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CASE REPORT

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An adult case of NOTCH3 mutation in pulmonary artery hypertension

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressively fatal disease process affecting the small distal arteries of the pulmonary vasculature. It is characterized by vascular remodeling, proliferation, and subsequent vasocontraction leading to right heart failure.[1] In idiopathic pulmonary artery hypertension (IPAH), an increasing number of culprit genes have been identified, with BMPR2 being the most common.[2] However, emerging literature has noted that the genetic basis for IPAH development is different between adults and children.[3,4] *NOTCH3* is one such example, with all reported cases seen only in children. Missense mutations in *NOTCH3* lead to pulmonary artery smooth muscle cell proliferation, vasoconstriction, and reduced apoptosis.[5] Here, we report the first identified case of *NOTCH3* mutation in an adult patient with IPAH.

CASE DESCRIPTION

A 48-year-old Caucasian female with a known history of hypertension, hyperlipidemia, tobacco use, and substance abuse (methamphetamine, Adderall, marijuana) initially presented at an outpatient setting with complaints of shortness of breath and exertional dyspnea for 6 months duration. Additional symptoms included lightheadedness with exertion, dyspnea when bending down, and swelling of her hands and legs. She reported prior use of fen-phen 20 years ago for about 2 years. Her family history was significant for her mother with pulmonary artery hypertension. She underwent a diagnostic echocardiogram revealing left ventricular ejection fraction 55%–60%, moderately enlarged right ventricular (RV) with impaired function and RV systolic pressure ~50 mmHg. A diagnostic right heart catheterization (RHC) showed right atrial pressure (RAP) 6 mmHg, RV pressure 84/2/10 mmHg, pulmonary artery pressure (PAP) 87/47/54 mmHg, pulmonary capillary wedge pressure (PCWP) 7 mmHg, cardiac output (CO)/cardiac index (CI) 3.23/1.7 and pulmonary vascular resistance (PVR) 14 Wood units, with no response to vasoreactivity testing.

On her initial evaluation, she was in World Health Organization (WHO) Functional Class IV. Admission B-type natriuretic peptide was 214 pg/ml. A 6-minute walk test noted a walked distance of 449 m. Patient was initiated on intravenous prostacyclin in addition to an

oral soluble guanylate cyclase stimulator and an endothelin receptor antagonist. But she was transitioned to oral prostacyclin analog from intravenous prostacyclin as per patient's request as she did not want to continue intravenous prostacyclin. Etiological evaluation for connective tissue disease, HIV, and hepatitis was negative and a genetic test panel from a commercially available laboratory was sent (Blueprint Genetics). At this point, genetic testing suggested heterozygous missense mutation for *NOTCH3* c.5990A>G, p.(Asn1997Ser) gene. Family member screening could not be performed as her siblings refused and she did not have any biological offspring. Since diagnosis, her symptoms have improved with treatment and she is now in WHO Functional Class III. A follow-up RHC after initial diagnostic catheterization showed improved PVR of 8.1 Wood units and hemodynamics of RAP 8 mmHg, RV 46/7/12 mmHg, PAP 74/35/48 mmHg, PCWP 13 mmHg, TD CO/CI 4.3/2.2.

DISCUSSION

The *NOTCH3* gene encodes a single-pass transmembrane protein receptor expressed almost exclusively in pulmonary vascular smooth muscle cells. It plays a key role in regulating cell phenotype and proliferation. The protein itself consists of 34 epidermal growth factor (EGF)-like repeats, three Notch/Lin-12 repeats, a transmembrane domain, seven ankyrin repeats, and a sequence rich in proline, glutamic acid, serine, and threonine.[6] Canonical notch signaling is relayed when cell membrane-bound ligands (Jagged-1) on nearby cells interact and bind with EGF-like repeats, which compose the extracellular domain of the receptor. This triggers protease-driven cleavage of the Notch intracellular domain that translocates to the nucleus and activates transcription of gene family *HES*. The downstream effect is phenotypical switching of vascular smooth muscle cells to a prosurvival, proliferative phenotype causing PAH (Figure 1).[5]

The importance of NOTCH3 overexpression and its mechanistic links with HES-5 signaling as the cause of PAH has been demonstrated in prior rodent and human studies.[7] Human and rodent pulmonary tissues affected by PH were noted to have elevated Notch3 and HES5 protein levels. Subsequent rodent studies used DAPT (N-[N-(3,5-diflurophenacetyl)-L-alanyl]-S-phenylglycine *t*-butyl ester), a γ-secretase inhibitor, to block Notch3 activation which led to PH reversal. This suggested that the NOTCH3-HES signaling pathway is important in the development of PH.[7]

Majority of the previously identified missense NOTCH3 mutations have been implicated in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).[8] Medical literature on the association of *NOTCH3* and pulmonary artery hypertension remains limited. A previous study in pediatric pulmonary hypertension patients demonstrated two *heterozygous* missense *NOTCH3* variants c.2519G>A, p.(Gly840Glu) and c.2698A>C, p.(THr900Pro).[6] These mutations were located in Exons 16 and 17, affecting the extracellular EGF-like repeat domains responsible for ligand binding. This same domain has been implicated in CADASIL mutations, however, these are typically found on exons 3 and 4. It is hypothesized that this difference in exon function is why PAH has not been reported in CADASIL.

Our report is the first demonstrating heterozygous *NOTCH3* mutation as a culprit mutation for PAH in an *adult* patient. Furthermore, our patient had the missense variant c.5990A>G, p.(Asn1997Ser), which has not been described in prior medical PAH literature nor has it been reported in the medical ClinVar and Human Gene Mutation Database (HGMD) databases. This variant resulted in the conservative substitution of asparagine (Asn) by serine (Ser) at protein position 1997, located in the EGF-like domain 28. This corresponds with mutations in exons 20 and 21, which have not been described previously in PAH. The implication of this mutation and its downstream effects remain unclear, but we hypothesize that it likely affects the Notch3-Jagged-1 binding domain and consequently, the downstream NOTCH3-HES5 signaling pathway given the development of PAH in our patient.

Our patient provides a strong history of methamphetamine, tobacco, and fen-phen use, which are known risk factors for PAH. However, not all patients with these risk factors develop PAH.[9,10] In a study, Notch3-knockout (Notch3−/−) mice subjected to hypoxia over a 6-week period were resistant to developing hypoxia-induced PAH.[7] Given that our patient had known mutation, there is a possibility that environmental stressors might have played a role in causing disease when genetically predisposed explaining the late manifestation of PAH. This observation needs further validation and careful assessment.

This is the first-ever reported case of *NOTCH3* mutation causing PAH in an adult, with a noted novel mutation not previously described in the medical literature. The mutation was found to affect the EGF-like repeat domain of the Notch signaling protein, which has been implicated in prior studies examining *NOTCH3* mutations and PAH.[5,6] Our case contributes to the

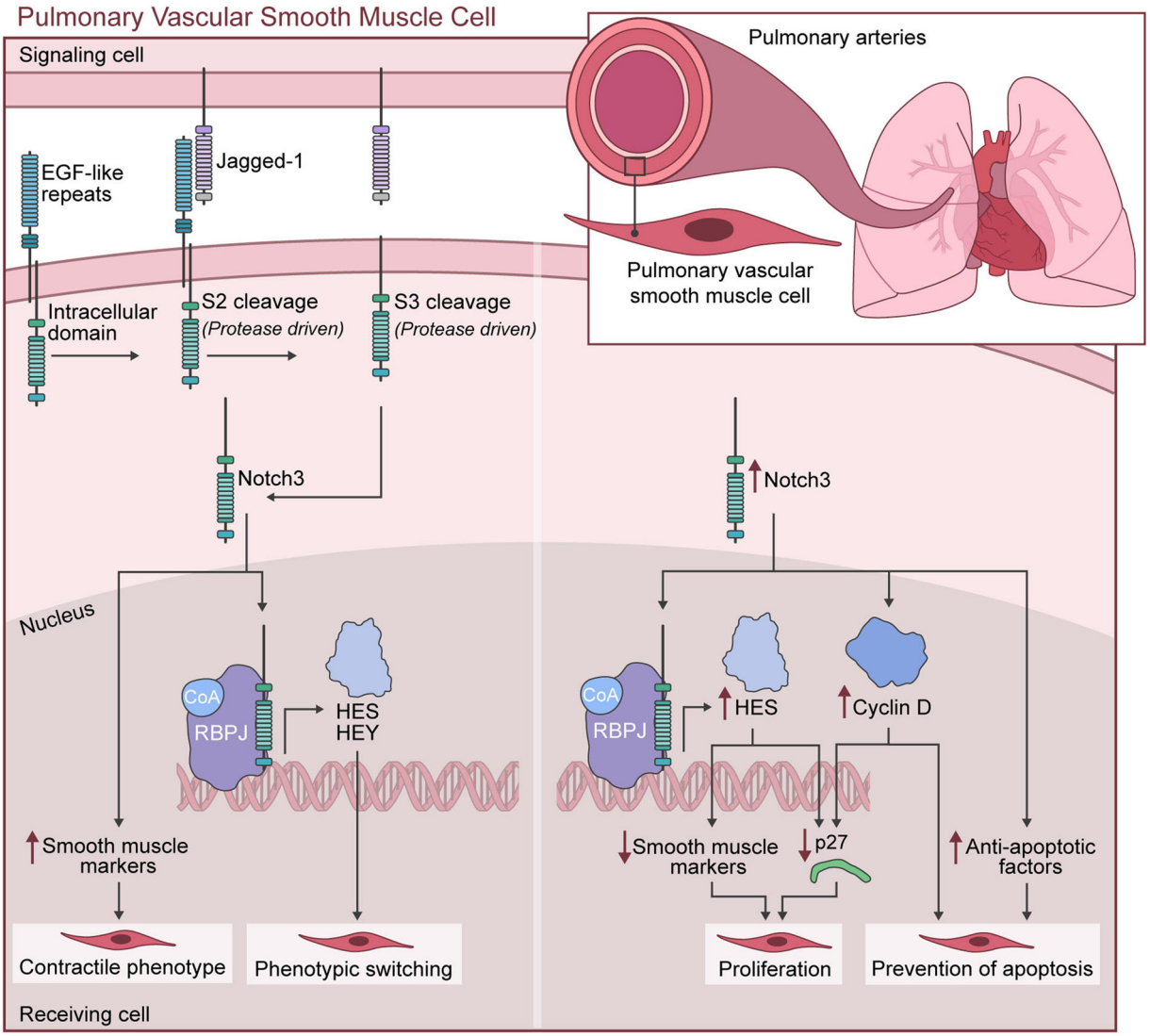

FIGURE 1 Notch3 signaling pathway in a pulmonary vascular smooth muscle cell. Left figure is the canonical pathway and right figure is the proposed pathway with Notch3 mutation. Canonical pathway description (left pathway): Cell membrane-bound ligands (Jagged-1) on the signaling cell physically bind with the extracellular domain (epidermal growth factor [EGF]-like repeats) of the NOTCH protein receptor. This triggers protease-driven cleavage of the intracellular domain of the NOTCH protein which then translocates to the nucleus, binding with recombining binding protein suppressor (RBPJ) to active HES gene transcription. This subsequently leads to downstream effects on the vascular smooth muscle cells. Mutation pathway (right pathway): mutations can lead to improper signaling of the Notch3 protein and overexpression of downstream antiapoptotic factors and cell cycle promoters (cyclin D) leading to proliferation, prosurvival pulmonary vascular smooth muscle cells

limited literature on *NOTCH3* in PAH and is the first to demonstrate that this association can be found in adults.

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CONFLICT OF INTERESTS

Akhilesh A. Padhye reports no conflict of interests. Sandeep Sahay is a speaker and consultant for Actelion, Bayer, and United Therapeutics; advisor for Boehringer Ingelheim, Liquidia Technologies, Gossamer Bio, and Altavant Sciences; Clinical trial end point adjudication committee member for a GSK sponsored RCT, Research grant from ACCP CHEST Foundation.

ETHICS STATEMENT

None.

AUTHOR CONTRIBUTIONS

Akhilesh A. Padhye contributed to the writing of the whole report and data collection. Sandeep Sahay mentored the first author in writing this report and is the

guarantor of this case report and clinician managing this patient.

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