Editorial (Journal of Hepatology, 2024)

Planar cell polarity is crucial for proper morphogenesis of the bile ducts

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Funding: supported by the Belgian Fund for Scientific Research FRS-FNRS, grants # T.0158.20 and J.0117.23; and by the D.G. Higher Education and Scientific Research of the Fédération Wallonie-Bruxelles, Belgium, grant ARC 23/28-133.

Conflicts of interest: None declared by the author.

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Abbreviations: PCP, planar cell polarity; Vangl2, Van Gogh-like 2

Introduction

While apical-basal polarity refers to the distribution of cellular components between the apical and basal poles of a cell, planar cell polarity (PCP) refers to the coordinated orientation of epithelial cells within the plane of a tissue, perpendicular to the apico-basal polarity axis.[1] Organs like kidney, pancreas or lung have tubular structures, the proper branching and elongation of which are perturbed in the presence of dysfunctional PCP. In kidneys, mutation of PCP regulators causes cystic kidneys, suggesting that cystic bile ducts relie on similar PCP deficiencies. Therefore, the proposition that PCP plays a role in normal bile duct development was introduced several years ago and initially tested using zebrafish (*Danio rerio*) as an animal model. Inhibition of genes known to be involved in PCP led to profound alterations of the biliary tree, characterised by reduced numbers of bile ducts with fewer terminal ductules and interconnecting ducts.[2] Although subsequent efforts were made to characterise the function of PCP genes in biliary morphogenesis in mammals, elucidating the mechanism of PCP in this process still remains elusive.[3,4] In this issue of the *Journal of Hepatology*, Boulter and coworkers fill this gap of knowledge by offering new insight into the normal embryonic development of the intrahepatic bile ducts and its regulation by PCP in mouse liver.[5]

Vangl2, a PCP core component, is required for bile duct development

In the nineties, a screen carried out in *Drosophila melanogaster* to search for PCP regulators identified a gene whose mutation causes hair swirling in cells of the wings.

Inspired by the swirling brushstrokes that embellish the portraits and landscapes painted by Vincent Van Gogh, the gene was named *Van Gogh* (*Vang*).[6] An independent group simultaneously characterised the same gene and called it *Strabismus* because of the misalligned eye lattice observed in the *Drosophila* mutants.[7] Homologous genes were found in vertebrates and named *Van Gogh-like* (*Vangl*) 1 and 2. Boulter and coworkers now re-analysed a published single cell RNA sequencing dataset [8] and highlighted that *Vangl2* is expressed in developing cholangiocytes.

There were three main reasons for pursuing the study of *Vangl2* in mammalian biliary development. First, *Vangl2* mutations are associated with a wide range of tubulogenic anomalies across several organs in mammals,[9] raising the possibility that disrupted function of *Vangl2* causes biliary dysmorphogenesis. Second, although differentiation of hepatoblasts to cholangiocytes is fairly well understood, how the cholangiocytes organise in three dimensions to form the biliary tree is less well known. Since knockdown of *Vangl2* in zebrafish disrupts the biliary architecture,[2] *Vangl2* appeared as a good candidate to regulate mammalian biliary morphogenesis. Third, Boulter and colleagues found predominant expression of *Vangl2* in cholangiocytes during their terminal maturation phase. At this stage, cholangiocytes, having already formed separate primordial ductules within the embryonic liver, undergo three-dimensional reorganisation to establish a continuous and branched biliary tree.[10-12] This suggested the involvement of PCP and *Vangl2* at the final stage of morphogenesis and branching.

Boulter and colleagues initiated their work by resorting to a classical approach. They first illustrated that maturing cholangiocytes express Vangl2 protein at their apical and lateral membranes, a subcellular distribution compatible with PCP (Fig. 1A). Next, they phenotyped a mouse mutant that carries a homozygous hypomorphic mutation *Vangl2*$^{S464N/S464N}$. The phenotype was spectacular: the number of bile ducts was significantly reduced and the biliary tree consisted of a network of small and disconnected ductules not linked with larger ducts (Fig. 1B). This was conceptually similar to the biliary defects detected in zebrafish PCP morphants,[2] demonstrating that the function of PCP in biliary morphogenesis is conserved between species. Unfortunately, perinatal lethality of the mouse mutants prevented further analysis of biliary morphology and function.

During embryonic development, cholangiocytes differentiate near the portal vein and form a discontinuous sheet or cellular network around the vein which is called ductal plate. This further develops along the vein as separate luminal spheres and ductules which elongate and fuse to constitute a continuous and branched network of bile ducts.[10-14] The presence of disconnected primordial ductules in the *Vangl2* mutant mice suggested that the phenotype results from changes in cell proliferation and from the failure of these initially separated ductules to fuse. Elegant *in vitro* experiments using cholangiocyte organoids derived from the embryonic livers provided good evidence that the rate of fusion of *Vangl2*-deficient organoids is lower than that of wild-type organoids, and that *Vangl2*-deficient cells are progressing more slowly through the cell cycle.

How does Vangl2 control biliary morphogenesis ?

Vangl2 is a membrane-embedded protein comprising four transmembrane domains and C- and N-terminal ends residing on the cytoplasmic face of the cell. It belongs to the PCP core components and mediates the exchange of polarity cues between adjacent cells.[1] Here, Boulter and co-workers showed in co-immunoprecipitation assays that Vangl2 interacts with cell junction proteins. This was perfectly in line with the observation that the *Vangl2*$^{S464N/S464N}$ cholangiocytes display tight junctional defects, thereby affecting the normal patterning of cell-cell interactions. PCP-dependent cell movements normally require the activation of Rho or JNK kinases. Interestingly, single cell RNA sequencing revealed that the *Rhoc* and *Mapk8* genes are increasingly expressed during normal biliary maturation.[8] Vangl2-deficient mice expressed reduced levels of Rhoc and displayed decreased phosphorylation of JNK. This likely influenced actin organisation, as evidenced by the poor polarisation of actin in *Vangl2*$^{S464N/S464N}$ cholangiocyte organoids, contrasting with the wild-type organoids where actin was predominantly observed at the apical membrane.

It is overly simplistic to consider that Vangl2 is solely required for PCP. Profound cell alterations inevitably also impact apical-basal polarity. The mutant organoids' ability to transport rhodamine 123 via the apical transporter MDR1 was reduced in Vangl2 mutants. Along the same lines, a significant proportion of mutant cholangiocytes failed to display a primary cilium normally emerging from the apical membrane. Therefore, while Boulter and colleagues provided compelling evidence regarding the significance of PCP in bile duct morphogenesis, distinguishing the effects of Vangl2 on PCP from its impact on apical-basal polarity remains challenging. Ectopic

localisation of Vangl2 in cholangiocytes, like was performed in the study of Vangl2 in pancreatic ducts, would help in clarifying this issue.[15]

Vangl2 defects in human biliary disease ?

While dysfunction of PCP can cause a number of developmental disorders affecting the nervous system, the kidneys, the heart or lungs, the involvement of PCP in biliary tract diseases is less well documented. Fabris et al. described how PCP is perturbed in a mouse model of congenital hepatic fibrosis.[4] Luke Boulter's team investigated Wnt-regulated PCP in mouse liver and illustrated how PCP components are activated in cholangiocytes following biliary injury, initiating a cascade of events leading to periductal fibrosis.[16] Also, PCP genes were identified as biliary atresia susceptibility genes.[17] As for *VANGL2*, mutations found in miscarried foetuses were associated with severe neural tube anomalies and were nonviable.[18] Therefore, although no clear link can yet be established between VANGL2 function and human biliary disease, the new findings by Boulter's team represent a leap forward in our understanding of a fundamental process occuring during embryonic liver development.

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Author names in bold designate shared co-first authorship.

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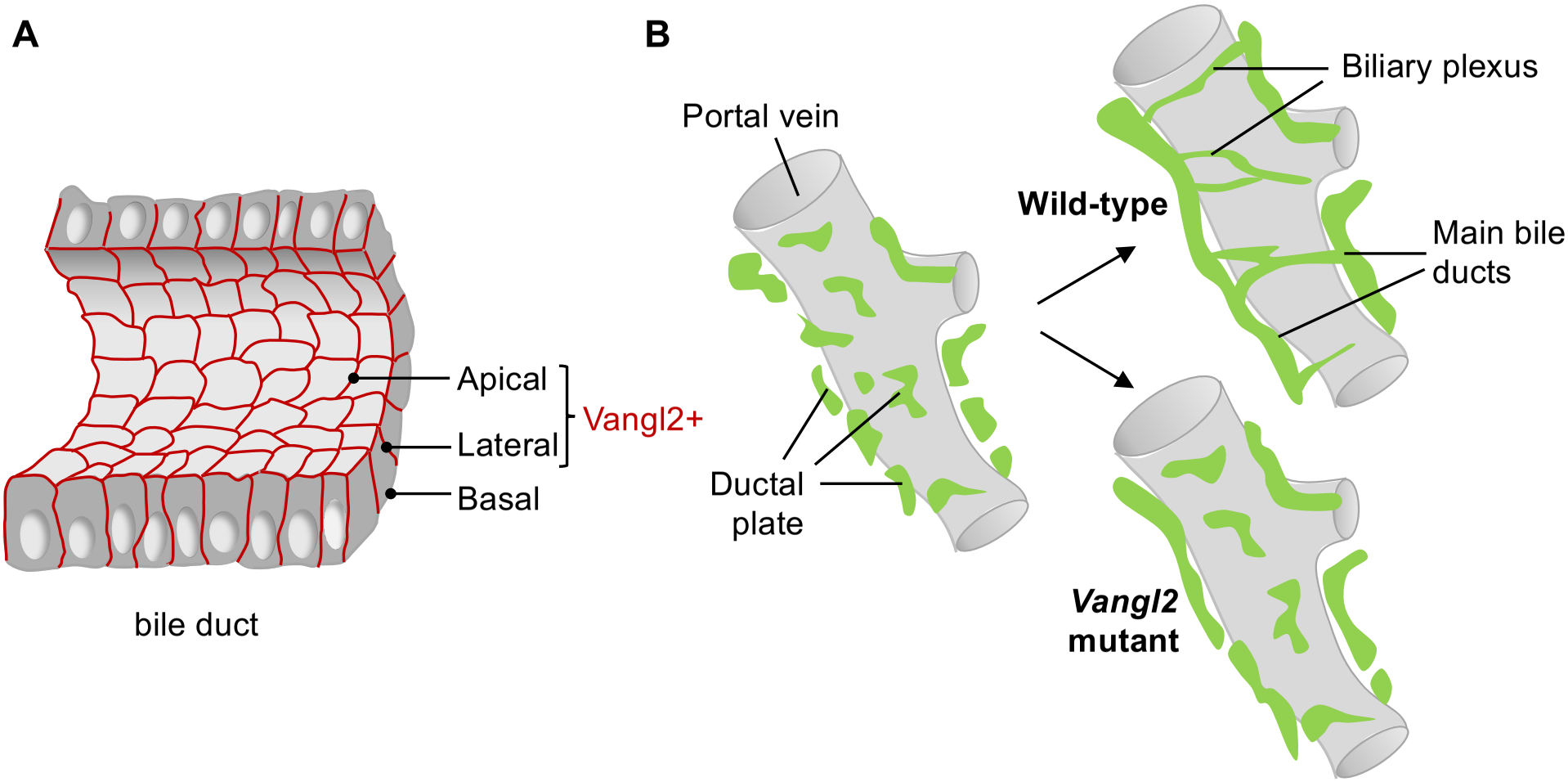
Figure Legend

Fig. 1. Subcellular distribution of Vangl2 in cholangiocytes and biliary phenotype of *Vangl2*$^{S464N/S464N}$ mice. (A) Scheme of a longitudinal section of a bile duct showing how cholangiocytes in their maturation phase express Vangl2 protein (red) at the apical and lateral membranes (B) During biliary maturation the primordial ductules forming the ductal plate around the portal vein (left) are remodeled to constitute a network of bile ducts in which one or two main ducts are connected via a biliary plexus constituted of small ductules (upper right). *Vangl2*$^{S464N/S464N}$ mice displayed a reduced bilary network with disconnected tubules (lower right).


A
Apical
Lateral
Basal
Vangl2+
bile duct
B
Portal vein
Ductal plate
Wild-type
Biliary plexus
Main bile ducts
Vangl2 mutant