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FOXA2 controls *Pkd1l1* expression in the mouse node during left-right determination

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Objectives

The embryonic node is a ciliated pit-like structure that is important in left-right (L-R) determination. *Pkd1l1*, encoding a membrane-bound ciliary protein, is expressed specifically in the node and is required to establish normal L-R patterning. Given the importance of this gene, we set out to understand its regulation.

Methods

Pkd1l1 and *FoxA2* expression was compared through whole-mount *in situ* hybridization and β-galactosidase staining in 7.5-9.5 dpc mouse embryos. Bioinformatic analysis was used to characterise FOXA2 binding regions upstream of *Pkd1l1* in the human-mouse lineage. Finally, luciferase assays were used to test the functionality of these potential regulatory regions.

Results

Our expression studies indicate that a regulatory relationship might exist between FOXA2 and *Pkd1l1*. Furthermore, $Shh^{-/-}$ and $FoxA2^{c/c}$;$ShhcreER^{T2}$ embryos display abnormal *Pkd1l1* expression. Indeed, previous studies identified FOXA2 binding regions upstream of *Pkd1l1*, in both human and mouse. Our work has revealed the two regions to be non-homologous, but conserved in the human-mouse lineage, in some cases to the binding site level. Although bioinformatic analysis predicts lower binding affinities for the conserved mouse-identified region in human and *vice versa*, luciferase assays indicate that the conserved regions are capable of driving gene expression.

Conclusion

Our study implicates FOXA2 in the regulation of *Pkd1l1* and characterises two FOXA2 binding regions upstream of this gene. Both regions are conserved across the human-mouse lineage to varying degrees. Furthermore, in both mouse and human, the two regions are able to activate gene expression.

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