

CLINICAL REPORT

The *VANGLI* P384R variant cause both neural tube defect and Klippel-Feil syndrome

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Abstract

Background: Neural tube defect (NTD) is a common birth defect causing much death in the world. Variants in *VANGLI* lead to NTD and caudal regression syndrome. NTD displays a complex phenotype encompassing both genetic and environmental factors.

Methods: The fetus was diagnosed by prenatal ultrasound examination. Postnatal CT and autopsy were performed. Genetic testing was conducted in the family and Sanger sequencing was validated. Multiple prediction soft-wares were used to predict the pathogenicity of the variant.

Results: The *VANGLI* gene variant c.1151C>G (P384R) was detected in a fetus diagnosed with tethered spinal cord and sacrococcygeal lipoma. The *VANGLI* variant c.1151C>G (P384R) was reported in a Klippel-Feil syndrome patient. The *VANGLI* variant was validated in the trio-family but the mother showed no abnormalities.

Conclusion: Overall, this study presents fetal NTD caused by the same *VANGLI* variant found in a Klippel-Feil syndrome patient with complete clinical information of prenatal ultrasound, postnatal CT, and genetic results as early as 25 GW. Our study not only expands the *VANGLI* mutational spectrum but also sheds light on the important role of the *VANGLI* P384R variant in human development.

KEY WORDS

Klippel-Feil syndrome, neural tube defect, prenatal diagnosis, *VANGLI*

1 | INTRODUCTION

Neural tube defect (NTD) is a serious birth defect involving anencephaly, encephalocele, and spina bifida. (Chen, 2008) NTD cause approximately 88,000 deaths per year worldwide. (Zaganjor et al., 2016) The incidence rate of NTD in China is 11.6 per 10,000, ranking second in 2000, and ranking the 10th after 2015. (National center for birth defects monitoring website, <http://www.mchscn.org>) Oral folic acid reduces the

incidence of NTD. NTD occurs when the neural tube fails to close properly during embryogenesis. Environmental and genetic factors both contribute to the NTD. *VANGLI* is the pathogenic gene of NTD (OMIM: 182940) and caudal regression syndrome (OMIM:600145) (Bartsch et al., 2012; Merello et al., 2015). The inheritance of both is autosomal dominant. *VANGLI* gene locates to chromosome 1p13, with a length of 8671 bp, 524 amino acids containing eight exons. *VANGLI* is a planar cell polarity protein encoding a member

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of the tetraspanin family, which is widely expressed in massive organs and tissue of the body. Mutations in this gene are associated with NTD.

Here, in this study, we report a 24.5 gestational week (GW) fetus was diagnosed with tethered cord and spinal bifida occulta through ultrasound. CT scan was performed on the fetus. Genetic testing was carried out in the trio-family.

2 | MATERIAL AND METHODS

2.1 | Ethics

This study was approved by HBFY Ethics Committee. The parents gave written informed consent for sampling, genetic testing, postnatal clinical examination, and autopsy.

2.2 | Ultrasound examination

The ultrasound examination was performed by qualified sonographers and was confirmed by two prenatal diagnostic specialists using Samsung ultrasound WS80A with Elite machine and the CA1-7A proband.

2.3 | Genetic testing

Low depth Whole Genome Sequencing was performed to detect a chromosome aneuploidy, deletion/duplication of more than 100 Kb CNV.

Genomic DNA from parental blood and fetal umbilical cord blood was used to perform the WES sequencing. First, DNA was interrupted and prepared for library. Then, the exon of the target gene and the DNA in the adjacent shear region were captured and enriched by the BGI V4 chip. Finally, the MGISEQ-2000 sequencing platform was used for mutation detection. The quality control index of sequencing data was as follows: the average sequencing depth of the target region was $\geq 180X$, and the loci with the average depth of $>20X$ in the target region accounted for 95%. Sequenced fragments were aligned to the UCSC hg19 human reference genome by BWA to remove duplicates. Classification of

variant pathogenicity was based on the American College of Medical Genetics and Genomics (ACMG) and the American Molecular Pathology Society (AMP) Sequence Variation Interpretation Guidelines (Appendix), with references to the ClinGen Sequence Variation Interpretation Working Group, the British Clinical Genomic Sciences Society (ACGS) and other refinements of the guidelines.

2.4 | Sanger sequencing

The variant found by WES was validated in the family by Sanger sequencing. F primer sequencing was GGACAGCTAAGGATGCAAGC and the R primer sequencing was TGTCCTCCAAGCGAAATTCT.

3 | RESULTS

3.1 | Clinical findings

A 32-year-old pregnant, G2P0 woman was referred to Maternal and Child Health Hospital of Hubei Province for a routine ultrasound examination. She was at 24.5 gestational weeks (GW). The couple was healthy and non-consanguineous. The pregnant woman had ten years of HBV, tested positive in HBsAg, HBeAg, HBcAb. She took oral Viread for treatment.

3.2 | US examination

The pregnant woman underwent a routine ultrasound examination. The biparietal diameter (BPD) was 6.0 cm and the equivalent US GW was 24.4 GW; head circumference (HC) was 23.2 cm and the equivalent US GW was 25.1 GW, abdominal circumference (AC) was 20.7 cm and the equivalent US GW was 25.1 GW, femur length was 4.5 cm and the equivalent US GW was 24.6 GW, tibia length was 4.1 cm and the equivalent US GW was 24.6 GW. The fetus was detected in the lower position of the conus medullaris below the L5 level. (Figure 1a) The sacrococcygeal segment of the spinal cord was ill-defined with fatty infiltration visible to

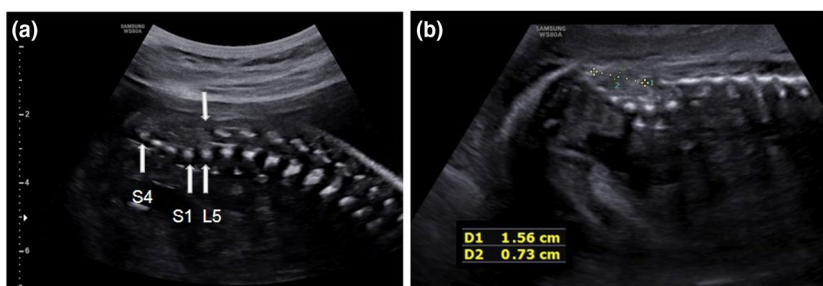


FIGURE 1 Prenatal two-dimensional ultrasound imaging of the spine. (a) The location of the fetal conus medullaris was below L5 (white arrow); (b) The sacrococcygeal segment of the spinal cord was ill-defined with fatty infiltration visible to an extent of about 1.6×0.7 cm

an extent of about 1.6×0.7 cm. (Figure 1b) Tethered spinal cord and sacrococcygeal lipoma were diagnosed.

3.3 | CT and autopsy

The fetus after the termination of pregnancy took computed tomography (CT) examination. The results showed physiological curvature. (Figure 2) No abnormality was found in vertebral morphology. The position of the conus medullaris was below the L5. The fetus was examined after TOP, the diagnosis was confirmed through an autopsy. (Figure 3) No myelocele or meningocele was observed.

3.4 | Genetic testing

The low-pass pipeline result showed no chromosome aneuploidy. (Figure 4a) Moreover, no pathogenic, likely pathogenic, or VUS CNV consistent with recessive genetic carrier status was detected in this fetus. Then the clinical trios-WES detected one variant in the *VANGL1* gene associated with NTD susceptibility/anterior sacral meningocele, which was partially associated with the clinical phenotype of the subject. No other variants associated with the clinical phenotype were detected. No unexpectedly identified pathogenic or likely pathogenic variants were detected. c.1151C>G *VANGL1* (NM_138959.2) was a missense variant causing the amino

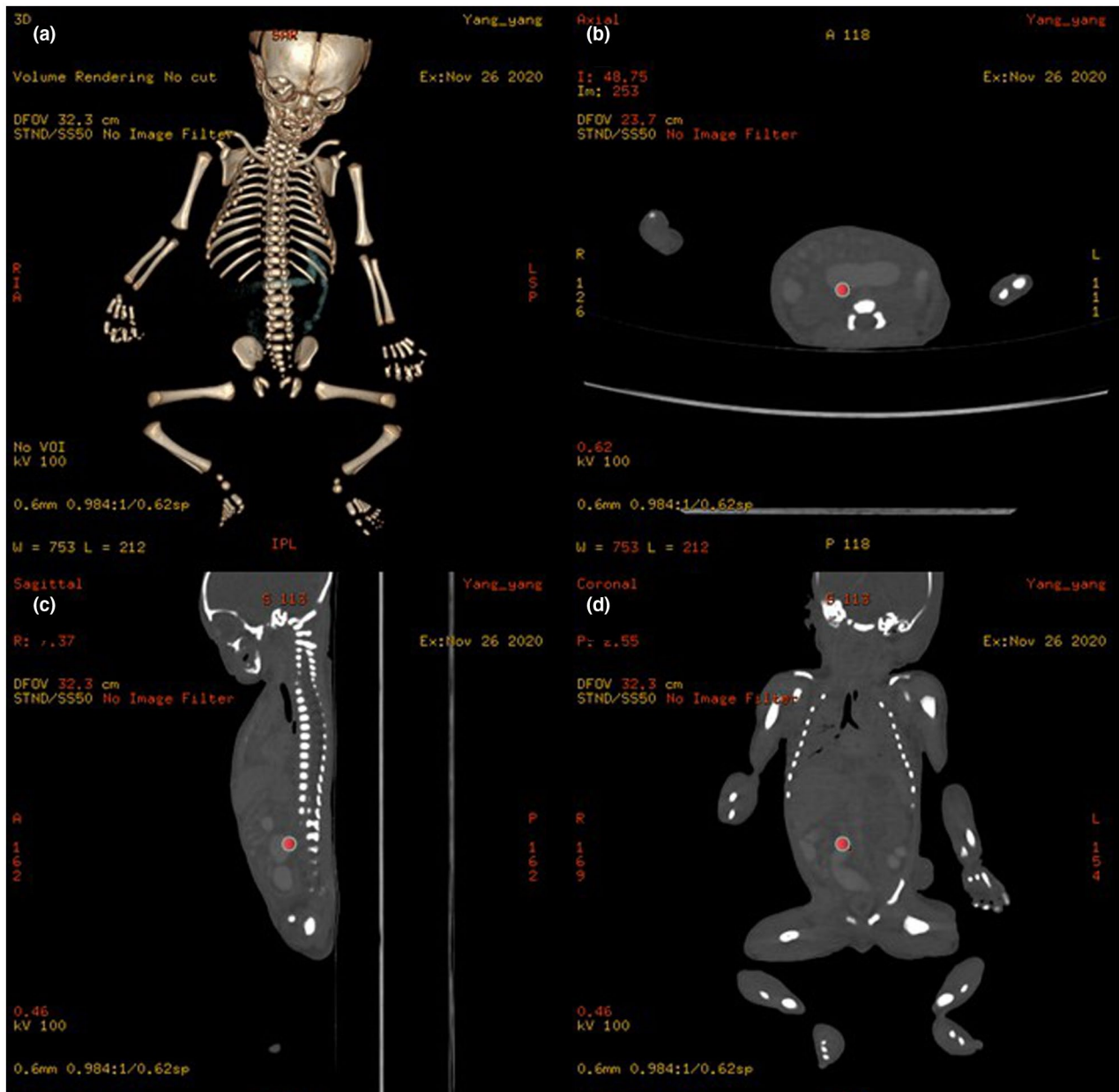


FIGURE 2 Postnatal CT scan of the upper abdomen. CT image shows spinal bifida; (a) The fetal spine was displayed; (b–d) The transverse, sagittal, and coronal plane of the L5 position. (Pointing out by the red spot)

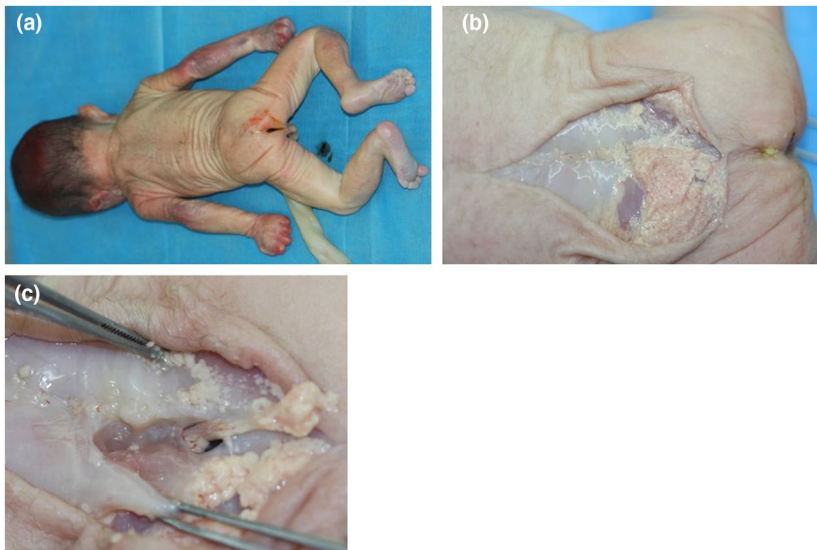


FIGURE 3 The fetus was examined after TOP, (a) The backside of the fetus; (b) Lipoma of the sacrococcygeal segment was appeared after opening the skin; (c) The tethered spinal cord was confirmed

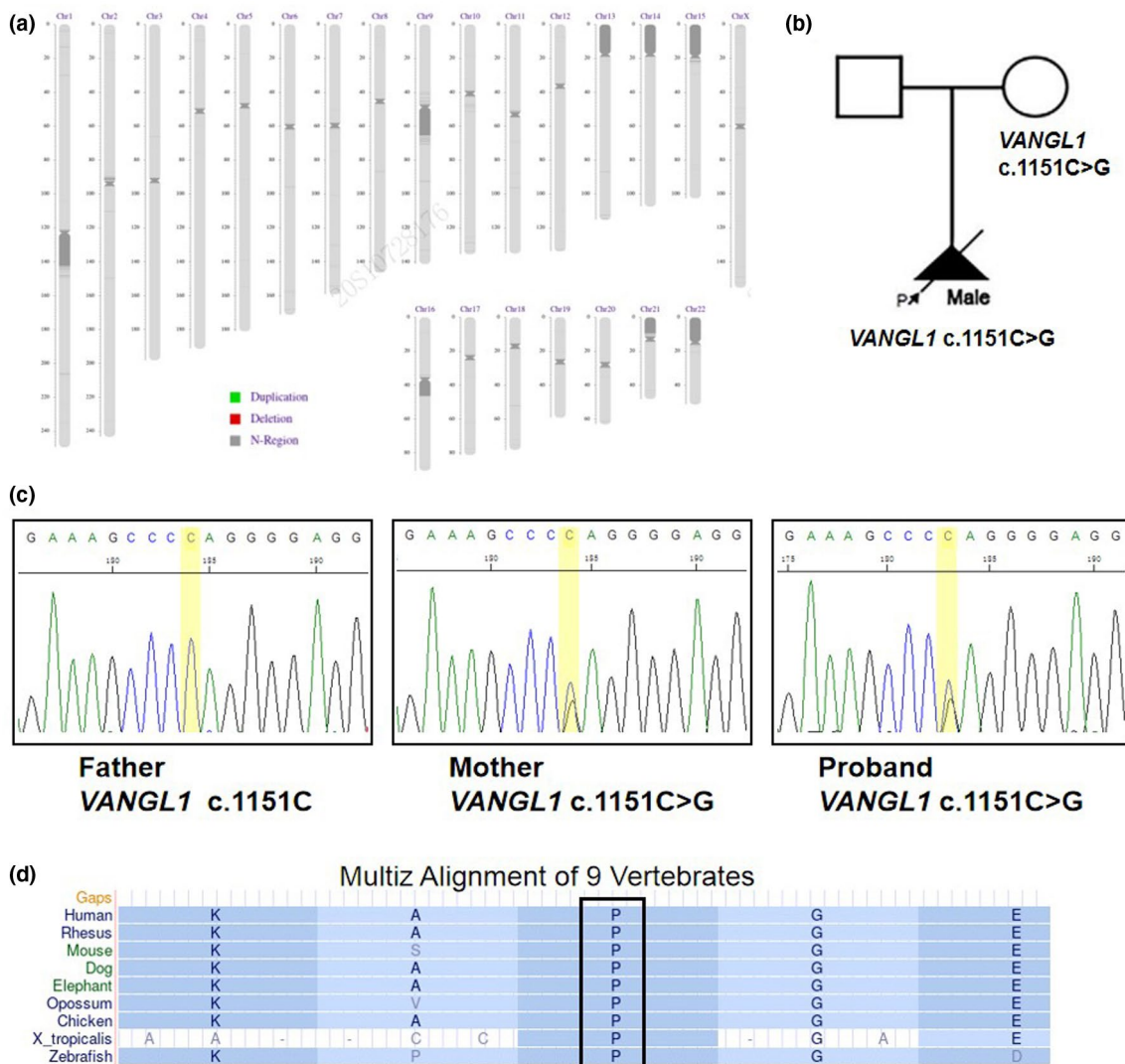


FIGURE 4 Genetic testing result in the family. (a) No Chromosome aneuploidy, or deletion/duplication above 100 Kb in the low depth whole-genome sequencing of the fetus; (b) Pedigree chart of the trio-family, p: proband; (c) *VANGL1* variant c.1151C>G were validated by Sanger sequencing in the trio-family; (d) Multiple alignments of nine vertebrates of the variant c.1151C>G(P384R)

acid alteration from proline to arginine in the 384 positions. Both the fetus and the mother carried a heterozygous state of the *VANGL1*. (Figure 4b) The variant had not been detected in the father of the proband. The pathogenicity of the variant has been once reported in a Klippel-Feil syndrome patient. According to ACMG guidelines, this variant was classified as a VUS with PP2 and PP3. Multiple statistical methods predicted deleterious effects of the variant on the gene or gene product. The variant found by WES was validated in the family by Sanger sequencing. Both the mother and fetus carried the heterozygous variant c.1151C>G *VANGL1*, the father does not. (Figure 4c) Multiple alignments of nine vertebrates showed that the variant c.1151C>G (P384R) was a conserved locus. (Figure 4d).

4 | DISCUSSION

The *VANGL1* variant c.1151C>G (P384R) was present in the mother and the fetus but absent in the father. The variant was probably inherited from the mother, however, maternal germline mosaicism cannot be excluded. The proline is a hydrophobic amino acid, while the arginine is a hydrophilic amino acid and positively charged. The 524-amino acid *VANGL1* has four transmembrane domains and a C-terminal ser/thr-x-val motif. The proline residues in the 384 position located in the C-terminal cytoplasmic region and showed perfect conservation in the above nine species. Many computational analysis tools have predicted that the Pro-Ala substitution exhibited “Possibly damaging” (PolyPhen-2), “Disease-causing” (MutationTaster), “Damaging” (SIFT), “Deleterious” (PROVEAN), “Deleterious” (CADD), “Deleterious” (FATHMM-MKL), “Probably damaging” (PANTHER). (Table 1) P384R variant probably affected the protein function through the interaction with other proteins in the cytoplasmic region. Allele frequency in gnomAD was 0.000007959 in total populations. The allele showed no frequency in populations other than East Asian, allele counting

2 in 18384 allele number, heterozygous with a frequency of 0.0001088 in gnomAD. This allele might be an Asian-Specific locus. Our findings added a piece of East Asian report to the population frequencies.

The mother of the proband carried the variant while manifested nothing abnormal, we held the opinion that multifactorial conditions caused the phenotype of the disease, including environmental and genetic factors. This was supported by previous researches. R181Q was present in the proband and mother but the mother was unaffected. Within a history of familial NTD, R274Q mutation in a patient had an open NTD while mother and aunt have mild NTD vertebral schisis. (Iliescu et al., 2014) V239I was a de novo mutation in the mother, but had no phenotype for an NTD, while the proband showed a severe form of caudal regression. Her sibling had a milder form of the disease, dermal sinus. (Kibar et al., 2007) Combining our study, more clues pointed out that penetrance expressivity of NTD caused by *VANGL1* varied in different individuals.

The *VANGL1* gene variant c.1151C>G (P384R) was firstly reported in NTD disease, however, this variant was once reported in a Klippel-Feil syndrome patient. (Li et al., 2020) Klippel-Feil syndrome is a rare anomaly characterized by congenital fusion of the cervical vertebrae, leading to the improper segmentation of the cervical spine. Synonymous c.249G >A and missense variant c.1151C>G of *VANGL1* were reported in Klippel-Feil syndrome patients. Previous reports of Klippel-Feil syndrome showed the overlapping manifesting of tethered cord. It was known that *VANGL1* variants were associated with NTD and Caudal regression sequence. The planar cellpolarity genes *VANGL1* played a role in neural tube closure and ependymal ciliary movement. (Yamasaki & Kanemura, 2015) Most of the *VANGL1* variants were in the UTR region. Most missense variants in this gene caused diseases, except for one frameshift was found. The P384R may cause a functional change of the *VANGL1* protein or affect the protein-protein interaction, leading to function abnormality. Klippel-Feil syndrome might cause

TABLE 1 Computational analysis tools predict the pathogenicity the *VANGL1* c.1151C>G variant

Variant	c.1151C>G(p.Pro384Arg)	Reference
SNP ID	rs763973351	http://asia.ensembl.org/index.html
Allele frequency in gnomAD	0.000007959	http://gnomad-old.broadinstitute.org/
PolyPhen-2	Possibly damaging (Score: 0.939)	http://genetics.bwh.harvard.edu/pph2/
MutationTaster	Disease causing	http://www.mutationtaster.org/
SIFT	Damaging (Score: 0.007)	http://provean.jcvi.org/protein_batch_submit.php?species=human
PROVEAN	Deleterious (Score: -5.562)	http://provean.jcvi.org/seq_submit.php
CADD	Deleterious (Score: 28)	https://cadd.gs.washington.edu/snv
FATHMM-MKL	Deleterious (Score: 0.98242)	http://fathmm.biocompute.org.uk/fathmmMKL.htm
PANTHER	Probably damaging (Preservation time: 1036)	http://www.pantherdb.org/tools/csnpscoreForm.jsp

neurological consequences, such as radiculopathy, myelopathy, quadriplegia, and spina bifida. Spondylosis of the cervical spine or instability of vertebrae adjacent to the fused segment could compress the spinal cord or spinal nerves. (Kaplan et al., 2005) Interestingly, we found an obstetric patient who was diagnosed with Klippel-Feil syndrome and also indicated the presence of a potential underlying NTD. She had cord injury after spinal anesthesia and was found a low-lying tethered spinal cord terminating at the level of L5 and congenital fusion of the C7/T1 vertebrae. (Stevens et al., 2019) There was one similar case report that a 14-year-old patient was presented with bilateral Sprengel's deformity, spina bifida occulta, and a tethered spinal cord. (Mittal et al., 2013) Our report added a piece of clue showing the potential association of Klippel-Feil syndrome and NTD, however, the mechanism involved in how the variant caused the two diseases was still unclear, which might be revealed by functional researches further.

Few studies reported the *VANGL1* variant in the fetal stage. To the best of our knowledge, our study first reported the prenatal diagnosis of NTD associated with *VANGL1* variant as early as 25 GW with complete clinical information of prenatal ultrasound, postnatal CT, and genetic results. Our study shed light on the important role of the *VANGL1* variant c.1151C>G in human development.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

C.C. and X.L.C. designed the study. F.Y. performed the pathological examination. Q.F, W.Y.W., Y.L., and H.H. helped with clinical information. C.C. wrote the paper. S.Z., X.Z., and X.L.C revised the manuscript critically. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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