Mutations in KIF7 auto-inhibitory region is associated with upregulation of inflammatory markers in AIS associated variants

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Disclosures: The authors have no disclosures.

Introduction: Idiopathic Scoliosis (IS) is a developmental disorder marked by a structural lateral spinal curvature ≥10° affecting 3% of otherwise healthy children. Genetics is a major risk factor for IS; however, the specific etiology remains unknown. Exome sequencing of 28 multigenerational IS families (3 individuals per family) followed by study of a secondary cohort of 1221 affected and 1397 unaffected individuals revealed multiple damaging variants in the ciliary kinesin gene *KIF7* that segregate with the IS phenotype. *KIF7* plays critical roles in sonic hedgehog signaling (ShH) and organization of the primary cilia. KIF7 has an inhibitory-coiled coil (inhCC) domain responsible for auto-inhibition of the N-terminal motor domain, which binds microtubules. To determine the functional consequences of these IS-associated mutations, we created knockout mouse embryonic fibroblast (MEFSV40) cell lines and reintroduced *Kif7* with three known human variants each suspected of affecting the inhCC domain, found in three different AIS families.

Methods: Immortalized MEFSV40 *Kif7*$^{-/-}$ lines were generated using CRISPR/Cas9. Lentiviral constructs were generated using the pLVX-TetOne-Puro vector with a doxycycline inducible c.*Kif7-SNAP* insert. We used in-Fusion cloning to create three *Kif7* variants identified in IS-affected individuals: p.H1115Q, p.Q1079X, and p.Q1001X (see **Figure 1**), which were then introduced into MEFSV40 *Kif7-/-* by infection. Immunofluorescent ɣ tubulin and α-tubulin stains were used to visualize cilia morphology. Bulk RNA sequencing was used to compare differentially expressed genes (DEG) constituting log2 fold change ±1 and padj <0.005 across the *Kif7* mutations using NovaSeq X Plus Series (PE 150, Novogene). Unaltered c.*Kif7-SNAP* was used as a control. Differential expression analysis was done using DESeq2 (R package 1.20.0). GO enrichment analysis was done using PANTHER Overrepresentation Test of all DEGs for each mutation (GO Consortium).

Results: All mutants showed longer cilia compared to wild-type following 48 hours of serum starvation (p.H1115Q: 0.4103 ± 0.1208; p.Q1079X: 0.4573 ± 0.1213; p.Q1001X: 0.5302 ± 0.1263, p<0.0001 for all, Welch's t-test). No genes within the ShH pathway met the cutoff criteria. Of 163 DEGs meeting cutoff criteria, a total of 22 transcripts were upregulated in all mutants compared to control. No transcripts in common were downregulated across mutants. GO enrichment analysis for biological process of these upregulated genes revealed various significant GO terms pertaining to response to stimulus and immune response. Notably, 21 genes are part of response to stimulus (GO:0050896, FDR 5.89E-05) while 19 genes fell under response to stress (GO: 0006950, 9.34E-10) and immune system process (GO: GO:0002376, FDR 1.70E-12). Fold enrichment of >100 are found in GO terms pertaining to interferon signaling and inflammasome regulation. Of the GO terms with this fold enrichment, response to interferon beta (GO:0035456) had 10 genes in common among all mutants: *Irgm2, Gbp2, Mndal, Xaf1, Iigp1, Ifit3, Gbp7, Oas1a, Ifit1, Gbp3*. This result is summarized in **Table 1**.

Discussion: Lacking autoinhibition of Kif7 within the cellular complex could result in potential excessive binding of microtubules, disruption of cilia organization and aberrant ShH signaling. The current work illuminates a potential intersection of cellular signaling mechanisms and inflammatory responses in AIS. Elevated immune system response may be exacerbated by the lack of intact *Kif7* inhCC domain. Furthermore, aberrant inflammatory response may be an important element in AIS disease pathogenesis. Studying known mutations in *KIF7* in individuals affected with IS provides unique insights into the biological mechanisms that may contribute to disease etiology. Functional studies in the MEFSV40 cell model support complex signaling dysregulation that may play a role in disease pathogenesis. Further research in the interplay of disruption of inhCC domain, sonic hedgehog pathway changes, and the upregulation of interferons and guanylate-binding proteins in these *Kif7* mutations could help us better understand AIS mechanism.

Significance/clinical relevance: The study of mutations in the *KIF7* gene in individuals affected with AIS, the associated signaling pathways and the regulatory elements of *Kif7* provides unique insights into the biological mechanisms that may contribute to the etiology and/or disease progression of AIS. Collectively, results suggest that further studies of known human mutations in ciliary genes may lead to a better understanding of the underlying pathophysiology of AIS and elucidate the impacts of cellular signaling dysregulation on inflammation.

Acknowledgements: This work was supported by the SRS Cotrel Research Grant (1153467) and the University of Colorado Anschutz Department of Orthopedics. Figure 1 was generated using Biorender.com.

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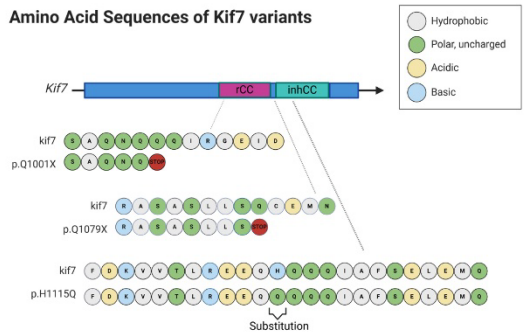

Figure 1: Schematic of amino acid change of KIF7 in AIS patients. p.Q1079X and p.Q1001X both produce a premature stop codon prior to inhCC domain. P.H1115Q produces a substitution from basic amino acid (H) to polar (Q) within the inhCC domain.

Table 1: Summary of genes in GO:0035456: response to interferon beta

	p.Q1079X		p.Q1001X		p.H1115Q	
Gene Name	**logfold 2**	**padj**	**logfold 2**	**padj**	**logfold 2**	**padj**
Irgm2	1.00	1.08E-05	3.14	2.62E-104	2.17	1.31E-38
Gbp2	1.09	7.62E-06	2.80	2.89E-85	2.12	5.75E-41
Mndal	1.17	0.00599	2.86	3.60E-41	2.15	1.46E-19
Xaf1	1.07	3.24E-05	2.71	1.50E-79	1.95	7.16E-31
Iigp1	2.83	8.37E-06	5.33	4.27E-48	4.29	5.74E-26
Ifit3	1.50	3.37E-08	3.91	9.17E-24	2.80	1.45E-51
Gbp7	1.33	3.88E-06	3.36	4.87E-21	2.53	1.03E-11
Oas1a	1.96	2.87E-05	4.38	7.14E-69	3.93	5.89E-54
Ifit1	1.38	1.85E-29	3.33	1.20E-37	2.41	1.33E-97
Gbp3	1.68	9.97E-16	3.77	5.72E-54	2.94	1.00E-74