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ORIGINAL ARTICLE

Genetic mutations in ribosomal biogenesis gene *TCOF1* identified in human neural tube defects

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Abstract

Background: Rare mutations in multiple genes have been associated with human neural tube defects (NTDs), but their causative roles in NTDs disease are poorly understood. Insufficiency of the ribosomal biogenesis gene treacle ribosome biogenesis factor 1(*Tcof1*) results in cranial NTDs and craniofacial malformations in mice. Here, we aimed to identify genetic association of *TCOF1* with human NTDs.

Methods: High-throughput sequencing targeted on *TCOF1* was performed on samples from 355 human cases affected by NTDs and 225 controls from a Han Chinese population.

Results: Four novel missense variants were found in the NTD cohort. Cell-based assays indicated that the p.(A491G) variant carried by an individual, who shows anencephaly and single-nostril abnormality, attenuates production of total proteins, suggesting a loss-of-function mutation in ribosomal biogenesis. Importantly, this variant promotes nucleolar disruption and stabilizes p53 protein, highlighting an unbalancing effect on cell apoptosis.

Conclusions: This study explored the functional impact of a missense variant in *TCOF1*, implicating a set of novel causative biological factors involved in the pathogenicity of human NTDs, particularly whom combined with craniofacial abnormality.

KEYWORDS
apoptosis, missense mutation, neural tube defects, ribosomal biogenesis, *TCOF1*

1 | INTRODUCTION

Neural tube defects (NTDs, OMIM 182940) are characterized by anencephaly, encephalocele, spina bifida and craniorachischisis, all of which result from failure of the neural tube to close. These diseases are common, affecting an average of one in every 1000 established pregnancies worldwide (Copp et al., 2013; Mitchell, 2005), and show an extremely high prevalence (19.93 per 1000 births) in the Shanxi Province of China (Gu et al., 2007). NTDs are multifactorial, with a polygenic or oligogenic pattern of inheritance and an important role for non-genetic factors

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(Copp et al., 2013); however, up to 70% of the variance in NTDs prevalence is apparently attributable to genetic factors (Leck, 1974). Recent research showed that accumulation of singleton loss-of-function variants contribute to the occurrence of NTDs, regardless of the patient's genetic background or ethnicity (Chen et al., 2018). Many rare loss-of-function variants have been found, which include genes that function in one-carbon metabolism (Marini et al., 2011), and the non-canonical wingless-type MMTV integration site family (WNT)/planar cell polarity, sonic hedgehog, and bone morphogenetic protein (BMP) pathways (Wang et al., 2019). In view of more than 300 genes that are essential in neural tube closure in mice, it is likely that causative genetic factors are largely unknown for human NTDs.

The molecular machines known as ribosomes serve as the site of biological protein synthesis in all living cells. In eukaryotes, ribosomal biogenesis takes place both in the cytoplasm and in the nucleolus, which is extremely demanding in terms of energy and resources, with virtually any type of severe cellular stress resulting in an immediate shutdown of ribosomal RNA (rRNA) transcription (Grummt, 2013). The treacle ribosome biogenesis factor 1 gene (*TCOF1*, OMIM 606847) encodes a serine/alanine-rich nucleolar phosphoprotein called Treacle that serves as a link between ribosomal DNA (rDNA) gene transcription and pre-rRNA processing in the nucleolus (Gonzales et al., 2005; Valdez et al., 2004). Treacle comprises a 213-residue N-terminus that is followed by 11 repeated units containing potential phosphorylation sites and a nucleolar localization signal (Wise et al., 1997). The pathogenetic role of *TCOF1* in Treacher Collins syndrome (TCS, OMIM 154500), which features a group of severe craniofacial deformities, is well documented (Dixon et al., 2007). Variations in *TCOF1* have been implicated in 81%–93% of TCS cases (Conte et al., 2011), indicating that it plays an essential role in craniofacial development. Additionally, whole-mount in situ hybridization experiments in mice indicated that *Tcof1* is highly expressed in the first branchial arch and rostral to the region of neural tube fusion in the cranial region in E8.5 embryos (Dixon et al., 1997). *Tcof1*$^{+/-}$ mice remained unturned with a patent rostral neuropore at E9, no division of the forebrain into telencephalic or optic vesicles at E9.5, no medial or lateral nasal processes, and exencephaly with neuroepithelium protruding through the open rostral neuropore (Dixon et al., 2000), implicating that *TCOF1* is involved in both neural tube closure and craniofacial development.

In the present study, we report four novel missense variants of *TCOF1* in a cohort of pediatric NTD cases, and in vitro analyses showing the involvement of one of these variants, p.(A491G), in tumor protein p53 (TP53)-associated cell apoptosis. Our results suggested that this novel rare mutation participates in the pathogenesis of human NTDs.

2 | MATERIALS AND METHODS

2.1 | Ethical Compliance

All of the pregnant mothers whose fetuses were diagnosed with NTDs by trained local clinicians using ultrasonography provided informed consent before entering the study.

2.2 | Participants

Our study enrolled patients with NTDs who were assessed by clinical geneticists and placed into at least one of the following diagnostic groups: anencephaly, spina bifida (aperta or cystica), craniorachischisis and encephalocele. Genomic DNA samples were collected from 355 human NTD cases (270 from Shanxi province, 25 from Tianjin province, 17 from Jiangsu province, 28 from Liaoning province, and 15 from Heilongjiang province) from the Han Chinese population in China (age: gestational week 12–10 years) for high-throughput sequencing. Ethnicity-matched control samples were collected from 225 (202 from Shanxi and 23 from Shanghai province) non-medically related pregnancy terminations and were free of any NTDs. We carried out the study in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for medical research involving humans, as well as the approved guidelines. Written informed consent was obtained from the patients' parents. In NTD cases, the examination was performed during mid-term pregnancy and the materials were obtained at the same period when the mother decided to terminate the pregnancy in mid-term pregnancy. In controls, the examination was performed during early pregnancy, and the materials were obtained at the same period when the woman decided to abort in early pregnancy.

2.3 | Genomic DNA sequencing

High throughput targeted genome DNA sequencing were performed on 280 neural tube closure-related genes in 355 NTDs cases and 225 ethnicity matched controls as described in our previous study (Zou et al., 2020). In the present study, we focus on the possible pathogenic variant in *TCOF1* gene (GI: 568815593). Genomic DNA was enriched for both coding regions and highly conserved regions in *TCOF1* (Table S1). DNA samples were prepared using a Truseq DNA Sample preparation kit (Illumina

Inc.) following the manufacturer's standard protocol. Detailed sequencing information was published in our previous paper (Chen et al., 2016). The variants were filtered using dbSNP in NCBI, Genome 1000, Exome Sequencing Project, Exome Aggregation Consortium, and shared variants in cases and controls.

2.4 | DNA constructs

Human *TCOF1* tagged with green fluorescent protein (TCOF1-GFP; NM_001135243; cat. RG227457) in pCMV6 and three mutants (pT353M, pA491G, pR667G) were purchased from Origene. Primer sequences used for cloning are shown in Table S2. All mutations were confirmed by Sanger sequencing.

2.5 | Transient transfection into human embryonic kidney 293T (HEK293T) cells

To assess the functional impacts of the mutations found in humans, and in view of the essential nature of ribosomal biogenesis in all living cells, we transiently transfected the wild type (WT) or mutant *TCOF1*-pCMV6 plasmids into HEK293T cells in a 1:3 ratio using Lipofectamine® 2000 (Life Technologies) according to the manufacturer's protocol. Transfection efficiencies were assayed using flow cytometry, counting the percentage of GFP-positive cells. 24 or 48 h after transfection, the cells were harvested for the following experiments.

2.6 | Treatment of cycloheximide on transfected HEK293T cells

After a 36 h transfection, the cycloheximide (200 μg/ml, aladdin-C112766) was added to culture medium, and after 12 h treatment, cells were collected. Proteins DNA were extracted according to manufacturer's protocols (Solarbio-BC3711, TIANGEN-DP304). Total RNA was extracted from cells with Trizol (Invitrogen) according to the manufacturer's protocol (Qiagen-74106).

2.7 | Real-time quantitative PCR

Real-time Real-time quantitative PCR (qPCR) was performed using an Applied Biosystems 7500 PCR machine with Ultrasybr mix (CW Bio). The cycling parameters were as follows: 2 min at 50°C, and 40 cycles of 15 s at 95°C and 1 min at 60°C. The relative mRNA expression level of each target gene is shown following normalization to the *ACTB* gene. The primer sequences are shown in Table S2.

2.8 | Immunofluorescence staining

The cells were fixed and stained 48 h after transfection. The locations of treacle-GFP fusion proteins were confirmed by detection with the following: anti-TCOF1 serum diluted to 1:1000 (ab65212, Abcam); mouse monoclonal turboGFP antibody, clone OTI2H8 GFP at 1:5000 (TA150041; Origene); and TP53 mouse monoclonal antibody, clone DO-1 at 1:1000 (TA804804; Origene). Images were obtained with a laser scanning confocal microscope (FV-1000, Olympus, Japan).

2.9 | Total protein/RNA/DNA detection in one sample

An Allprep DNA/RNA/Protein Mini Kit (80004, Qiagen) was used to detect the influence of each mutation on total protein production. HEK293T cells (10^5) were harvested and processed as per the manufacturer's instructions. Total genomic DNA and total RNA were used as loading controls for assessment of total protein production. Ponceau S was used to visualize total protein.

2.10 | Statistical analysis

Results from all cell-based assays were analyzed by Student's *t*-test (two-tailed). Data are presented as means ± standard errors. A $p < 0.05$ was considered to show statistical significance. We used SPSS (version 16.0) for statistical analysis.

3 | RESULTS

3.1 | Rare missense variants in *TCOF1* in patients with NTDs from a Han Chinese population

We enrolled 355 sporadic, unrelated NTD cases and 225 ethnicity-matched controls from a local Han Chinese population between 2005 and 2011. Among the NTDs were 19 cases of craniorachischisis, 17 anencephaly, 57 anencephaly with spina bifida, 57 encephalocele, 27 encephalocele with spina bifida, and 178 spina bifida. As shown in our previous data, SIFT and Polyphen2 were used to identify putatively damaging SNVs, and totally case-specific 791 putatively damaging rare variants (including missense

mutations, splicing mutations, frameshift mutations or nonsense mutations) were identified in 213 genes, including *TCOF1* gene. Thirty-five novel, case-specific rare variants (Table S3) and 17 novel rare variants in controls (Table S4) were identified in *TCOF1* gene. Four missense variants and one synonymous variant were found among NTD cases, but only two synonymous variants were found among the controls. Four missense variants are singleton. Five cases showed burdening, NTDs-specific rare variants in exonic regions: three with anencephaly and two with spina bifida aperta (Table 1). Notably, the case T31 had a complex of anencephaly and single-nostril abnormality (Table 1), which is similar to the phenotypic traits of *Tcof1* mutant mice (Dixon et al., 2000), implying that the p.(A491G) missense mutation could be a new pathogenic variant for such phenotypes. A schematic of the TCOF1/Treacle protein indicates variants p.(A491G) and p.(T353M) are located in the so-called treacle repeats, variant p.(R667G) located in the POLI binding domain, and variant p.(Q1251L) located in the UBF/NOP40/rDNA binding domain (Figure 1a). Sanger sequencing further validated that the three rare missense variants were heterozygous (Figure 1b), and showed certain conservation among multiple species (Figure 1c).

To determine the functional characteristics of the rare variants found in the NTD cases, HEK293T cells were transiently transfected with GFP-fused WT or mutant *TCOF1* expression plasmids. Due to the novel variant p.(Q1251L) occurs at the same loci as p.(Q1251R), a known rare variant in the ExAC database, it was filtered out in the following functional experiments. The percentages of GFP-positive cell numbers indicated that the transfection efficiencies were similar for the WT and mutant plasmids (Figure 2a). Furthermore, the real-time qPCR assays showed no obvious differences in *TCOF1* mRNA levels among the different genotypes (Figure 2b).

3.1.1 | Rare missense variant p.(A491G) impacts ribosomal biogenesis and protein production

To understand the impact of the rare variants on ribosomal biogenesis, total protein, genomic DNA and total RNA were extracted from transfected HEK293T cells and fractionated using an Allprep DNA/RNA/Protein Mini Kit, with genomic DNA and total RNA used as loading controls to detect possible changes in the amount of total protein. Ponceau S staining results in cells carrying the p.(A491G) mutant indicated that the levels of proteins larger than 70 kD, especially the bands indicated by an asterisk in Figure 2c, were obviously reduced compared with WT. By contrast, there were no differences between

TABLE 1 Rare variants in exonic regions of *TCOF1* genes in the present cohort.

Sample ID	Genomic position (Chr5)	cDNA change (NM_001135243)	Amino acid change	SIFT score	POLYPhen score	Clinical phenotype of NTDs	Sex	Gestational weeks	Other clinical phenotype
T30	149753924	exon8:c.1058C>T	p.(T353M)	0.01	0.166	Sacral spina bifida aperta	F	34 W	Hydrocephaly
T31	149754710	exon10:c.1472C>G	p.(A491G)	0.01	0.403	Anencephaly	F	20 W	Single nostril abnormality
D137	149755750	exon13:c.1999C>G	p.(R667G)	0.02	0.99	Anencephaly	F	19 W	Short neck
D151	149773086	exon23:c.3752A>T	p.(Q1251L)	0.01	0.105	Lumbosacral spina bifida aperta	F	19 W	Hydrocephaly; Pulmonary lobe malformation
D160	149754263	exon9:c.1167G>A	p.(A389A)			Anencephaly	M	18 W	Pulmonary lobe malformation
A1882	149776341	exon24:c.4278G>A	p.(G1426G)			Normal	F	24 W	Normal
A1896	149759130	exon17:c.2694T>C	p.(A898A)			Normal	M	16 W	Normal

Note: TCOF1 (OMIM 606847, GI: 568815593).

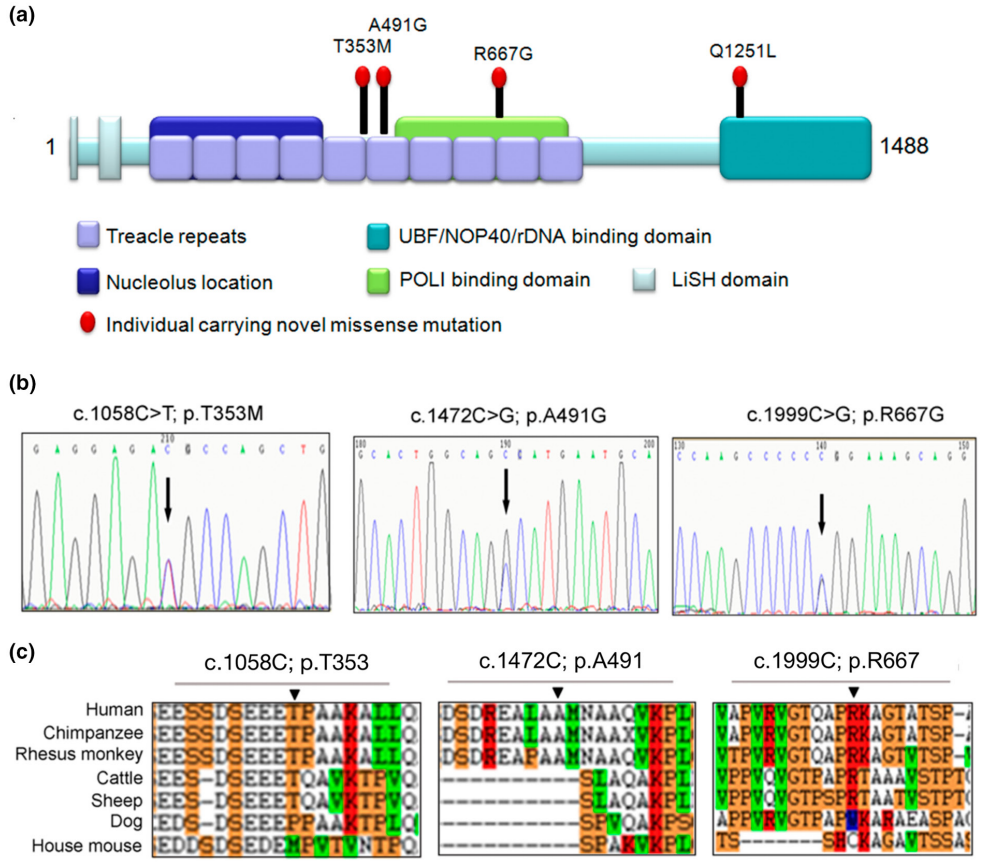

FIGURE 1 Rare missense variants in *TCOF1* in patients with NTDs from a Han Chinese population. (a) Schematic of the TCOF1/treacle protein profile showing the locations of missense variants found in the present study. (b) Representative Sanger sequencing results of the missense heterozygote variants. (c) Conservation analysis of missense variants.

the WT and the mutants p.(T353M) and p.(R667G) (Figure 2c), hinting that the p.(A491G) mutant impacts protein processing specifically. To confirm the reduced protein is due to loss of function in protein synthesis but not protein degradation, a protein synthesis inhibitor cycloheximide was used to treat transfected HEK293T cells, Indeed, after cycloheximide treatment the total protein bands, particularly which are larger than 70 kD, were similarly attenuated compared to dimethyl sulphoxide (DMSO) (Figure 2d). The reduced protein owing to the p.(A491G) mutant show similar variation in DMSO and cycloheximide treatment compared to vs. WT.

Next, to further confirm whether p.(A491G) affects ribosomal biogenesis, we detected the expression of 45 S pre-rRNA to evaluate the effect of *TCOF1* gene mutation on ribosomal biogenesis. Since ribosome biogenesis starts with transcription of 45 S pre-rRNA from rDNA in mammals, which then can be processed into mature 18S, 5.8S and 28S rRNA(Boisvert et al., 2007), meanwhile, the product of *TCOF1* gene serves as a link between rDNA gene transcription and pre-rRNA processing in the nucleolus. As we expected, the p.(A491G) variation induced a significant reduction of 45 S pre-rRNA level compared with the WT (Figure 2e), while the p.(T353M) and p.(R667G) did not display the statistic difference. We estimate the p.(A491G) variation-induced dysfunction of ribosomal biogenesis might contribute to the defects of protein synthesis.

3.2 | Rare missense variant p.(A491G) promotes TP53 stabilization

Impairment of pre-rRNA transcription induces nucleolar stress. Nucleolar disruption (ND) is an early response to nucleolar stress (Avitabile et al., 2011; Hariharan & Sussman, 2014), causing TP53 stabilization and cell apoptosis (Rubbi & Milner, 2003). Immunofluorescent staining results indicated that the TCOF1-GFP-positive cells with ND were obviously more in the p.(A491G) mutant compared with those in WT (Figure 3a,b). Importantly, the numbers of TP53-positive cells with TCOF1-GFP overexpression were significantly increased in the mutant (1.35 ± 0.13-fold difference compared with those in WT; $p = 0.023$) (Figure 3c). By contrast, the mutant p.(T353M) appeared to have no significant influence on

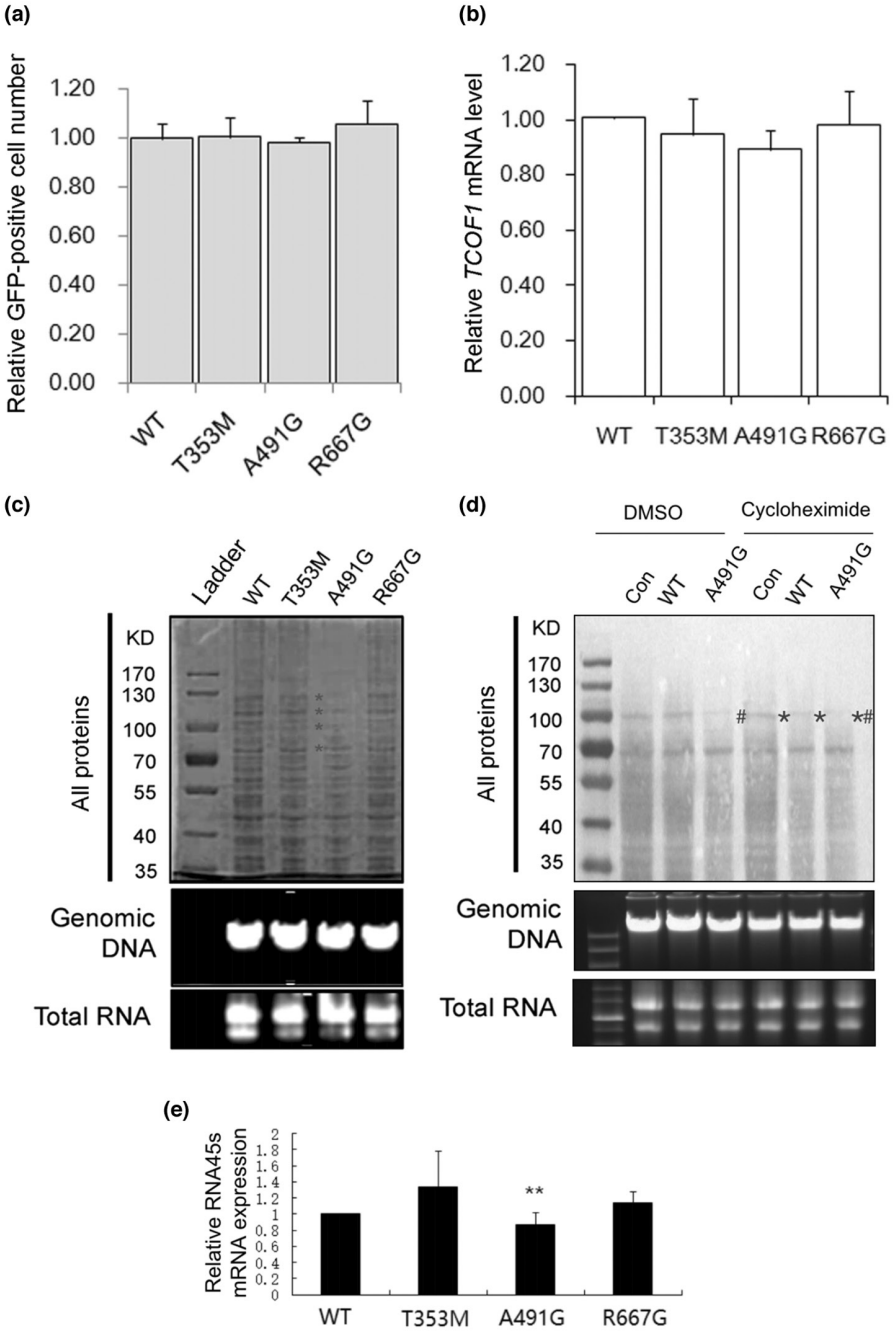

FIGURE 2 Rare missense variant p.(A491G) impacts ribosomal biogenesis and protein production. (a) Flow cytometry assay of the percentage of green fluorescent protein (GFP)-positive cells. (b) Real-time quantitative PCR results showing similar levels of *TCOF1* mRNA between cells carrying the WT and mutant expression plasmids. β-Actin was used as a loading control. (c) A representative image showing an impact of mutant p.(A491G), but not the other mutants, on total protein levels compared with that of WT *TCOF1* in HEK293T cells. Genomic DNA and total RNA were used as loading controls. Asterisks indicate the bands with obvious decreases in protein levels. (d) The total protein levels were decreased after Cycloheximide treatment and the asterisks indicate the bands with obvious decreases in protein levels compared to DMSO-treated. Genomic DNA and total RNA were used as loading controls. (#$p < 0.05$, p.A491G vs. WT; *$p < 0.05$, Cycloheximide vs. DMSO). (e) The relative expression of 45s RNA between WT and *TCOF1* mutation. **$p < 0.01$.

TP53 stabilization (1.19 ± 0.14-fold higher compared with WT, Figure 3c). The western blot assay further confirmed higher TP53 protein level in the p.(A491G) mutant (Figure 3d,e). As expected, cycloheximide treatment diminished total protein level; as a matter of fact, the higher TP53 protein owing to the p.(A491G) mutant show similar variation in DMSO and cycloheximide treatment compared to vs. WT. Combining the result of immunofluorescent staining, in which the numbers of TP53-positive cells were also greater in p.(A491G) mutant group, we speculated that the p.(A491G) mutation is likely to lead to loss of function, impacting ribosome biogenesis and resulting in nucleolar disruption and TP53 stabilization.

In order to understand that the p.(A491G) mutation of the case T31 is inherited, or a de novo mutation. Sanger sequencing was performed on a maternal sample due to absence of a paternal sample. The result showed p.(A491G) is acquired from maternal inheritance (Figure S1). Then we consult the questionnaire of sample T31, and the father denied the family history of NTDs, these implying that the *TCOF1* p.(A491G) is not the unique causative factor in this case. NTDs are complex traits with a multifactorial etiology encompassing both genetic and environmental components. In fact, our high-throughput sequencing also found five novel rare missense variants in exon regions of *CREBBP*, *FZD4*, *SHROOM2*, *TCTA* and *TCOF1* genes in the T31 case (Table S5).

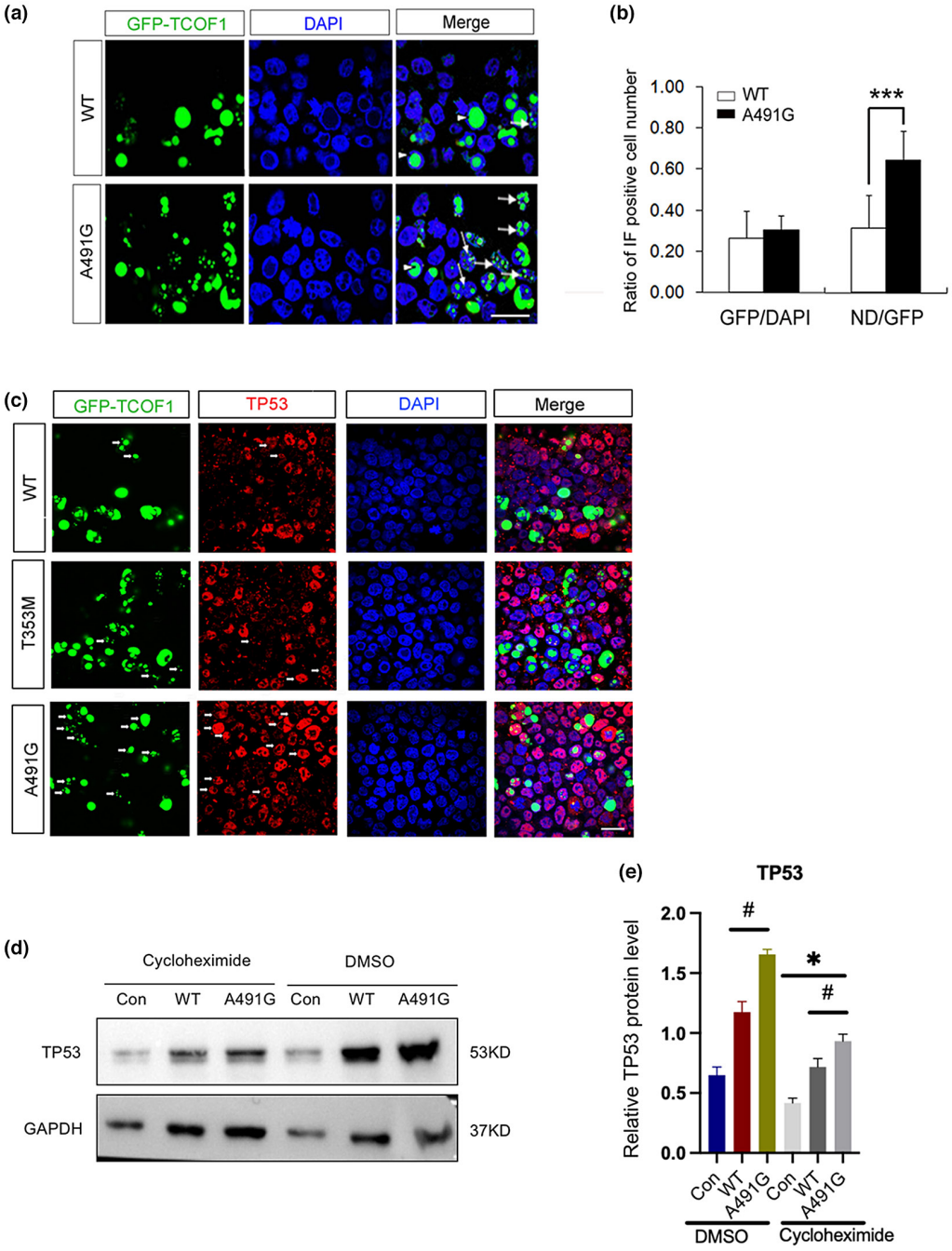

FIGURE 3 Rare missense variant p.(A491G) promotes TP53 stabilization. Representative image (a) and quantification (b) of increased nucleolar disruption (ND) in HEK293T cells with p.(A491G) mutant-green fluorescent protein (GFP)-positive expression (ND/GFP) compared with that in cells with wild type (WT)-GFP-positive expression (GFP/DAPI). The percentages of GFP-positive cells were similar in the mutant and WT cultures. The arrow indicates ND; the arrowhead indicates nucleolar enlargement. ***$p < 0.0001$. Data are from three independent experiments. (c) Representative images showing that, in p.(A491G)-GFP-positive cells, but not p.(T353M)-GFP-positive cells, the percentage of TP53-positive cells was increased compared with that in WT-GFP cells. The arrow indicates TP53-positive cells with TCOF1-GFP positivity. Scale bar: 20 μm. Western blot images (d) and quantification (e) indicated that TP53 protein level was increased in the A491G group, and all the protein expression level decreased after cycloheximide treatment. (#$p < 0.05$， p.A491G vs WT group; *$p < 0.05$, Cycloheximide vs DMSO).

4 | DISCUSSION

TCOF1/Tcof1 is highly conserved in humans and mice (Dixon et al., 1997). In our study, four novel rare missense variants of *TCOF1* (p.T353M, p.A491G, p.R667G, p.Q1251L) were identified in NTD cases in a Han Chinese population. It has been reported that TCOF1 takes part in the migration process of neural

crest and mesenchymal stem cells (Serrano et al., 2019). *Tcof1*$^{+/-}$ mice exhibit perturbation of ribosome biogenesis, increased apoptosis of neuroepithelial cells in the cranial neural fold, cell cycle arrest, and impaired migration of neural crest cells(Dixon et al., 2006; Jones et al., 2008). Inhibition of p53 prevents apoptotic elimination of the neural crest and rescues the craniofacial abnormalities associated with *Tcof1* deficiency, which is independent of the effects on ribosome biogenesis, suggesting that p53-dependent neuroepithelial apoptosis might be the primary pathogenic mechanism of TCS (Jones et al., 2008). Particularly, we found that a NTD case that exhibited a phenotype of anencephaly and craniofacial malformation, and carry the *TCOF1* p.(A491G) variation, our cellular data verified that the mutation increased TP53-associated apoptosis (Figure 3c). Proper apoptosis in the cranial neural fold is a well-known determinant of neural tube closure (Yamaguchi et al., 2011) and excessive apoptosis could cause failure of neural tube closure (Li et al., 2018). Therefore, we propose that the *TCOF1* A491G missense mutation may induce a p53-related enhancement in cell apoptosis, which might be one causative factor in risk of NTDs.

It is believed that the causes of NTDs are multifactorial, having both genetic and environmental components, the gene–environment interactions contribute to the defect on neural tube closure. Folic acid fortification has reduced the prevalence of NTDs at 30% in spina bifida and 16% in anencephaly (Williams et al., 2015). In the present study, majority of the NTD samples (270/355) were collected from Shanxi Province from 2005 and 2011. Our previous study during 2002 to 2004 has documented a high prevalence of NTDs in Shanxi province with the incidence of 199.38 per 10,000 births (Gu et al., 2007). Lacks of local average consumption of foods including dark green vegetables, fruits, fat and meat, and nutrient intake are more common than the national average level, especially folic acid, zinc, vitamins A and B12 (Zhang et al., 2008). At the same period, Shanxi province harbors a lots of coal mines and prevalence of NTDs was also reported (Liao et al., 2010). Therefore, regarding that the p.(A491G) mutation is not a de novo mutation, and featured loss of function, we estimated the interactions of these non-genetic factors with the genetic variations, and/or complex mutations, are component of the pathogenecity of local NTDs prevalence. Further studies on gene–gene interaction (eg. *EMG1* gene) or gene–environment interaction might be performed in the future.

The present study demonstrated that the presence of the p.(A491G) missense mutant in HEK293T cells disturbed the processing of high-molecular-weight proteins, suggesting a dysfunction in ribosome biogenesis in the patient who carries this mutation. However, the reason of only proteins larger than 70kD are affected is unclear, which is interesting and needs further investigation. We recently reported that the expression levels of rRNA are dramatically decreased in folate insufficiency-related NTDs brain tissues (Xie et al., 2016), highlighting that disruption in the functioning of ribosome biogenesis genes is a possible etiological factor in human NTDs. Apart from the *TCOF1* gene, model mice featuring a knock-in of the p.(D86G) substitution that induces deficiency in *EMG1*, a gene encoding a methyltransferase that modifies the nascent 18S rRNA, exhibit exencephaly in the midbrain, and a distorted but closed spinal cord (Armistead et al., 2015). This phenotype is similar to that of *Tcof1*$^{+/-}$ mice, which exhibit exencephaly in the forebrain and midbrain (Dixon et al., 2000). Furthermore, deficiency in either *Tcof1* or *Emg1* has no apparent impact on neural tube closure in spinal regions, indicating the essential role of ribosome biogenesis on cranial neural tube closure. Notably, deficiency in either *Tcof1* or *Emg1* can result in incremental cell apoptosis (Armistead et al., 2015; Dixon et al., 2000; Jones et al., 2008), taken together with the rescue effect of Tp53 inhibition on *Tcof1*$^{+/-}$ mice (Jones et al., 2008), we deduce that excessive cell apoptosis is a large component of the consequence of the loss of function of genes involved in ribosome biogenesis in neural tube closure. Besides impairment of pre-rRNA transcription-induced nucleolar stress, many ribosomal proteins interact with murine double minute2 (MDM2), a nuclear-localized E3 ubiquitin ligase, thereby inhibiting MDM2-mediated p53 ubiquitination and degradation (Golomb et al., 2014; Toledo & Wahl, 2006).

In summary, we have provided a line of evidence that a novel missense mutation of the ribosome biogenesis gene *TCOF1* promotes TP53 stabilization and increases cell apoptosis, ultimately promoting human anencephaly with craniofacial abnormality. Our study offers a peek into the contribution of ribosomal biogenesis genes in the formation of human NTDs and highlights a new etiology for clinical consideration.

AUTHOR CONTRIBUTIONS

Fang Wang performed the genomic DNA and total RNA as well as total protein extraction, and also carried out the statistical analysis of the data, Haiqin Cheng conducted the Transient transfection experiments on HEK293T cells and the immunofluorescence staining study, Qin Zhang performed the qPCR experiments, Fang Wang and Haiqin Cheng wrote the draft, Jin Guo edited the manuscript. Fang Wang and Jin Guo conceived the research.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the article.

ETHICS STATEMENT

All participants signed for written informed consent. This study was approved by the Committee of Medical Ethics of the Capital Institute of Pediatrics.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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