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# Temporal–Spatial Expression of Inversin and Dishevelled-1 in Yotari (Dab1−/−) Mouse Stomach Suggests Dab1-Linked Gastrointestinal Developmental Alterations

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**ABSTRACT**

**Background:** We investigated the temporal and spatial expression of inversin and Dvl-1 in the developing stomach of yotari mice at embryonic day (E) 13.5 and E15.5 to assess their potential involvement in Dab1-related gastrointestinal developmental alterations.

**Methods**: Protein expression was assessed by immunofluorescence and quantitative image analysis in stomach sections from yotari (Dab1−/−) and control C57BL/6N embryos at E13.5 and E15.5, corresponding to mid- and late-gestational developmental stages. Group and stage differences were analyzed using two-way analysis of variance with Tukey's post hoc test.

**Main findings**: Hematoxylin-eosin staining showed no morphological differences between the groups. At E13.5, inversin was strongly expressed in mesenchyme in both groups, but was significantly lower in yotari compared to controls ($p < 0.01$). By E15.5, epithelial inversin expression was significantly higher in yotari mice than in controls ($p < 0.00001$), with mesenchymal signal increasing from E13.5 to E15.5 ($p < 0.01$). Dvl-1 expression was reduced in yotari mesenchyme at E13.5 ($p < 0.01$), but exhibited significantly higher epithelial expression at E15.5 compared with controls ($p < 0.01$), indicating distinct temporal–spatial expression shifts associated with Dab1 deficiency.

**Principal conclusion:** Yotari (Dab1−/−) mice exhibit stage- and compartment-specific alterations in inversin and Dvl-1 expression during stomach development. These changes likely reflect Dab1-linked disturbances in Wnt signaling dynamics, providing new insight into the potential molecular mechanisms underlying gastrointestinal developmental abnormalities in this model.

**Key words**: yotari, dishevelled-1, inversin, Dab1, Wnt signaling, stomach

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## INTRODUCTION

The gastrointestinal (GI) tract develops through a series of tightly regulated morphogenetic events that coordinate epithelial and mesenchymal growth, patterning, and differentiation (1). Multiple signaling pathways, including Hedgehog, bone morphogenetic protein (BMP), and Wnt, orchestrate these processes to establish the structural and functional organization of the gut during embryogenesis (1–3). In particular, Wnt signaling plays a crucial role in gastrointestinal development by regulating stem cell maintenance, epithelial–mesenchymal interactions, and regional patterning along the gut tube (4–6). Perturbations in these pathways can result in developmental anomalies affecting multiple organ systems, including the GI tract.

During the generation of mice carrying a mutation in the gene encoding the receptor for inositol-1,4,5-trisphosphate, a new autosomal recessive mutant, named yotari (Dab1−/−), was identified (7). The yotari phenotype closely resembles that of reeler (reelin−/−) mice, which exhibit an unstable gait and tremors, as well as premature death during weaning (8). Yotari mice harbor a mutation in the murine disabled-1 (Dab1) gene, a critical intracellular adaptor in the reelin signaling pathway, which explains the strong phenotypic overlap with the reeler mutant (8, 9). The close similarity between the phenotypes of yotari and reeler, as well as the expression of reelin in yotari, suggests that the gene mutated in yotari encodes a molecule acting downstream of reelin in the same signaling cascade (7).

The reelin–Dab1 axis plays a pivotal role in neuronal migration, cortical layering, and synaptic organization, and its disruption leads to profound neurodevelopmental defects (10). In addition to their neurological phenotype, yotari mice also develop severe renal abnormalities. They typically die prematurely by the end of the third postnatal week due to chronic kidney disease (CKD), which arises from congenital anomalies of the kidney and urinary tract (CAKUT) and associated structural defects (11, 12). These findings highlight Dab1 as a central developmental regulator affecting both neural and visceral organ systems.

Among the signaling pathways regulating gut development, Wnt plays a central role. It controls stem cell maintenance, proliferation, and axis patterning through canonical (β-catenin–dependent) and non-canonical (planar cell polarity [PCP] and calcium-mediated) branches (4, 13). Crosstalk between Wnt and other developmental pathways ensures proper epithelial–mesenchymal organization and tissue morphogenesis during stomach development. Dysregulation of this pathway has been linked to congenital gastrointestinal malformations and abnormal epithelial patterning (13).

In this context, inversin and dishevelled-1 (Dvl-1) are two key intracellular regulators of Wnt signaling that may serve as molecular links between Dab1 deficiency and altered GI development (14). The INVS gene encodes inversin, which localizes to primary cilia and functions as a molecular switch between canonical and non-canonical Wnt pathways by promoting the degradation of cytoplasmic dishevelled proteins (14, 15). Mutations in INVS lead to nephronophthisis type 2, a ciliopathy characterized by defective tubular morphogenesis and left–right axis determination, highlighting its essential role in embryonic patterning (16).

DVL-1 encodes a cytoplasmic phosphoprotein that acts as a central transducer of Wnt signals, coordinating canonical and non-canonical pathways to regulate planar cell polarity, segmentation, and organogenesis (17, 18). Dishevelled proteins are critical for the organization of primary cilia and tissue polarity; their dysregulation has been associated with developmental defects in multiple organ systems, including the kidney (19).

Although the roles of inversin and Dvl-1 have been extensively characterized in renal and neural development, their spatiotemporal expression patterns in the gastrointestinal

system, and how these are affected by Dab1 deficiency, remain unknown. Given the pivotal role of Wnt signaling in GI tract development, altered expression of these proteins in yotari mice may reveal previously unrecognized molecular mechanisms linking Dab1 signaling to gastrointestinal morphogenesis.

The aim of this study was therefore to investigate the temporal and spatial expression patterns of inversin and Dvl-1 in the developing stomach of yotari (Dab1−/−) mice at embryonic day (E) 13.5 and E15.5. We hypothesized that Dab1 deficiency would be associated with compartment-specific alterations in these Wnt pathway regulators, reflecting disrupted developmental signaling during stomach organogenesis.

## MATERIALS AND METHODS

### Sample collection

This study utilized homozygous yotari (Dab1−/−) mutant mice, which carry an autosomal recessive mutation of the Dab1 gene. Affected mice exhibit various phenotypes, including tremors and an unsteady gait, as well as premature death around the time of weaning. Yotari (yot) and C57BL/6N control specimens (ctrl) were housed separately in standard polycarbonate cages with free access to food and water. The environmental temperature was maintained at 23 ± 2°C, with a photo period set to 12 hours of artificial light followed by 12 hours of darkness.

The following polymerase chain reaction (PCR) primers were used for genotyping:

- For yotari: GCCCTTCAGCATCACCATGCT and CAGTGAGTACATATTGTGTGAGTTCC;
- For control specimens: GCCCTTCAGCATCACCATGCT and CCTTGTTTCTTTGCTTTAAGGCTGT.

Gravid mice were sacrificed on embryonic days 13.5 (E13.5) and 15.5 (E15.5) to obtain their embryos. Upon examination at the designated time points, three mice from each genotype (yotari and ctrl) were analyzed. First, the animals were deeply anesthetized with pentobarbital. They were then transcardially perfused with phosphate-buffered saline (PBS, pH 7.2), followed by 4% paraformaldehyde (PFA) in 0.1 M PBS. The stomachs of the mice were fixed separately in 4% PFA in 0.1 M PBS overnight for hematoxylin-eosin (H&E) and immunofluorescence staining.

### Immunofluorescence staining

The tissue was initially fixed and dehydrated using graded ethanol solutions (Sigma Aldrich, St. Louis, MO, USA). It was then serially sliced into 5 µm-thick sections, embedded in paraffin blocks, and mounted on slides. Proper tissue preservation was confirmed by performing hematoxylin-eosin (H&E) staining on every 10th section. The mounted tissue samples were heated in sodium citrate buffer (Sigma Aldrich, St. Louis, MO, USA) at 95°C for 20 minutes in a water steamer. After deparaffinization in xylene and rehydration utilizing graded ethanol and distilled water, the samples were progressively cooled to room temperature. Following washes with 0.1 M PBS, a protein-blocking buffer (ab64226, Abcam, Cambridge, UK) was applied for 20 minutes to prevent non-specific staining. The sections were then incubated overnight in a humidity chamber with primary antibodies (Table 1). The next day, the samples were washed with PBS and incubated for an hour with the respective secondary antibodies (Table 1). After rinsing with PBS, the samples were stained with 4′,6-diamidino-2-phenylindole (DAPI) and covered with a mounting medium (Immuno-Mount, Thermo Shandon, Pittsburgh, PA, USA).

### Data acquisition and analysis

We captured hematoxylin-eosin (H&E) images of the stomachs from both yotari and control specimens to identify potential differences in tissue morphology. The H&E slides were analyzed using a light microscope (BX40, Olympus, Tokyo, Japan) equipped with a camera (DP27, Olympus, Tokyo, Japan). We observed morphological differences between yotari mice and the control specimens at embryonic day 13.5 (E13.5) and embryonic day 15.5 (E15.5) through hematoxylin-eosin staining

Table 1. Antibodies used for immunofluorescence

| | Antibodies | Catalog number | Host | Dilution | Source |
|---|---|---|---|---|---|
| Primary | Anti-inversin | ab65187 | Rabbit | 1:100 | Abcam (Cambridge, UK) |
| | Anti-dishevelled-1 (3F12) | sc-8025 | Mouse | 1:50 | Santa Cruz Biotechnology (Dallas, TX, USA) |
| Secondary | Anti-rabbit IgG, Alexa Fluor® 488 | 711-545-152 | Donkey | 1:300 | Jackson Immuno Research Laboratories, Inc. (Baltimore, PA, USA) |
| | Anti-mouse IgG, Rhodamine Red™-X | 715-295-151 | Donkey | 1:300 | Jackson Immuno Research Laboratories, Inc. (Baltimore, PA, USA) |

(Figure 1). The stomach sections were analyzed with an immunofluorescence microscope (BX51, Olympus, Tokyo, Japan), which was equipped with a Nikon DS-Ri2 camera (Nikon Corporation, Tokyo, Japan). To quantify the immunoexpression of the proteins of interest, we captured non-overlapping visual fields at 40x magnification with constant exposure times. At least 10 images of the embryonic stomach structures – both epithelium and mesenchyme – were collected. These were then processed with ImageJ software (National Institutes of Health, Bethesda, MD, USA) and Adobe Photoshop (Adobe, San Jose, CA, USA). Initially, Adobe Photoshop version 21.0.2 (Adobe, San Jose, CA, USA) was employed to remove the background signal utilizing the "levels" function. Next, we selected the epithelium with the Lasso tool, isolating it from the mesenchyme by cutting it from the original image and placing it into a blank image of the same dimensions. The separated images were then opened in ImageJ software version 1.53o (NIH, Bethesda, MD, USA), duplicated, and the channels were split. We closed the green and blue channels, leaving only the red. The red-color channel was subtracted to purify the green signal.

The images were duplicated again, and a median filter with a radius of 10 was applied to one of the images for both inversin and Dvl-1. The filtered images were subtracted from the non-filtered images to isolate the positive signal. The resulting images were converted into 8-bit images and thresholded with the "triangle" method. The area percentage of the thresholded images was determined with the "analyze particles" function. Upon examination of the analyzed images, it was noted that certain regions lacked tissue, leading to an area percentage measurement that was lower than the true area percentage. To address this issue, we used the Magic Wand tool in Adobe Photoshop to calculate the total number of pixels (px) present in the images, as well as the number of pixels corresponding to empty spaces. The corrected area percentage was then calculated utilizing the following formula:

*Corrected area percentage=(Uncorrected area percentage × total px)/(total px -empty space px),*

and it was used for the statistical analyses.

This information was recorded in an Excel table. The staining intensity of various parts of the stomach was evaluated on a semi-quantitative scale of four degrees: no reactivity (-), mild reactivity (+), moderate reactivity (++), and strong reactivity (+++).

**Statistical analysis**

The statistical analysis was conducted using GraphPad Prism 9.0.0 software (GraphPad Software, San Diego, CA, USA). To identify significant differences in the percentage of positive cells, we compared immunoexpression with a two-way analysis of variance (ANOVA) test followed by Tukey's multiple comparison test. This was performed for both the epithelium and mesenchyme at E13.5 and E15.5

in control specimens and yotari mice. The mean value and standard deviation (SD) were utilized to represent the percentage of positive cells, with a threshold for statistical significance set at $p < 0.05$.

## RESULTS

This study used yotari (yot) and control specimens (ctrl) mice stomach to examine the protein expression patterns of inversin and Dvl-1 in the epithelium (e) and mesenchyme (m) of mice at gestation days E13.5 and E15.5. The research focused on the co-expression of inversin with Dvl-1.

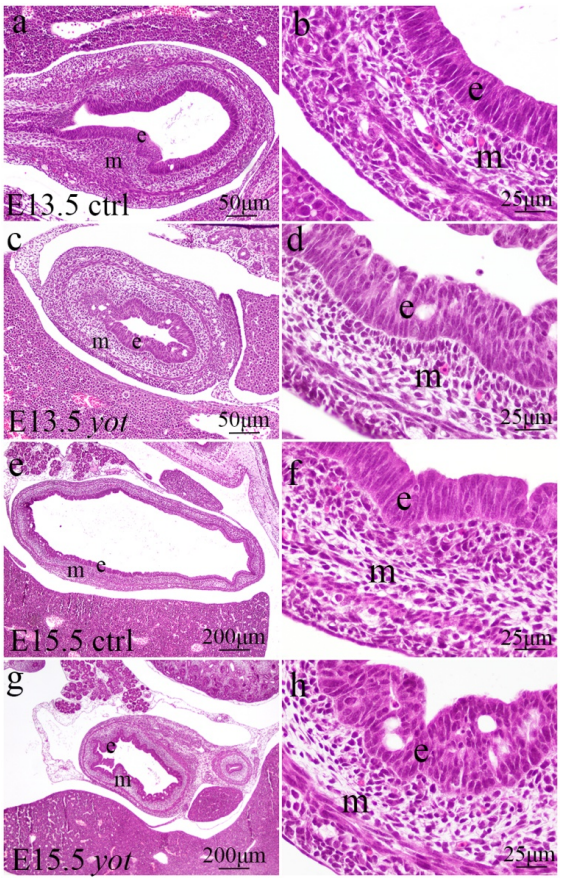


Figure 1. Hematoxylin-eosin (H&E) staining of the control specimens (ctrl) and *yotari* (*yot*) stomach. H&E staining of the ctrl (a,b,) and *yot* (c,d,) stomach at the embryonic day 13.5 (E13.5). H&E staining of the ctrl (e,f) and *yot* (g,h) stomach at embryonic day 15.5 (E15.5). Images were taken at a magnification of ×4 (e,g), ×20 (a,c), and ×40 (b,d,f,h)

The obtained results showed positive expression patterns, but with variations in intensity, distribution, and quantity. A semi-quantitative analysis of these differences is described in Table 2. Hematoxylin-eosin (H&E) staining did not reveal any morphological differences between the stomachs of the control specimens and yotari (Figure 1).

### Inversin displays distinct temporal–spatial expression patterns in yotari stomach

Semi-quantitative immunostaining revealed strong inversin reactivity (+++) in the mesenchyme at both E13.5 and E15.5, and mild reactivity (+) in the epithelium at both developmental stages in control and yotari stomachs (Table 2). This indicates that inversin is consistently abundant in the mesenchyme during mid- and late gestation, whereas its epithelial expression remains lower but detectable throughout these stages.

Quantitative image analysis further demonstrated distinct temporal–spatial differences between yotari and control mice (Figure 3). In the mesenchyme, the area percentage of inversin expression was significantly lower in yotari compared to controls at E13.5 ($p < 0.01$). By E15.5, inversin expression in the mesenchyme of yotari stomachs increased significantly, surpassing control levels. This was accompanied by a significant rise in the mesenchymal inversin area percentage in yotari from E13.5 to E15.5 ($p < 0.01$), whereas control mesenchymal expression decreased over this interval (Figure 3).

In the epithelium, inversin expression was higher in yotari than in controls, with this difference reaching statistical significance at E15.5 ($p < 0.00001$). Moreover, epithelial inversin expression in yotari increased significantly from E13.5 to E15.5 ($p < 0.01$), while control epithelial expression decreased (Figure 3).

Together, these findings indicate that although overall immunostaining intensity appeared similar between groups, quantitative analysis uncovered pronounced temporal–spatial shifts in inversin expression in Dab1-deficient mice, particularly a redistribution from mesenchyme to epithelium between mid- and late gestation,

reflecting altered developmental regulation during stomach organogenesis (Figure 2).

Table 2. Specific antibody staining intensity in the stomach of *yotari* mice (*yot*) and control specimens (ctrl) at embryonic days 13.5 (E13.5) and 15.5 (E15.5)

| | Inversin | | Dishevelled-1 | |
|---|---|---|---|---|
| | E13.5(ctrl) | E15.5(*yot*) | E15.5(ctrl) | E15.5(*yot*) |
| Mesenchyme (m) | +++ | +++ | + | + |
| Epithelium (e) | + | + | ++ | ++ |

+++ strong reactivity; ++ moderate reactivity; + mild reactivity; E—day of embryonic development; ctrl—control specimens; *yot*—*yotari*

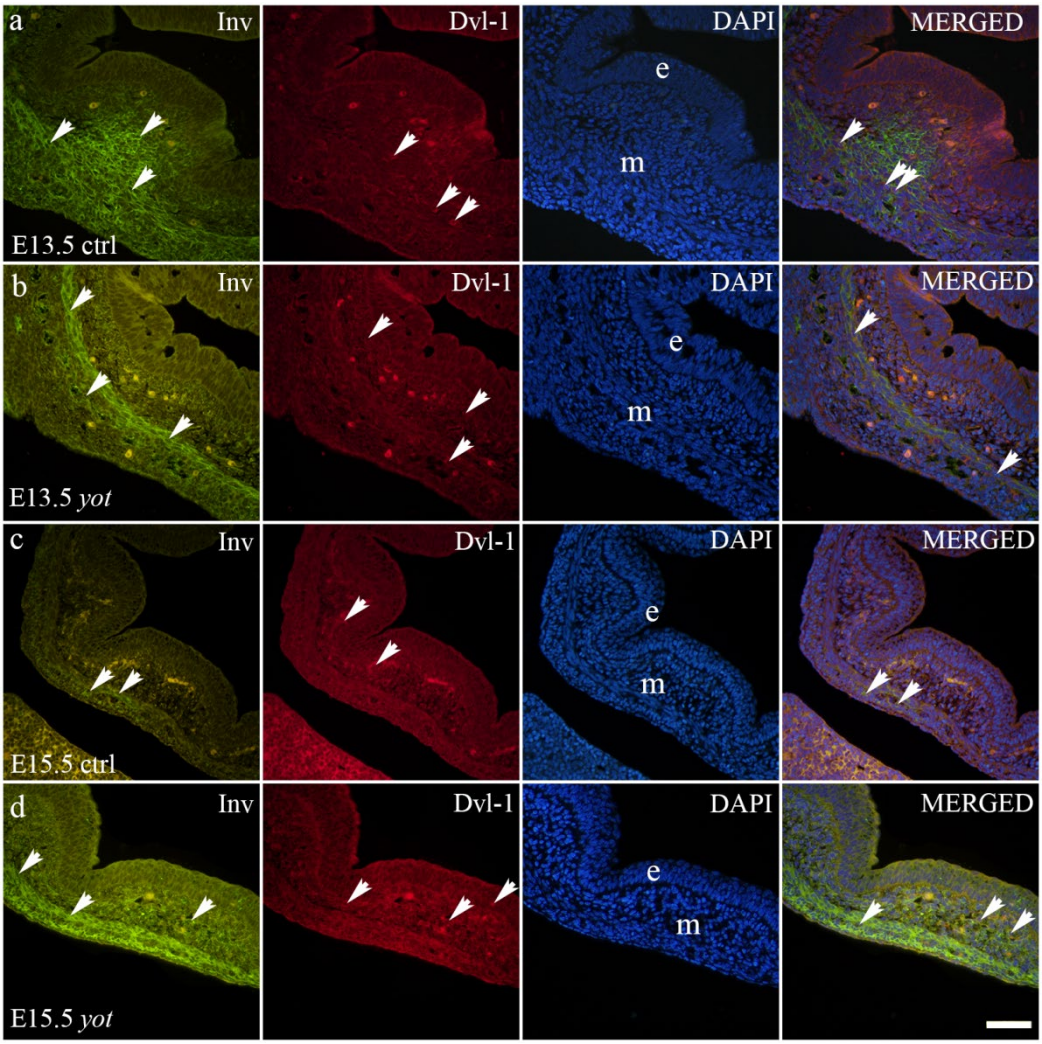


Figure 2. Immunofluorescence staining of inversin (inv), dishevelled-1(Dvl-1) merged with 4′,6-diamidino-2-phenylindole (DAPI) in the developing control specimens (ctrl) and *yotari* (*yot*) stomach (a–d). Inv and Dvl-1 comparison between stomach at embryonic days 13.5 (E13.5) and 15.5 (E15.5) (a–d). Positive staining of inv and Dvl-1 (arrows) is shown in each substructure in the stomach (a–d). Co-localization of merged images of all panels (arrows). Epithelium (e) and mesenchyme (m) at E13.5 and E15.5 (a–d). Magnification ×40, scale bar 100 µm

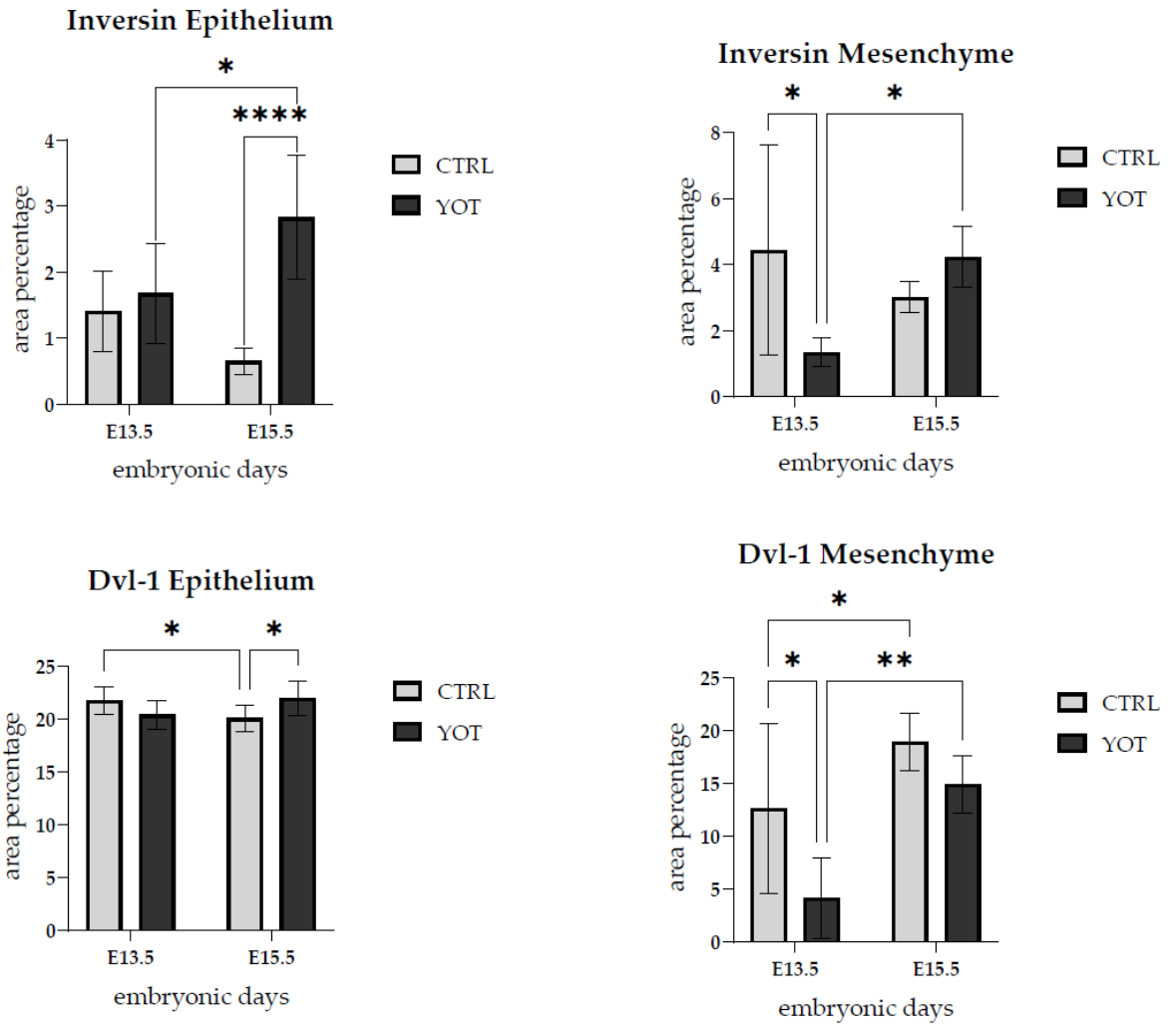


Figure 3. The area percentage of inversin and Dvl-1 in control specimens (ctrl) and *yotari* mice (*yot)* per structure (epithelium and mesenchyme) throughout different stages of developing stomach. Two substructures were assessed at E13.5 and E15.5. Data were displayed as the mean ± SD (vertical line) and analyzed by a two-way ANOVA test followed by Tukey's multiple comparison test. Significant differences are indicated by the following: * $p < 0.05$; ** $p < 0.01$; **** $p < 0.00001$

**Dvl-1 expression exhibits subtle temporal–spatial alterations in yotari mice**

Dvl-1 immunostaining revealed no major differences in intensity between the groups, with both control and yotari samples showing mild (+) epithelial and moderate (++) mesenchymal reactivity (Table 2). However, quantitative image analysis uncovered significant temporal and spatial differences (Figure 3). In the epithelium, yotari mice displayed significantly higher Dvl-1 area percentages compared to controls at E15.5 ($p < 0.01$). Additionally, control epithelial Dvl-1 expression decreased significantly between E13.5 and E15.5 ($p < 0.01$), whereas yotari samples maintained their levels across this developmental window (Figure 3). In the mesenchyme, Dvl-1 expression was significantly lower in yotari than in controls at E13.5 ($p < 0.01$). From E13.5 to E15.5, Dvl-1 mesenchymal area percentage increased significantly in both groups ($p < 0.01$ for controls; $p < 0.001$ for yotari), reflecting normal developmental upregulation with subtle quantitative differences between genotypes (Figure 3, Figure 2).

These findings indicate that although overall staining intensity patterns were similar, temporal–spatial regulation of Dvl-1 is measurably altered in Dab1-deficient mice,

complementing the more pronounced shifts observed for inversin.

## DISCUSSION

This study investigates the effect of genetic mutations, particularly those affecting the Wnt signaling pathway, on the growth of essential organs such as the kidneys and stomach. The research used yotari mutant mice to investigate the roles of proteins like inversin and dishevelled-1 in stomach development. By analyzing epithelial and mesenchymal compartments at mid- (E13.5) and late-gestational (E15.5) stages, we revealed coordinated but genotype-specific shifts in protein distribution that coincide with key stages of gastric morphogenesis. The discoveries highlight unique patterns in the expression of inversin and Dvl-1 during embryonic stomach development, indicating potential implications for congenital anomalies and disease progression linked to dysregulated Wnt signaling. Immunofluorescence staining and quantitative analysis were utilized to observe detailed changes in protein expression levels during critical stages of stomach development in yotari mutant mice and controls. Notably, routine H&E histology showed no gross morphological differences, suggesting molecular alterations precede overt anatomical change in this model.

Overall, this study lays the groundwork for future research aimed at understanding how gene mutations disrupt normal stomach development, which may result in targeted therapeutic interventions in congenital anomalies and diseases.

For inversin, we observed strong mesenchymal expression at E13.5 in both groups, followed by a significant epithelial upregulation by E15.5 in yotari mice compared to controls. In the mesenchyme, inversin was initially reduced in yotari at E13.5 but increased by E15.5 to exceed control levels, indicating a biphasic "catch-up and surpass" trajectory. This pattern points to altered regulatory dynamics between tissue compartments during mid- to late gestation, a developmental window critical for epithelial folding, stromal reorganization, and regional specification of the stomach. Although there is no available research on the expression of inversin during stomach development in yotari mice, there are significant studies on mice and zebrafish kidney development.

Moreover, there are a few articles that describe the iv mutation that is linked to defective cilia, specifically the absence of dynein arms, which leads to impaired ciliary function. Pennekamp et al. have put forward a theory stating that normal ciliary function is crucial in determining proper organ placement during embryonic development (20). Additionally, Okada et al. found that the inversin mice mutation displays abnormal nodal flow, indicating that cilia at the node are important during left–right axis development and share a common genetic basis with iv (21). Our findings align with these observations by demonstrating that inversin expression in yotari stomach is both temporally and spatially altered, particularly with increased epithelial and mesenchymal expression at E15.5. This suggests that the same cilia-dependent mechanisms implicated in organ positioning may also influence compartment-specific signaling during gastrointestinal development, linking Dab1 deficiency to disrupted Wnt–cilia interactions in the stomach.

Simons et al. positioned inversin as a molecular switch that promotes non-canonical Wnt signaling by targeting cytoplasmic dishevelled for degradation, thereby shaping axis formation (14). Taken together, these studies align with our data: the late epithelial rise and mesenchymal rebound of inversin in yotari likely reflect a Dab1-linked disturbance of cilia-dependent Wnt pathway selection during a period when epithelial–mesenchymal crosstalk patterns the stomach. Links between cilia and Wnt signaling further strengthen this model. Lee et al. showed that canonical Wnt/β-catenin outputs are disrupted when ciliary function is impaired, and Oh et al. emphasized that ciliary dysfunction perturbs multiple developmental pathways, including Wnt. Integrating these

insights with our findings suggests Dab1 deficiency alters the timing and compartmentalization of canonical vs. non-canonical Wnt balance, with inversin acting as a nodal control point in the developing stomach.

The yotari mice, also known as Dab1−/− mice, have been used as a mice model to study congenital anomalies of the kidney and urinary tract (CAKUT). Perutina et al. discovered that the overall expression of inversin increases during normal kidney development, with higher expression in yotari mice as the kidney acquires a mature morphology (22). Otto et al. demonstrated that mutations in INVS (inversin) cause nephronophthisis type 2 and link ciliary function to left–right axis determination (16).

Consistent with this cilia-centric role, our late-gestation epithelial rise and mesenchymal rebound of inversin in yotari stomach suggest a compartment-specific retuning of cilia-dependent Wnt pathway selection during gastric morphogenesis. Dvl-1 displayed subtler but biologically meaningful temporal–spatial differences. Semi-quantitative intensities were similar between groups, yet quantitative image analysis revealed that mesenchymal Dvl-1 was lower in yotari at E13.5 and increased by E15.5 in both genotypes, while epithelial Dvl-1 rose in yotari from E13.5 to E15.5 but declined in controls. These findings highlight that sensitive image-based metrics can detect regulatory shifts that conventional scoring may miss. Although there is currently no research available on the expression of dishevelled-1 (Dvl-1) during stomach development in yotari mice. Dvl-1 is a protein that plays a significant role in the Wnt signaling pathway, which is involved in different cellular processes such as embryonic development, tissue homeostasis, and cancer. While there is no direct connection between Dvl-1 and the stomach, research has suggested that the Wnt signaling pathway, in which Dvl-1 is a crucial component, is linked with gastric cancer.

Liao et al., in a study on hepatocellular carcinoma (HCC), found that Wnt signaling, regulated by Dvl-1, is variably activated in HCC and contributes to cancer stem cell heterogeneity and tumor progression (23). This suggests that Dvl-1, as part of the Wnt signaling pathway, may play a role in the development and progression of gastric cancer.

Perutina et al. also reported that an increase in β-catenin and cytosolic Dbl-1 levels, indicating a switch from non-canonical to canonical Wnt signaling, has been found in the postnatal kidney of yotari mice. This suggests that the defective Dab1 gene product in yotari mice may promote CAKUT due to interfering with the switching between canonical and non-canonical Wnt signaling (22). To complement these findings, Krylova et al. provided direct evidence that Dvl-1 stabilizes microtubules via a GSK3β-dependent mechanism, linking dishevelled to cytoskeletal organization and cell polarity (24). In this context, the epithelial increase in Dvl-1 we observed at E15.5 in yotari may reflect enhanced cytoskeletal/polarity programming during late gastric remodeling, consistent with a shift toward canonical Wnt routing.

However, specific research on the direct relationship between Dvl-1 and the stomach is limited and further studies are needed to understand the role of Dvl-1 in stomach development. Bringing these strands together, our results support a unified view: Dab1 loss disrupts a cilia–Wnt regulatory hub that includes inversin (pathway selector) and Dvl-1 (signal transducer/cytoskeletal modulator). inversin's late epithelial elevation alongside mesenchymal recovery in yotari implies the retuning of the canonical/non-canonical balance precisely when epithelial patterning and stromal cues must be coordinated; in parallel, Dvl-1's quantitative shifts indicate more nuanced adjustments to intracellular Wnt routing and polarity control. This division of labor offers a plausible explanation for why gross morphology remains unchanged at these stages (H&E) despite clear molecular divergence.

While immunofluorescence offers high-value spatial information, it is semi-quantitative and sensitive to staining conditions. To strengthen

causal inference, future work should pair immunofluorescence with Western blotting and/or quantitative PCR (qPCR) to confirm the magnitude and direction of change, and incorporate pathway activity readouts (e.g., β-catenin localization/target genes; planar cell polarity reporters). Mechanistic perturbations (e.g., transient inhibition of β-catenin–T cell factor (TCF) or modulation of PCP effectors) in organoid or ex vivo stomach explant systems would directly test whether the inversin/Dvl-1 shifts we observe translate into altered epithelial–mesenchymal signaling and morphogenesis.

## CONCLUSION

Yotari (Dab1−/−) mice exhibit stage- and compartment-specific alterations in inversin and Dvl-1 expression during stomach development. These changes likely reflect Dab1-linked disturbances in Wnt signaling dynamics, providing new insight into potential molecular mechanisms underlying gastrointestinal developmental abnormalities in this model.

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None declared.

**CONFLICT OF INTEREST**
The authors state no conflict of interest.

**AUTHORS' CONTRIBUTIONS**

PT and KV: contribution to study conception and design, literature review, supervision, writing of the paper, interpretation of data, critical revision of the paper; NK: acquisition of data, contribution to study conception and design, literature review, critical revision of the paper, assistance in writing the paper.

**ETHICAL BACKGROUND**

**Institutional review board statement:** The Shiga University of Medical Science's Guidelines for the Care and Use of Laboratory Animals permitted the use of animals in this research. The University of Split School of Medicine's Ethical Committee granted its approval for the study, which was conducted according to the Declaration of Helsinki's criteria (class: 003-08/16-03/0001, approval number: 2181-198-03-04-16-0024).

**Data availability statement:** The authors are responsible for all aspects of the work and assure that any questions regarding the accuracy or integrity of the work are properly investigated and resolved.

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