Identification of Novel Variants in Hedgehog Signaling Pathway Genes Associated with Familial Osteoarthritis

718

Shivakumar R. Veerabhadraiah, Kendra Novak, Ying Ma, Nikolas H. Kazmers, Michael J. Jurynec

[1]Department of Orthopaedics, University of Utah, Salt Lake City, UT 84112

THE UNIVERSITY OF UTAH®

juryneclab.org

Introduction

Osteoarthritis (OA) is a common joint disease characterized by abnormal remodeling of joint tissue, affecting 32.5 million adults in the United States. There is no cure for OA and no therapies prevent disease initiation or progression. We lack effective therapeutics because of our limited understanding of the genes and biological processes underlying or conferring susceptibility to OA. Identifying genetic risk factors is central to understanding the causation of OA and will help guide rational drug design. The **goal** of this study is to identify recurrent novel gene variants that are associated with susceptibility to OA. To do this, we analyzed the exomes from >170 families with multiple forms of OA and identified candidate gene variants in each family. Here we describe the identification of the Hedgehog (Hh) pathway as a major risk factor for OA susceptibility.

Methods

We used a unique medical genetics resource, the Utah Population Database, to identify independent families with dominant inheritance patterns of OA. Within each family, a distinct set of joints are affected, encompassing distal and proximal interphalangeal OA, erosive hand OA, glenohumeral osteoarthritis OA, and 1st metatarsophalangeal (MTP) joint OA. Whole exome sequence (WES) analysis was performed on informative members of families, and rare coding variants that invariably segregated with OA and were predicted to alter gene function were identified.

The SMO plasmid was kindly obtained from Benjamin R. Myers, University of Utah, in which the human *SMO* gene was cloned into the pGEN vector backbone. The variant *SMO* was generated using *in vitro* mutagenesis (NEB Q5 Site-Directed Mutagenesis Kit). GLI-luciferase assays were performed as described elsewhere [1]. Briefly, *SMO*$^{-/-}$ MEF cells were seeded into 24-well plates and transfected (TransIT 2020) with wildtype or variant *SMO* expression constructs, 1:1 mixture of 8xGli-Firefly and nanoluc plasmids and GFP. Transfected cells at 100% confluency stimulated for 48 to 60 hours with 1:20 dilutions of control or ShhN-containing conditioned medium in low-serum medium (2% FBS), collected from stably transfected HEK293-ShhN cells as described elsewhere [1]. Luminescence was determined using a Nano-Glo Luciferase Assay Kit (Promega) on a Berthold Centro XS3 luminometer with automated injection. The ratio of Nanoluc to Firefly luciferase ("GLI-Luc Activity") is reported as relative luciferase units (RLUs).

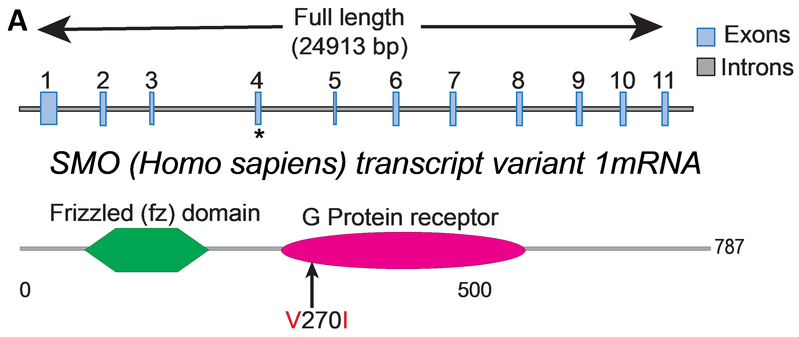
Results

WES revealed recurrent novel variants in the Hh signaling pathway (Table 1). Variants were identified in *SMO* (which encodes Smoothened), *IHH* (which encodes Indian hedgehog), *GLI1* (which encodes Glioma-associated oncogene homolog-1), *GLI3* (which encodes Glioma-associated oncogene homolog-3) genes. The *SMO* gene variant allele, identified in a family with erosive hand OA, encodes significant amino acid change (p.Val270Ile) in the G protein receptor, indicating possible alteration to SMO protein function. Similarly, *IHH*, identified in a family with erosive hand OA, and *GLI1*, identified in a family with 1st MTP OA, gene variants encode (p.Gly233Arg) and (p.Pro690Thr), respectively. *GLI3* gene was found to have two variant alleles, identified in families with interphalangeal joint and 1st MTP OA, encoding (p.Ser297Thr) and (p.Pro1222Ser) in our variant analysis. The two *GLI3* variants are located in separate domains of the protein.

In cultured SMO$^{-/-}$ fibroblasts, the variant SMO plasmid significantly increased GLI-dependent transcription in response to Hh ligands in comparison to wildtype SMO plasmid (Figure 1). These data indicate that the OA-associated SMO variant increases Hh signaling in response to ligand. Future work includes validation of SMO variant and analyzing remaining candidate gene functions in human cell lines and zebrafish and generating mice carrying human disease alleles to understand the contribution of these gene variants to the osteoarthritis phenotype.

Gene	OA Phenotype (Family)	Variant	Minor Allele Frequency	Protein Domain Affected by Variant
SMO	Erosive Hand OA (ERO 369)	c.G808A:p.V270I	0.007084	G Protein Receptor Domain
GLI1	1st MTP Joint OA (MTP 23)	c.C2068A:p.P690T	0.000016	NA
GLI3	Finger Interphalangeal Joint OA (FIJ 6)	c.T889A:p.S297T	Novel	NA
GLI3	Erosive Hand OA (ERO 30)	c.C3664T:p.P1222S	0.002656	NA
IHH	Erosive Hand OA (ERO 15)	c.G697C:p.G233R	Novel	Intein N-terminal Splicing Motif

Table 1: Data represent Hedgehog signaling pathway variants identified in independent osteoarthritis families.



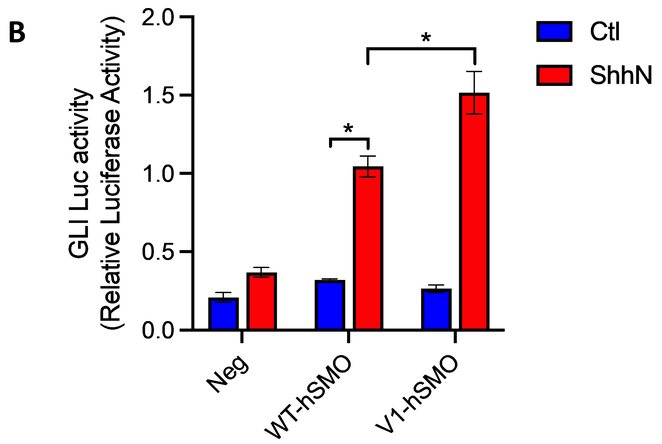


Figure 1: **(A)** Novel Smoothened (*SMO*) gene variants identified in the Hh signaling pathway. **(B)** Hh signal transduction was significantly increased by variant SMO plasmid. GLI transcriptional reporter assay in SMO−/− MEFs expressing wild-type SMO ("WT-hSMO") or variant SMO plasmid, treated with conditioned medium containing the N-terminal signaling domain of Sonic hedgehog (ShhN, gray) or control, non-ShhN-containing conditioned medium (Ctl, white). GFP serves as a negative control ("Neg.").

Discussion

Gene variants in the Hh pathway are associated with OA susceptibility across a diverse range of joints, suggesting that alterations in Hh signaling is a major risk factor for OA susceptibility.

We anticipate that identifying these novel gene variants will advance our understanding of OA initiation and progression. This study could provide a scientific basis for establishing new targets for the prevention and treatment of OA.

References

1. Myers, B. R., Neahring, L., Zhang, Y., Roberts, K. J. & Beachy, P. A. Rapid, direct activity assays for Smoothened reveal Hedgehog pathway regulation by membrane cholesterol and extracellular sodium. *Proc. Natl. Acad. Sci.* 114, E11141–E11150 (2017).

Acknowledgment

This project was supported by National Institutes of Health, Arthritis National Research Foundation, and the Utah Genome Project. We would like to acknowledge the technical assistance of Dr. David Grunwald, Dr. Kazuyuki Hoshijima, Matthew Honeggar, and Ying Ma.