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A View on the Contribution of Hedgehog Signalling to Ventricular Septal Development

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The ventricular septal defect (VSD) is the most frequent congenital heart disease in humans. It is defined as an opening in the septum separating the left and the right ventricle. This gap results in a mixture of oxygenated and deoxygenated blood and in an enhanced blood flow towards the lung and the left ventricle [1], a condition that leads to severe diseases such as left ventricular hypertrophy as well as pulmonary edema and dilatation [2, 3]. The molecular mechanisms underlying the development of VSDs are poorly understood. The recently published review article entitled "The Role of Hedgehog Signalling in the Formation of the Ventricular Septum" discusses the importance of the Hedgehog (HH) signalling pathway in the formation of the ventricular septum (VS) [4]. HH signalling begins with the binding of the HH protein to its receptor Patched (PTC1) which localises in the membrane of primary cilia, little cellular protrusions dedicated to signal mediation. Low-density lipoprotein receptor related protein 2 (LRP2) participates in this binding event [5]. The HH/PTC1 complex leaves the cilium and, in turn, Smoothened (SMO) enters the ciliary membrane. Subsequently, SMO releases the full-length Glioblastoma 2 (GLI2) and Glioblastoma 3 (GLI3) proteins from a complex with Suppressor of Fused (SUFU) and converts them into transcriptional activators (GLI2-A and GLI3-A) [6,7]. Proteins such as Broad-Minded (BROMI), Ellis Van Creveld 1 (EVC1) and Ellis Van Creveld 2 (EVC2) are involved in the activation of GLI2 and GLI3 [8-14]. GLI2-A and GLI3-A enter the nucleus and induce the expression of HH target genes. The Intraflagellar transport proteins 25 and 27 (IFT25 and IFT27) ensure the transport of several HH signaling components and the deficiency of IFT25 or IFT27 results in reduced HH target gene expression [15-17]. Without the HH ligand, PTC1 remains in the ciliary membrane and blocks the ciliary entry of SMO. In the absence of SMO, the full-length GLI2 and GLI3 proteins are proteolytically processed into transcriptional repressors (GLI2-R and GLI3-R) by the cilia-regulated proteasome [18-20]. Further proteins such as Protein kinase A (PKA), Casein kinase 1 (CK1), Glycogen synthase kinase 3-β (GSK3-β), Kinesin family member 7 (KIF7) and Fuzzy (FUZ) are required for GLI2 and GLI3 processing [21-26].

The fact that HH signalling mouse mutants such as *Shh*, *Lrp2*, *Sufu*, *Bromi*, *Ift25*, *Ift27*, *Gsk3-β*, *Kif7* and *Fuz* mutant mice display defects in VS outgrowth implies an essential role of HH signalling in VS development [15,16,27-30]. Furthermore, mutations of *EVC1* and *EVC2* cause VSDs in humans [31-33]. Since mutations in genes whose products positively regulate HH signalling and mutations in genes which encode proteins negatively controlling HH signalling lead to the occurrence of VSDs, our review article rises the question which role HH signalling plays in the development of the VS and of VSDs.

As outlined above, GLI3 is able to act as a transcriptional activator and induce HH target gene expression or as a transcriptional repressor and inhibit HH target gene expression. Previously, we demonstrated that the absence of the ciliary protein Retinitis pigmentosa GTPase Regulator Interacting Protein 1 Like (RPGRIP1L) leads to a disturbed GLI3 processing and also to the development of VSDs [19,34] suggesting that the inhibition of HH signalling might be important to ensure proper VS formation. Remarkably, loss of GLI3 does not affect VS development [35]. In our very recent preprint [36], we provide further insight into this topic by analysing mouse embryos which produce GLI3-R but lack GLI3-A (*Gli3*$^{\Delta 699/\Delta 699}$ embryos) [37,38]. Importantly, these embryos display reduced HH signalling and VSDs proposing that proper HH signalling is essential for the development of the VS. The finding of VSDs in *Rpgrip1l*$^{-/-}$ mouse embryos might also be traced back to a potential role of RPGRIP1L in

the transformation of GLI3 into GLI3-A [34]. Taking up a question from our review article, namely whether it is possible to prevent the development of VSDs by targeting HH signalling in pregnancy, our new results suggest that the activation of HH signalling (e.g. by using small molecules) could avoid the occurrence of VSDs.

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