprotein, Ro/LA, rheumatoid

factor/cyclic citrullinated peptide, erythrocyte sedimentation rate, and C-reactive protein were all negative or within normal limits.

A right heart catheterization (RHC) showed severe precapillary PH. Mean pulmonary arterial pressure (mPAP) was 50 mmHg, with pulmonary capillary wedge pressure 4 mmHg, pulmonary vascular resistance (PVR) 15.2 Wood Units, and cardiac index 1.56 L/min/min^2. Vasoreactivity testing was negative. A diagnosis of idiopathic pulmonary arterial hypertension (IPAH) was made. Her REVEAL 2.0 score was 10, consistent with high-risk IPAH. We recommended up-front combination therapy with ambrisentan and tadalafil. A literature review revealed teriflunomide to be a metabolite of leflunomide, a drug possibly associated with PAH.[1] We therefore recommended switching teriflunomide to an alternative therapy for her MS. In accordance with the latest guidelines for management of PH,[2] we referred the patient for genetic testing (Blueprint Genetics PAH Panel version 5, October 19, 2019), which revealed a heterozygous missense mutation in the *neurogenic locus notch homolog protein 3* (*NOTCH3*) gene, c.5015G>A, (p.Ser1672Asn), currently characterized as a variant of uncertain significance (VUS).

One year after discontinuing teriflunomide and beginning PH-specific therapy, repeat RHC showed mPAP improved to 26 mmHg, with PVR improved to 3.36 WU. Her symptoms and exertional tolerance improved on therapy.

DISCUSSION

The NOTCH3 protein is a transmembrane protein found in the distal pulmonary arteries that, upon ligand binding, initiates a cascade of intracellular events resulting in transcription of downstream genes influencing proliferation, differentiation, and contractile phenotypes of vascular smooth muscle cells (VSMCs).[3–5] NOTCH3 therefore plays a crucial role in vascular integrity and function and has been implicated in the pathogenesis of various vascular disorders, including PAH. NOTCH3 overall protein structure (via UniProt.org) and the specific residue affected by our patient's variant are shown in Figure 1.

Several observations link NOTCH3 to clinical PAH. The NOTCH3 intracellular domain is more abundant in human PAH lungs (and specifically, in pulmonary artery smooth muscle cells) relative to control lungs.[3] NOTCH3 mutations have been observed in small cohort studies of children[6] and adults with PAH.[7] However, due to a paucity of reports over time, in the most recent International Consortium for Genetic Studies in PAH (PAH-ICON) report, NOTCH3 was listed among genes with "disputed" associations with PAH.[8]

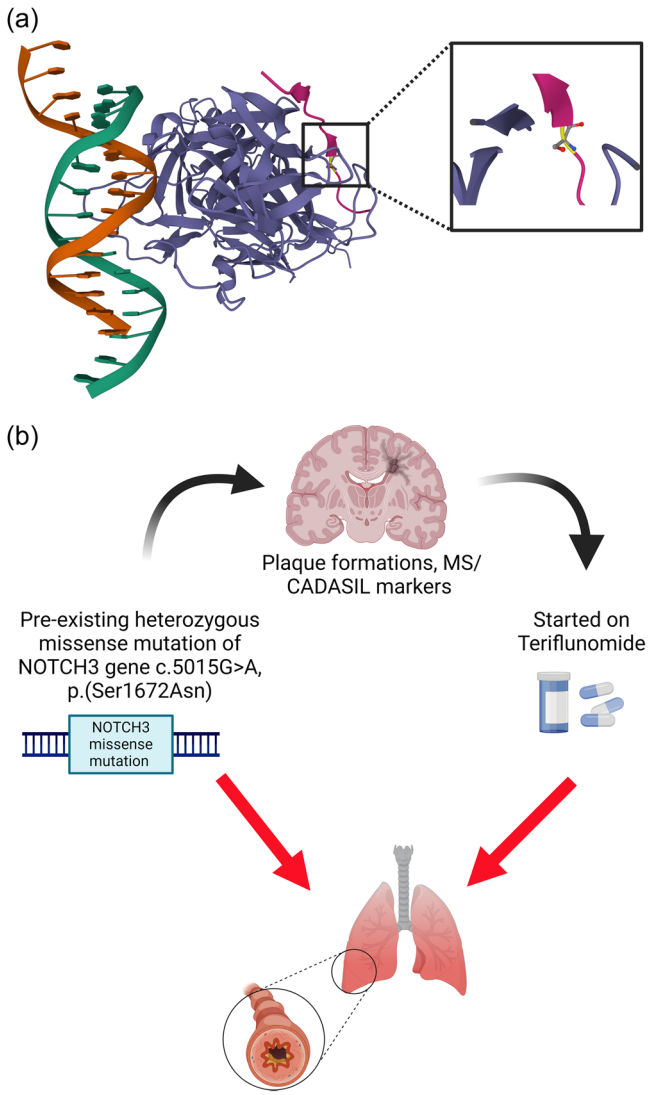

FIGURE 1 (a) Protein structure from UniProt. Inset shows residue modified by the variant. (b) A schematic of potential contributors to pulmonary vasculopathy in our patient with PAH. CADASIl, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy; MS, multiple sclerosis; PAH, pulmonary arterial hypertension.

NOTCH3 mutations have also been implicated in Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), a heritable small-vessel vasculopathy also characterized by vessel medial thickening, luminal narrowing, and VSMC degenerative changes.[9] CADASIL and MS have overlapping symptoms and similar diagnostic criteria. Whether a diagnosis of CADASIL should be considered for our patient is being considered by our neurology colleagues.

Our patient's mutation is located on the short (p) arm of chromosome 19 at position 15,281,241. Its dbSNP identifier (https://www.ncbi.nlm.nih.gov/snp) is rs2046722168, and it is rare, with an allele frequency of 1.240×10^{-6}. To the best of our knowledge, the functional consequence of

this variant is unknown. Though it is classified as a VUS, the CADD score for the variant is 27.0, considered "probably deleterious."

Teriflunomide treatment for MS represents a co-equal risk factor for PAH development in our patient. Pharmacovigilance disproportionality analysis has revealed a significant overrepresentation of leflunomide among drug associations with PAH reported to the WHO.[10] In a series of 28 patients from the French PH registry with a history of leflunomide exposure, 23/28 (82%) had additional risk factors for PAH. Only 5/28 patients had leflunomide as a sole risk factor.[10] The authors concluded that PAH related to leflunomide is rare and typically associated with other risk factors.

There are several unique aspects of our patient's clinical presentation, including her facial telangiectasias, low DLCO, unusual degree of hypoxia with no cardiopulmonary etiology identified aside from PAH, and the diagnostic uncertainty surrounding her neurologic disorder. She demonstrated substantial hemodynamic improvement with PH-specific therapy and withdrawal of teriflunamide. It is impossible to know to what degree, if at all, teriflunomide exposure and/or *NOTCH3* mutation contributed to her vasculopathy. Teriflunomide and *NOTCH3* are each possibly, though not conclusively, associated with PAH. It is reasonable to speculate that this patient's *NOTCH3* mutation may have enhanced her susceptibility to disease, with administration of teriflunomide acting as a "second hit" promoting development of her vasculopathy (Figure 1). It is equally reasonable to speculate that leflunomide exposure was the predominant cause, and withdrawal of teriflunomide was a large contributor to her hemodynamic improvement.

In conclusion, we present here a PAH patient with leflunomide exposure and a *NOTCH3* mutation discovered via routine genetic testing. We hope that the broad uptake of guideline-recommended genetic testing in the clinical setting will allow researchers and clinicians to develop an improved understanding of whatever role *NOTCH3* might play in human PAH.

AUTHOR CONTRIBUTIONS

Elizabeth G. Fenner wrote the report. Catherine E. Simpson is the clinician managing this patient and mentored Elizabeth G. Fenner in the writing of the report.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

The patient consented to the publication of this case report. Catherine E. Simpson had complete access to the data and takes full responsibility for the conduct of the work and overall content of the manuscript.

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