Novel Inheritable Caveolin-1 Mutations in Pulmonary Arterial Hypertension

Ankit A. Desai, MD


Study Hypothesis

Previous genetic studies of patients with idiopathic pulmonary arterial hypertension (PAH) and heritable PAH have identified mutations in several genes related to transforming growth factor-β signaling, including bone morphogenetic protein receptor type 2 (BMPR2), activin receptor-like kinase 1 (ALK1), endoglin (ENG), and mothers against decapentaplegic 9 (SMAD9). Furthermore, 25% of heritable PAH and 85% of idiopathic PAH cases have no identifiable genetic association. The study by Austin et al sought to identify additional genes responsible for idiopathic PAH and heritable PAH.

How Was the Hypothesis Tested?

Sequencing was performed on 2 cohorts. The first was a 3-generation family with autosomal dominant transmission of PAH and no identifiable mutations in BMPR2 by dideoxy sequencing or multiple ligation-dependent probe amplification and ALK1, ENG, or SMAD9 by exon sequencing. Exon sequencing was then performed on samples from 4 of 12 family members to identify the presumed causal mutation, and Sanger sequencing was used for the remainder 8 samples from family members to evaluate for the presence of the genotype. Sequencing of coding exons of caveolin-1 (CAV1) was then performed in 62 unrelated heritable PAH and 198 idiopathic PAH patients without detectable BMPR2 mutations. The authors used skin (from family cohort) and lung tissues (from 1 patient in replicate cohort) collected from study patients to characterize the effects of the mutations on Cav1 expression.

Principal Findings

After exome sequencing of samples from 4 family members with PAH, subsequent comparison of variation derived from these cases, and a screen using the Database for Single Nucleotide Polymorphism (DBSNP), Sorting Intolerant From Tolerant (SIFT), and 1000 Genomes Project, the study reports 11 novel variants that not only were present in all cases but also were predicted to be nonsynonymous and further verified with dideoxy sequencing. Sanger sequencing confirmed 3 of these variants, 2 of which were found to have neutral effects after analysis with Single Nucleotide Polymorphism Annotation Platform, Sorting Intolerant From Tolerant, and PolyPhen. The third, c.474delA (P158PfsX22) in CAV1, was predicted to cause a frameshift P158PfsX22 and to add 21 novel amino acids at the C-terminal domain of CAV1 protein. Several other family members who did not demonstrate any evidence of PAH also carried the c.474delA CAV1 mutation, suggesting incomplete penetrance with this CAV1 mutation. Western blot analysis of extracts from skin biopsies exhibited reduced expression of CAV1 in 3 of the family members compared with those derived from 2 healthy control subjects.

Sequencing coding exons in the replication cohort of unrelated patients with PAH revealed 2 variants in CAV1: c.463G_A (V155I) and c.473delC (P158HfsX22) in 1 pediatric case of PAH (confirmed by catheterization and lung biopsy) that was not inherited by family history. In silico analysis revealed a tolerable c.463G_A (V155I) substitution for protein function, identified in the clinically unaffected father. However, the latter deletion variant, c.473delC (P158HfsX22), was de novo, located in the same exon as the variant filtered from 3-generation family cohort, and not present in either parent.

Genotyping in 1000 ethnically matched white, European control subjects confirmed the absence of both novel variants (c.474delA and c.473delC). Finally, lung (derived from the biopsy specimens of the pediatric PAH patient and control subjects) immunohistochemistry revealed decreased CAV1 expression in small pulmonary artery endothelial cells of PAH.

Implications

The study highlights the utility of sequencing in identifying 2 novel mutations in CAV1 in human PAH and without involvement in transforming growth factor-β signaling. In one family, 1 mutation helped, in part, to identify an autosomal dominant pattern, whereas the second mutation was identified as de novo and helped to reclassify a patient with idiopathic PAH to heritable PAH. The findings of reduced Cav1 expression in tissue from patients with PAH support the evidence from in silico analyses.
which identified protein alteration with the presence of the mutation, and data from previous preclinical work that have established a PH phenotype in the murine model of Cav1 knockout. Ultimately, this work has set the stage for further work to identify the functional significance of the mutations, including potential for changes in Cav1 protein modification and its downstream impact on anchorage of caveolae to the plasma membrane and subsequent cell signaling pathways.

Acknowledgments

Dr Desai is a member of the Early Career Committee of the American Heart Association Functional Genomics and Translational Biology Council.

Disclosures

None.

Keywords: caveolin-1 ■ genetics ■ mutations ■ pulmonary hypertension ■ sequencing
Novel Inheritable Caveolin-1 Mutations in Pulmonary Arterial Hypertension
Ankit A. Desai

doi: 10.1161/CIRCGENETICS.112.965335
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/5/6/706

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Genetics can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org//subscriptions/