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ORIGINAL ARTICLE

Single nucleotide polymorphisms of PCP pathway related genes participate in the occurrence and development of neural tube defect

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Abstract

Background: To screen the single nucleotide polymorphisms (SNPs) in the coding regions of *VANGL* and *FZD* family members related to the plane cell polarity (PCP) signaling pathway in neural tube defects (NTDs) patients, so as to provide theoretical and experimental basis for the prevention and treatment of NTDs by intervening PCP signal transduction.

Methods: 112 NTDs patients were collected as the case group and 112 craniocerebral trauma patients as control. Afterwards, blood genomic DNA was extracted and sequenced. The distribution of SNP alleles and genotypes between case and control groups was analyzed. Finally, the NTD rat model was constructed, and the effect of SNPs on the expression level of *VANG*L and *FZD* genes was verified by qRT-PCR.

Results: GC genotype was newly found at *VANGL1* c.346G>A, as well as AT genotype in *FZD6* c.97A>G. The distribution of *VANGL1* c.346g>A allele and genotype was statistically different between the case and control groups ($p < 0.05$). The newly found genotype GC increased the risk of NTDs (OR = 9.918, 95% CI: 1.234%–79.709%). The results of qRT-PCR showed that the expression level of *FZD6* in E11 NTD fetuses were significantly increased ($p < 0.05$), but there was no obvious difference in the expression of *VANGL1*.

Yan Liu, Liang Dong, and Xiufang Zhi contributed equally to this work.

Conclusion: We found a new variant of *VANGL1* c.346G>A, whose GC genotype might play an important role in the pathogenesis of NTDs. The SNPs of *VANGL1* had no significant effect on its expression level, indicating that it may induce NTDs through other ways. *FZD6* was significantly overexpressed in NTDs fetuses.

KEYWORDS

FZD, neural tube defects, single nucleotide polymorphisms, *VANGL*

1 | BACKGROUND

Neural tube defects (NTDs) are a kind of congenital malformation of nervous system, which are caused by the incomplete closure of the neural tube from the 21st to the 28th day of embryonic development. The main clinical manifestations are anencephaly, meningoencephalocele, spina bifida (SB), meningomelocele (MMC), etc. (Endalifer & Diress, 2020). About 3% of the world's newborns suffer from this defect, most of them die after birth, and most of the survivors are accompanied by disability, which seriously affects the quality of life. NTDs are one of the most common birth defects in the world, second only to congenital heart birth defects (Inan & Saraydin, 2019). Researches have revealed that NTDs are one of the important causes of abortion, fetal death, and stillbirth in pregnant women, and also the main cause of infant death and lifelong disability (Kancherla & Black, 2018). China is a country with a high incidence of NTDs, especially in the northern region. The prevalence of NTDs in per 1000 newborns is about 3.2, which seriously affects the quality of the birth population, threatens the health of women and children, and places heavy burden on patients, families and the society (Liu et al., 2016; Zaganjor et al., 2016). Controlling the incidence of NTDs is an important measure to reduce perinatal and infant mortality and improve population quality. Therefore, both developed countries and regions, as well as developing countries, regard the disease as an important public health problem (Center for Disease Control, 2016).

NTDs are a type of multifactorial disease, the result of genetic and environmental factors (Abdullah et al., 2018). A large number of studies have shown that 20% of NTDs is caused by genetic factors and 80% by non-genetic factors, but up to now, no breakthrough has been made in the etiology and pathogenesis of NTDs (Wang, 2017). In recent years, it is generally believed that the occurrence of NTDs is the results of the interaction between environmental and genetic factors, which affects the determination of genotypes and the susceptibility of embryos to teratogens (Cai, 2010). Researches showed that the formation of neural tube is directly related to directional cell division and directional induced migration. One of the key factors in these processes is the regulation of planar cellular polarity (PCP) signaling pathway. *VANGL1*, *VANGL2*, *FZD3* and *FZD6* are the core genes of PCP signaling pathway (De Marco et al., 2012; Tian, Lei, Chen, Guo, et al., 2020). At present, it has been found that these genes play certain roles in neural tube formation. Mutations in these genes may cause NTDs, but extensive studies that have sought causative mutations for NTDs have yielded limited positive findings to date (Tian, Lei, Chen, Guo, et al., 2020; Tian, Lei, Chen, Karki, et al., 2020).

Based on this, this study screened SNPs of PCP signaling pathway genes *VANGL1*, *VANGL2*, *FZD3* and *FZD6* through Sanger sequencing of peripheral blood of NTD patients, and then analyzed the difference of genotype frequency of each SNP site between case group and control group. Furthermore, the effect of SNPs on gene expression level was verified by animal model construction (Sahin Inan & Unver, 2019) and quantitative real-time PCR (qRT-PCR), to reveal the role and potential mechanism of SNPs of *VANGL1*, *VANGL2*, *FZD3* and *FZD6* in the development of NTDs.

2 | METHODS

2.1 | Patient and sample collection

From November 2003 to October 2009, NTD patients who were hospitalized in neurosurgery department of Tianjin Children's Hospital were selected. Inclusion standard: patients whose mothers did not receive folic acid preparation during pregnancy (folic acid supplementation time ranged from 1 month to half a year) were excluded. All cases were Han families in North, Northwest and Northeast China. Finally, 112 patients were enrolled in the case group. Meanwhile, 112 patients with craniocerebral injury of similar age and gender were selected as the control group. Peripheral blood samples of patients were collected and sequenced with Sanger sequencing. All the guardians of the children participated in the study signed the informed consent voluntarily. This study was approved by the ethics committee of Tianjin Children's Hospital.

2.2 | DNA extraction and Sanger sequencing

The genomic DNA in blood samples was extracted using gDNA Mini Kit (Qiagen Co., Ltd., Germany) according to the manufacturer's instructions, and subjected to PCR amplification after passing the 1.5% agarose gel electrophoresis test. All PCR products were sequenced by GENEWIZ Inc. (Beijing, China) and the results were compared with the gene reference sequence hg19 on NCBI using Clustal W2.0 software (https://academic.oup.com/bioinformatics/article/23/21/2947/371686).

2.3 | Animal model construction and quantitative real-time PCR assay

24 Wistar rats, weighing 250–300 g, were raised in SPF grade animal room at 20–24°C and 50%–70% humidity. These animal models were provided by the Laboratory Animal Center of Shengjing Hospital of China Medical University (Beijing, China). After fertilization, the rats were randomly divided into two groups, 12 of them were injected with retinoic acid at dose of 150 mg/kg through gastric tube at embryonic 10 days (E10) as the experimental group, and the other 12 were given the same volume of olive oil as the control.

At E11, E12 and E13, fetuses were dissected and total RNA was extracted from spinal cord samples. The reverse transcription was carried out with the ABM 5X All-In-One RT MasterMix (Applied Biological Materials (ABM) Inc., Canada), the reaction conditions were 25°C for 10 min, 42°C for 15 min, 85°C for 5 min and 4°C for hold. Afterwards, the qRT-PCR was carried out with the EvaGreen 2X qRT-PCR MasterMix (ABM Inc., Canada) to detect the expression of *VANGL1*, *FZD3*, *FZD6* and *DACT1*, and *GAPDH* was used as internal control. The reaction conditions were 95°C for 10 min, 95°C for 15 s, 60°C for 1 min, 40 cycles. The primers were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and the sequences were listed in Table 1.

TABLE 1 Primer sequences of *VANGL1*, *FZD3*, *FZD6* and *DACT1*

Gene		Sequences	Size
VANGL1	F	5′-AGGACATTGCCAGGATTAGCA-3′	190 bp
	R	5′-GGATTTGGGGTAGCAGGATGAA-3′	
FZD3	F	5′-ATGGCTGTGAGCTGGATTGTC-3′	109 bp
	R	5′-GGCACATCCTCAAGGTTATAGGT-3′	
FZD6	F	5′-TCTTCCCTAACCTGATGGGTC-3′	174 bp
	R	5′-ACAATTTCCGACAGGGTAGAAC-3′	
DACT1	F	5′-AAGAGATGCCGGTTTGTTGAA-3′	70 bp
	R	5′-CACATCCAGTCTCAGGTCACTTA-3′	
GAPDH	F	5′-AGGTCGGTGTGAACGGATTTG-3′	123 bp
	R	5′-TGTAGACCATGTAGTTGAGGTCA-3′	

2.4 | Statistical analysis

The test for Hardy–Weinberg equilibrium was performed using HWE software (http://www.biology.ualberta.ca/jbrzusto/hwenj.html). SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. The comparison of alleles and genotypes distribution between case and control group was made by chi-square test or Fisher exact test. The odds ratio (OR) and 95% confidence interval (CI) of each genotype to NTDs were calculated. Student *t*-test was used to analyze the differences of qRT-PCR results among groups. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics

In the 112 NTDs patients, 47 were boys and 65 were girls, with an average age of 2.21 years old. A total of 9 kinds of NTD were included, such as meningocele, meningoencephalocele, meningomyelocele and lipomyelomeningocele. The clinical characteristics were shown in Table 2.

TABLE 2 Clinical characteristics of 112 NTD patients

Characteristics	Number ($n = 112$)
Sex	
Boy	47 (41.96)
Girl	65 (58.04)
Age	2.21 ± 2.71
Clinical classification of neural tube defects	
Meningocele	9 (8.04)
Meningoencephalocele	3 (2.68)
Meningomyelocele	21 (18.75)
Lipomyelomeningocele	32 (28.57)
Myelomeningocele	14 (12.50)
Secretory sinus	13 (11.61)
Diastematomyelia	12 (10.71)
Coccyx agenesis	5 (4.46)
Filum terminale lipomas	3 (2.68)

3.2 | Comparison of allele and genotype frequency of SNPs between case and control groups

A total of 8 SNPs of four genes (*VANGL1*, *VANGL2*, *FZD3*, and *FZD6*) were screened in NTDs patients (Table 3). A new GC genotype was found at *VANGL1* c.346G>A and AT genotype was found in *FZD6* c.97A>G. Table 3 showed the results of Hardy–Weinberg equilibrium test, the P value of genotype distribution of 8 SNPs within the case group and control group were all >0.05, suggesting that the genes were inherited according to the Mendel's genetic law. Besides, 3 out of the 8 SNPs (*VANGL1* c.1040A>C, *VANGL2* c.1209G>C, *FZD3* c.702T>G) were not detected for polymorphism, and the distribution of the *VANGL1* c.346G>A between case and control groups was statistically significant ($p = 0.022$).

Table 4 exhibited the comparison of allele and genotype frequencies of the 5 SNPs with polymorphism between the case group and the control group, as well as the relationship between each genotype and the risk of NTDs. The distribution of allele and genotype at *VANGL1* c.346G>A was statistically different between case and control group ($p = 0.034$ and 0.014, respectively), the newly found genotype GC increased the risk of NTDs (OR = 9.918, 95% CI: 1.234%–79.709%).

3.3 | Effect of the SNPs on gene expression

Since mutation may affect gene expression, we further constructed NTDs rat model, and detected the expression level of *VANGL1*, *FZD3*, *FZD6*, and *DACT1* in NTDs and normal fetuses by qRT-PCR. The results were presented in Figure 1. The mRNA levels of *FZD6* were remarkably higher in NTDs fetuses than normal fetuses at E11 ($p < 0.001$), however the differentially expression of *VANGL1*, *FZD3* and *DACT1* were not found. The results indicated that there were no significant

TABLE 3 Hardy–Weinberg equilibrium test of SNPs in case and control groups

Gene	SNPs	Genotype	Case group ($n = 112$)	Control group ($n = 112$)	*p*
VANGL1	c.346G>A	GG	71	83	**0.022**
		GA	27	23	
		GC	9	1	
		AA	0	2	
		p	0.34	0.69	
VANGL1	c.1040A>C	AA	112	112	1
		p	1	1	
VANGL2	c.1209G>C	GG	112	112	1
		p	1	1	
FZD3	c.435A>G	AA	12	10	0.738
		AG	53	56	
		GG	42	36	
		p	0.53	0.095	
FZD3	c.702T>G	TT	112	112	1
		p	1	1	
FZD6	c.97A>G	AA	92	87	0.535
		AG	1	2	
		p	1	0.71	
FZD6	c.1033A>C	TT	24	15	0.204
		TG	59	59	
		GG	25	33	
		p	0.44	0.23	
FZD6	c.1991C>A	CC	100	102	0.168
		CA	9	4	
		p	1	1	

Significant differences, $p < 0.05$ values are indicated in bold.

TABLE 4 Comparison of allele and genotype frequency between case and control groups

SNPs	Allele and genotype	Case group ($n = 107$)	Control group ($n = 109$)	χ^2	p	OR (95% CI)
VANGL1 c.346G>A	G (%)	178 (83.2)	190 (87.2)	6.755	**0.034**	–
	C (%)	9 (4.2)	1 (0.5)			
	A (%)	27 (12.6)	27 (12.3)			
	GG (%)	71 (66.4)	83 (76.2)	Fisher	**0.014**	0.618 (0.341, 1.121)
	GA (%)	27 (25.2)	23 (21.1)			1.262 (0.669, 2.379)
	GC (%)	9 (8.4)	1 (0.9)			9.918 (1.234, 79.709)
	AA (%)	0 (0)	2 (1.8)			–
FZD3 c.435A>G	A (%)	77 (36.0)	76 (37.3)	0.073	0.787	–
	G (%)	137 (64.0)	128 (62.7)			
	AA (%)	12 (11.2)	10 (9.8)	0.607	0.738	1.162 (0.479, 2.821)
	AG (%)	53 (49.5)	56 (54.9)			0.806 (0.468, 1.389)
	GG (%)	42 (39.3)	36 (35.3)			1.185 (0.676, 2.077)
FZD6 c.97A>G	A (%)	185 (99.5)	177 (98.3)	Fisher	0.429	–
	G (%)	1 (0.5)	2 (1.1)			
	T (%)	0 (0)	1 (0.6)			
	AA (%)	92 (98.9)	87 (96.7)	Fisher	0.427	3.172 (0.324, 31.08)
	AG (%)	1 (1.1)	2 (2.2)			0.478 (0.043, 5.369)
	AT (%)	0 (0)	1 (1.1)			–
FZD6 c.1033A>C	T (%)	107 (49.5)	89 (41.6)	2.738	0.098	–
	G (%)	109 (50.5)	125 (58.4)			
	TT (%)	24 (22.2)	15 (14.0)	3.176	0.204	1.752 (0.862, 3.563)
	TG (%)	59 (54.6)	59 (55.1)			0.980 (0.572, 1.676)
	GG (%)	25 (23.2)	33 (30.9)			0.675 (0.368, 1.239)
FZD6 c.1991C>A	C (%)	209 (95.9)	208 (98.1)	1.842	0.175	–
	A (%)	9 (4.1)	4 (1.9)			
	CC (%)	100 (91.7)	102 (96.2)	1.901	0.168	0.436 (0.130, 1.461)
	CA (%)	9 (8.3)	4 (3.8)			2.295 (0.685, 7.694)
	AA (%)	0 (0)	0 (0)			–

Significant differences, $p < 0.05$ values are indicated in bold.

relationship between SNPs and gene expression level, indicating that SNPs might affect the occurrence and development of NTDs through other ways rather than gene differentially expression. De Marco reported the highly penetrant occurrence of NTDs in double Fzd 3/Fzd 6−/− mutant mice, the role of human orthologues, FZD 3 and FZD 6, by re-sequencing a cohort of 473 NTDs patients and 639 ethnically matched controls (De Marco et al., 2012).

4 | DISCUSSION

Up to now, more than 300 NTDs related mutations have been found in mice (Wallingford et al., 2013). These genes exist in different pathways, including Wnt/PCP pathway, folate metabolism pathway, MAPK pathway and epigenetic regulatory factors, etc. (Wilde et al., 2014). The role of Wnt/PCP pathway in vertebrate cell movement during embryogenesis was first found in Xenopus and zebrafish (Heisenberg et al., 2000; Wallingford et al., 2000). During neurulation, when the neuraxis is elongated and eventually neural tube is closed, the convergent extension of the mid region of the neural plate occurs (Mukhopadhyay et al., 2020; Wallingford et al., 2020) which is mediated by PCP pathway. The core genes *Frizzled* (*Fzd*) and *VANGL* of PCP pathway encode transmembrane proteins (Nikolopoulou et al., 2017). The studies of vertebrate models have demonstrated a high level of evolutionary conservation at PCP core genes, and the functional variants of these genes have been identified in human NTDs

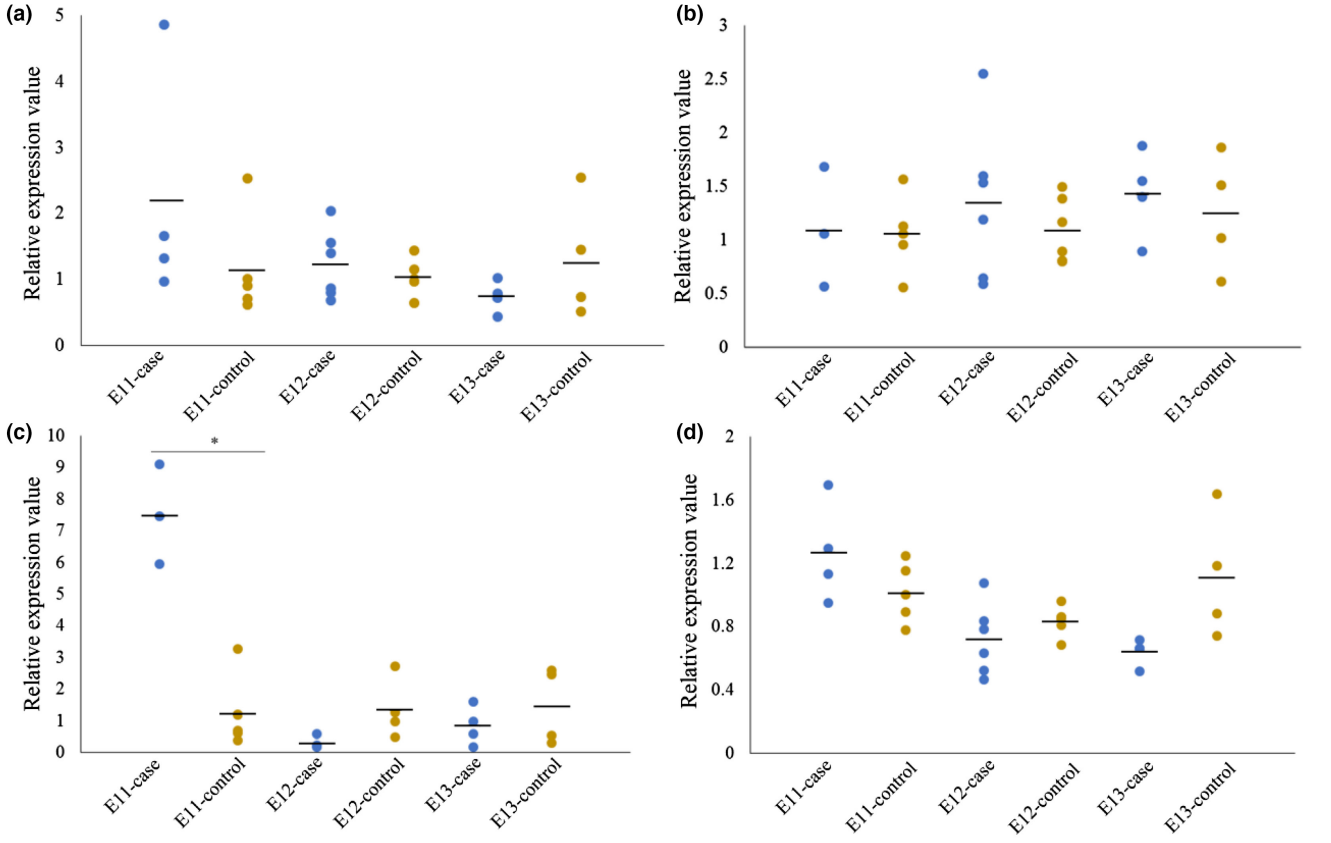

FIGURE 1 Scatter diagram of *VANGL1* (a), *FZD3* (b), *FZD6* (c) and *DACT1* (d) relative expression level in neural tube defects (case) and normal (control) fetuses at E11, E12 and E13, respectively.

(Chen et al., 2018; Wang et al., 2019). In addition, in our previous study, the deletion and mutation of PCP maternal effect gene *Fuzzy* (*Fuzz*) or noncore PCP related gene *DACT1* were also found in human NTDs (Cai & Shi, 2014). In this study, we observed a novel genotype GC in *VANLG1* c.346G>A, which resulted in the transformation of the 116th amino acid from Ala to Pro, and increased the risk of NTDs. In addition, the distribution of alleles and genotypes of *VANGL1* c.346G>A between the case and control groups was statistically different ($p < 0.05$).

Through this study and literature review, we found that SNPs of these genes are closely related to NTDs, and almost no mutation occurs in normal human body. It can be considered that SNPs of *VANGL1*, *FZD3* and *FZD6* genes are specific to NTDs, which may be the potential pathogenic factors of NTDs. Besides, our previous research found 5 missense heterozygote mutations of the PCP related gene *DACT1* specifically identified in 167 stillborn or miscarried Han Chinese fetuses with NTDs, suggesting that *DACT1* may constitute a great contribution to NTDs (Shi et al., 2012). Therefore, we continued to detect the expression of *VANGL1*, *FZD3*, *FZD6* and *DACT1* in NTDs fetuses, to explore whether the SNPs affect their expression level. After qRT-PCR, *FZD6* was overexpressed in E11 NTDs fetuses. *FZD6* is one of the ten Wnt receptors (*FZD1-FZD10*). It can activate Fzd/PCP pathway through activator activation. At the same time, *FZD6* is involved in the intercellular PCP signal transmission process together with *VANGL1*. It has been found to be differentially expressed in hepatocarcinoma, colorectal cancer, glioblastomas, prostate cancer and so on (Bengochea et al., 2008; Huang et al., 2016; Kim et al., 2015; Saramaki et al., 2006). Corda et al. (2017) found that high expression of *FZD6* was driven by gene amplification, and FZD6 protein expression was significantly associated with lower metastasis-free survival in breast cancer patients. De Marco et al. (2012) found that the significant increase in NTDs mutation burden was due to the overall incidence of predicted deleterious FZD6 variants being 5.1 times higher in cases than in controls. However, until now, there is no related report about the abnormal expression of *FZD6* in NTDs. Our study found for the first time that *FZD6* was highly expressed in NTDs fetuses, more mechanism exploration needs to be carried out to explore the mechanism of the differential expression of *FZD6* and its relationship with the occurrence and development of NTDs.

The results of qRT-PCR also showed that the expression of *VANGL1* was no statistical significance between

NTD and normal fetuses. It can be concluded preliminarily that the SNPs of *VANGL1* do not affect its expression level. We speculate the reason may be that the SNPs of *VANGL1* may lead to changes in protein spatial conformation through amino acid changes but not abnormal gene expression, and may further affect the biological function of *VANGL1*, thus affecting the process of neural tube closure. The conjecture is also meet with our previous research (Cai et al., 2013). However, more animal and clinical experiments need to be conducted to verify our results.

5 | CONCLUSION

In this study, the Sanger sequencing was performed on the SNPs selected from the genomic DNA of NTD patients, and 2 novel genotypes (GC genotype at *VANGL1* c.346G>A, AT genotype at *FZD6* c.97A>G) were found for the first time. The allele and genotype distribution of the *VANGL1* c.346G>A between the case and the control group were statistically different ($p = 0.034$ and 0.014, respectively). The newly discovered genotype GC increased the risk of NTDs (OR = 9.918). In NTD fetuses, *FZD6* expression was up-regulated, while *VANGL1* expression was not significantly altered, indicating that PCP signaling pathway members may affect the occurrence and development of NTDs at different levels.

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AUTHOR CONTRIBUTIONS

Concepualization: Yan Liu, Liang Dong and Xiufang Zhi; Methodology: Yang Liu, Linsheng Zhao and Xiaowei Xu; Formal analysis and investigation: Lu Wang, Jie Zheng, Linjie Pu and Chunyu Gu; Writing-original draft preparation: Yan Liu, Jianbo Shu and Chunquan Cai; Writing-review and editing: Jianbo Shu and Chunquan Cai. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

We confirm that we have read *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans* and affirm that this report is consistent with those guidelines. The study was approved by the medical ethics committee of Tianjin Children's Hospital.

CONSENT TO PARTICIPATE

All participants has signed the informed consent voluntarily.

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REFERENCES

Abdullah, N. L., Gunasekaran, R., Mohd-Zin, S. W., Lim, B. H., Maniam, P., Mohd-Salleh, A. S., Thong, M. K., Chik, Z., Nordin, N., Omar, Z., Engkasan, J. P., Ganesan, D., Aiezzah, Z. N., Ahmad-Annuar, A., & Abdul-Aziz, N. M. (2018). Cranial neural tube defect after trimethoprim exposure. *BMC Research Notes*, *11*(1), 475.

Bengochea, A., de Souza, M. M., Lefrançois, L., le Roux, E., Galy, O., Chemin, I., Kim, M., Wands, J. R., Trepo, C., Hainaut, P., Scoazec, J. Y., Vitvitski, L., & Merle, P. (2008). Common dysregulation of Wnt/frizzled receptor elements in human hepatocellular carcinoma. *British Journal of Cancer*, *99*(1), 143–150.

Cai, C., & Shi, O. (2014). Genetic evidence in planar cell polarity signaling pathway in human neural tube defects. *Frontiers in Medicine*, *8*(1), 68–78.

Cai, C. Q. (2010). *FZD/VANGL gene polymorphisms associated with neural tube defects*. Tianjin Medical University (In Chinese).

Cai, C. Q., Shi, O. Y., Guan, W., Shen, Y. M., Zhang, Q. J., & Ma, X. (2013). The association of *VANGL1* gene polymorphisms and neural tube defects in Han population of northern China. *Chinese Journal of Pediatric Surgery*, *34*(1), 10–13 (In Chinese).

Center for Disease Control. (2016). *Global situation of birth defects and initiatives for prevention*.

Chen, Z. Z., Lei, Y. P., Cao, X. Y., Zheng, Y. F., Wang, F., Bao, Y. H., Peng, R., Finnell, R. H., Zhang, T., & Wang, H. (2018). Genetic analysis of Wnt/PCP genes in neural tube defects. *BMC Medical Genomics*, *11*(1), 38.

Corda, G., Sala, G., Lattanzio, R., Iezzi, M., Sallese, M., Fragassi, G., Lamolinara, A., Mirza, H., Barcaroli, D., Ermler, S., Silva, E., Yasaei, H., Newbold, R. F., Vagnarelli, P., Mottolese, M., Natali, P. G., Perracchio, L., Quist, J., Grigoriadis, A., … Sala, A. (2017). Functional and prognostic significance of the genomic amplification of frizzled 6 (FZD6) in breast cancer. *The Journal of Pathology*, *241*(3), 350–361.

De Marco, P., Merello, E., Rossi, A., Piatelli, G., Cama, A., Kibar, Z., & Capra, V. (2012). FZD6 is a novel gene for human neural tube defects. *Human Mutation*, *33*(2), 384–390.

Endalifer, M. L., & Diress, G. (2020). Epidemiology and determinant factors of neural tube defect: Narrative review. *Surgical Neurology International*, *11*, 81.

Heisenberg, C. P., Tada, M., Rauch, G. J., Saúde, L., Concha, M. L., Geisler, R., Stemple, D. L., Smith, J. C., & Wilson, S. W. (2000). Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature*, *405*(6782), 76–81.

Huang, T. Z., Alvarez, A., Pangeni, R., Horbinski, C., Lu, S., Kim, S. H., James, C. D., Raizer, J. J., Kessler, J. A., Brenann, C. W., Sulman, E. P., Finocchiaro, G., Tan, M., Nishikawa, R., Lu, X., Nakano, I., Hu, B., & Cheng, S.-Y. (2016). A regulatory circuit of miR-125b/miR-20b and Wnt signaling controls GBM phenotypes through FZD6-mediated pathways. *Nature Communications*, *7*, 12885.

Inan, Z. D. S., & Saraydin, S. U. (2019). Immunohistochemical profile of CD markers in experimental neural tube defect. *Stain Technology*, *94*(8), 617–627.

Kancherla, V., & Black, R. E. (2018). Historical perspective on folic acid and challenges in estimating global prevalence of neural tube defects. *Annals of the New York Academy of Sciences*, *1414*(1), 20–30.

Kim, B. K., Yoo, H. I., Kim, I., Park, J., & Yoon, S. K. (2015). FZD6 expression is negatively regulated by miR-199a-5p in human colorectal cancer. *Bmb Reports*, *48*(6), 360–366.

Liu, J. F., Zhang, L., Li, Z. W., Jin, L., Zhang, Y. L., Ye, R. W., Liu, J., & Ren, A. (2016). Prevalence and trend of neural tube defects in five counties in Shanxi province of northern China, 2000 to 2014. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *106*(4), 267–274.

Mukhopadhyay, P., Greene, R. M., & Pisano, M. M. (2020). MicroRNA targeting of the non-anonical planar cell polarity pathway in the developing neural tube. *Cell Biochemistry and Function*, *3*, 905–920.

Nikolopoulou, E., Galea, G. L., Rolo, A., Greene, N. D., & Copp, A. J. (2017). Neural tube closure: Cellular, molecular and biomechanical mechanisms. *Development*, *144*(4), 552–566.

Sahin Inan, Z. D., & Unver, S. S. (2019). Immunohistochemical profile of CD markers in experimental neural tube defect. *Biotechnic & Histochemistry*, *94*(8), 617–627.

Saramaki, O. R., Porkka, K. P., Vessella, R. L., & Visakorpi, T. (2006). Genetic aberrations in prostate cancer by microarray analysis. *International Journal of Cancer*, *119*(6), 1322–1329.

Shi, Y., Ding, Y., Lei, Y. P., Yang, X. Y., Xie, G. M., Wen, J., Cai, C. Q., Li, H., Chen, Y., Zhang, T., Wu, B. L., Jin, L., Chen, Y. G., & Wang, H. Y. (2012). Identification of novel rare mutations of DACT1 in human neural tube defects. *Human Mutation*, *33*, 1450–1455.

Tian, T., Lei, Y. P., Chen, Y. Y., Guo, Y. N., Jin, L., Finnell, R. H., Wang, L., & Ren, A. (2020). Rare copy number variations of planar cell polarity genes are associated with human neural tube defects. *Neurogenetics*, *21*(3), 217–225.

Tian, T., Lei, Y. P., Chen, Y. Y., Karki, M., Jin, L., Finnell, R. H., Wang, L., & Ren, A. (2020). Somatic mutations in planar cell polarity genes in neural tissue from human fetuses with neural tube defects. *Human Genetics*, *139*(10), 1299–1314.

Wallingford, J. B., Fraser, S. E., & Harland, R. M. (2020). Convergent extension: The molecular control of polarized cell movement during embryonic development. *Developmental Cell*, *2*(6), 695–706.

Wallingford, J. B., Niswander, L. A., Shaw, G. M., & Finnell, R. H. (2013). The continuing challenge of understanding, preventing, and treating neural tube defects. *Science*, *339*(6123), 1222002.

Wallingford, J. B., Rowning, B. A., Vogeli, K. M., Rothbächer, U., Fraser, S. E., & Harland, R. M. (2000). Dishevelled controls cell polarity during Xenopus gastrulation. *Nature*, *405*(6782), 81–85.

Wang, L. (2017). *Study on inhibition of GARFT mice to establish NTDs animal model and its mechanism*. Shanxi Medical University (In Chinese).

Wang, M. Q., Marco, P., Capra, V., & Kibar, Z. (2019). Update on the role of the non-canonical Wnt/planar cell polarity pathway in neural tube defects. *Cell*, *8*(10), 1198.

Wilde, J. J., Petersen, J. R., & Niswander, L. (2014). Genetic, epigenetic, and environmental contributions to neural tube closure. *Annual Review of Genetics*, *48*, 583–611.

Zaganjor, I., Sekkarie, A., Tsang, B. L., Williams, J., Razzaghi, H., Mulinare, J., Sniezek, J. E., Cannon, M. J., & Rosenthal, J. (2016). Describing the prevalence of neural tube defects worldwide: A systematic literature review. *PLoS ONE*, *11*(4), e0151586.

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