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The CFTR K464N variant in fetuses potential increases premature birth risk in Chinese families

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Abstract

Background Global fertility decline has led to increased use of assisted reproductive technology (ART), raising concerns about genetic risks to offspring. This study aimed to investigate cystic fibrosis transmembrane conductance regulator (CFTR) variants in Chinese families and assess their association with pregnancy complications and neonatal outcomes.

Methods This prospective cohort study included 446 Chinese families (148 natural conceptions, 298 ART conceptions) who underwent whole genome sequencing. We analyzed the frequency of pathogenic/likely pathogenic CFTR variants and their association with preterm birth (PTB), pregnancy complications, and neonatal outcomes.

Results Twelve pathogenic/likely pathogenic CFTR variants were identified, with K464N (c.1392G > T) being the most prevalent (2.9% of cohort). PTB incidence was significantly higher in pregnancies with fetal CFTR variants (43.1%, 22/51) compared to those without (17.5%, 69/395; $p < 0.001$). Fetuses carrying the CFTR K464N variant exhibited a 3.39-fold increased risk of PTB (95% confidence interval (CI): 1.39–8.23, $p = 0.007$) after adjusting for confounders. Neither fetal nor maternal CFTR variants were significantly associated with other neonatal outcomes, including neonatal weight, Apgar scores, respiratory distress, or hyperbilirubinemia ($p > 0.050$).

Conclusion These findings suggest a potential association between fetal CFTR K464N variant and increased risk of preterm birth in Chinese families, highlighting the importance of considering CFTR genotyping in prenatal care.

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Introduction

The global decline in fertility represents a significant challenge and is increasingly being addressed through assisted reproductive technology (ART) [1, 2]. As ART becomes a more common solution, ensuring its safety is critical, particularly in terms of potential genetic risks to offspring [3, 4]. We initiated the Genetic Safety Study of Assisted Reproductive Technologies project (National Key Research and Development Plan, 2018YFC1004900), which focused on the comprehensive whole-genome sequencing (WGS) analysis of 469 family cohorts, to address this. Using this sequencing data, we placed particular emphasis on the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This gene plays important roles in the respiratory, digestive, and reproductive systems [5, 6], and its mutations can cause cystic fibrosis (CF), the most common lethal genetic disease among Caucasians [7].

CFTR is located on the long arm of chromosome 7 and functions primarily as a cAMP-activated ion channel that regulates bicarbonate and chloride exchange, glutathione transport, and epithelial Na + channel activity [8]. The regulation of these processes is essential for maintaining the balance of salt, fluid, and pH in many organ systems [9]. In the respiratory system, CFTR dysfunction leads to the production of thick, sticky mucus that accumulates in the airways, causing obstruction, inflammation, and increased susceptibility to bacterial infections [10]. In the digestive system, *CFTR* dysfunction in the pancreas, intestine, and biliary system results in exocrine pancreatic insufficiency, intestinal blockage, and malabsorption [11]. In the reproductive system, *CFTR* dysfunction can cause congenital bilateral absence of the vas deferens (CBAVD) in males [12]and impair fertility in females owing to thicker cervical mucus [13].

The severity and specific manifestations of *CFTR* mutations depend on the type and combination of pathogenic variants carried by an individual. Classical biallelic pathogenic variations in *CFTR* cause CF (OMIM: 219700), which is inherited in an autosomal recessive manner. In addition to CF, pathogenic variations in CFTR can give rise to CFTR-related disorders (CFTR-RD), which exhibit a less severe phenotype than classic CF and typically affect a single-organ system through autosomal dominant or recessive inheritance patterns, including CBAVD, recurrent/chronic idiopathic pancreatitis, and diffuse bronchiectasis [14, 15]. *CFTR* mutations can also have significant implications for pregnant women and their offspring. During pregnancy, women with CF have an elevated risk of gestational diabetes, hypertension, worsening respiratory symptoms, and preterm birth (PTB), which can increase the likelihood of complications in their babies [16]. In addition, babies born to mothers with CF are at higher risk of low birth weight, respiratory problems, and other health issues [17–19].

The impact of *CFTR* gene variants on disease severity and incidence varies globally with notable differences across countries, regions, and ethnicities [20–22]. In Caucasians, approximately 95% of patients with CF suffer from obstructive azoospermia caused by the congenital absence of the vas deferens [23]. However, the incidence of CF in other races is very low, and obstructive azoospermia caused by CBAVD is more common [23]. Studies reporting the CFTR variant frequency within a large cohort of healthy Chinese individuals are limited. Therefore, we used WGS data to investigate CFTR variants in Chinese individuals without CF or CFTR-RD. Focusing on these variants, we explored their association with pregnancy-related complications and newborn diseases, particularly in the context of ART use.

Methods

Participants

This prospective cohort study was conducted from December 2018 to October 2020 at Women's Hospital, School of Medicine Zhejiang University. The study included two groups of participants: families conceived through assisted reproductive technology (ART), specifically in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), and families who conceived naturally (NC). In the ART cohort, which comprised both IVF and ICSI families, mothers underwent various controlled ovarian stimulation (COS) protocols based on individual patient characteristics and clinical indications. These COS protocols included the GnRH agonist long protocol, GnRH antagonist protocol, Flare-up GnRH agonist protocol, luteal-phase stimulation protocol, and minimal stimulation protocol. Family relationship verification was performed using IBD (Identity By Descent) analysis to ensure authentic biological relationships within each trio.

The inclusion criteria for this study were as follows: [1] all adult family members signed medical informed consent forms; [2] the parents were unrelated individuals from different families; [3] family members had no history of malignancy; [4] infants were born alive after 28 weeks of gestation; [5] the parents had no serious genetic disorders; [6] the DNA quantity and quality met the following criteria: a total amount of DNA ≥ 1 μg, a concentration ≥ 10 ng/UL and no degraded DNA detected by agarose gel electrophoresis. The exclusion criteria were as follows: [1] parents with chromosomal abnormalities; [2] unwillingness to provide relevant medical information; [3] participants with CF, CBAVD, sweat chloride elevation, bronchiectasis, hypertyrosinemia, and pancreatitis; and [4] family members with weak kinship, defined as instances where one or both parents are not biologically related to the child.

The present study was conducted under a license from the Human Genetic Resource in China ([2021] CJ0522) and approved by the Institutional Review Board of the School of Medicine, Zhejiang University, China (20180127). All adults received oral and written information and signed a written consent.

Clinical data collection

Data were collected independently by three senior chief physicians. The clinical data included the following: [1] demographic characteristics, including age of the parents, race, birthplace, female body mass index (BMI), duration of pregnancy, birth weight of the fetus, and sex of the fetus; [2] infertility factors, including sperm parameters, fallopian factor obstruction, polycystic ovary syndrome (PCOS), primary ovarian insufficiency (POI) and endometriosis; [3] ART procedures and outcomes; [4] perinatal-related diseases including gestational diabetes, hypertension, cholestasis during pregnancy, placenta previa, placenta abruption, placenta accreta, and premature rupture of fetal membranes; and [5] neonatal characteristics, including neonatal weight, Apgar score at 10 min, neonatal respiratory distress, and neonatal hyperbilirubinemia. Sperm concentrations lower than 15 million spermatozoa per milliliter (mL) were defined as oligozoospermia. Azoospermia was defined as the absence of spermatozoa in the ejaculate after centrifugation. Fallopian tube obstruction was diagnosed by using hysterosalpingography, laparoscopy, and ultrasonography. PCOS is defined as a combination of signs and symptoms of androgen excess and ovarian dysfunction in the absence of other specific diagnoses. POI and endometriosis were diagnosed according to the European Society of Human Reproduction and Embryology (ESHRE) guidelines [24, 25].

Whole genome sequencing (WGS) variant filtration

We performed trio-based whole genome sequencing on father-mother- offspring groups to enable comprehensive variant analysis and inheritance pattern determination. Total genomic DNA (gDNA) was isolated from the peripheral blood samples of all participants using magnetic beads. WGS libraries were constructed using a Universal DNA Library Prep Set according to a standard protocol (MGI; Cat: 1000017571). Sequencing reads were acquired from the DNBSEQ-T1 platform of the China National GenBank. The bioinformatics analysis pipeline consisted of several steps: First, low-quality reads were filtered out, and the remaining sequencing reads were aligned to the human genome (GRCh37/hg19). Variants were then identified using the Sentieon pipeline. To obtain high-confidence variants, variant quality score recalibration (VQSR) was performed using the Genome Analysis Toolkit (GATK v3.4.6). Individual gVCF files were jointly genotyped using GenotypeGVCFs, and variants that passed through the VQSR filter were obtained for further analysis. For CFTR variant analysis, wANNOVAR was used to annotate the functional consequences of variants (https://wannovar.wglab.org/). Variants were compared between parents and offspring to confirm inheritance patterns. CFTR variants were classified as pathogenic or likely pathogenic according to multiple databases including ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), CFTR2 (https://cftr2.org/), Varsome (https://varsome.com/), and LOVD3 (https://databases.lovd.nl/shared/variants/CFTR/unique) databases.

Statistical analysis

One-way ANOVA or the Kruskal–Wallis test was used to compare quantitative parameters according to the normality of the data. Of the seven sets of twins, only one individual from each pair underwent WGS. Therefore, our analysis of the consequences of CFTR variants in fetuses with pregnancy-related complications excluded these families. The chi-square test or Fisher's exact test was used to examine the differences between categorical variables. Stepwise logistic regression analysis was used to select the variables (maternal and paternal age, maternal BMI, pregnancy-related diseases and complications, number of offspring, and CFTR mutation status of the offspring) that affected neonatal respiratory distress and premature birth. The Generalized Estimating Equations (GEE) model was used to analyze the association between offspring CFTR variants and neonatal diseases, accounting for the correlation between twins. $p < 0.05$ was regarded as statistically significant. All data analyses were performed using SPSS (version 30.0; IBM Corp., USA) and R 4.10 software (available at http://www.r-project.org).

Results

Patient characteristics

In total, 469 families were recruited for this study, including 157 families whose children were conceived naturally, 191 families whose children were conceived via IVF, and 121 families whose children were conceived via ICSI. Due to low-quality DNA that did not conform to the quality standards of WGS and confusing kinship relationships among family members, 15 families were excluded from the study. Eight families in which the male parents had CBAVD were also excluded. Ultimately, 446 families participated in this study (Fig. 1), with 148 families in the NC group, 189 in the IVF group, and 109 in the ICSI group. Notably, in twin pregnancies, only one fetus from each of six pairs in the IVF group and one pair in the ICSI group underwent whole genome sequencing analysis. Therefore, while there were 543 newborns in total, genomic analysis was performed on 536 samples.

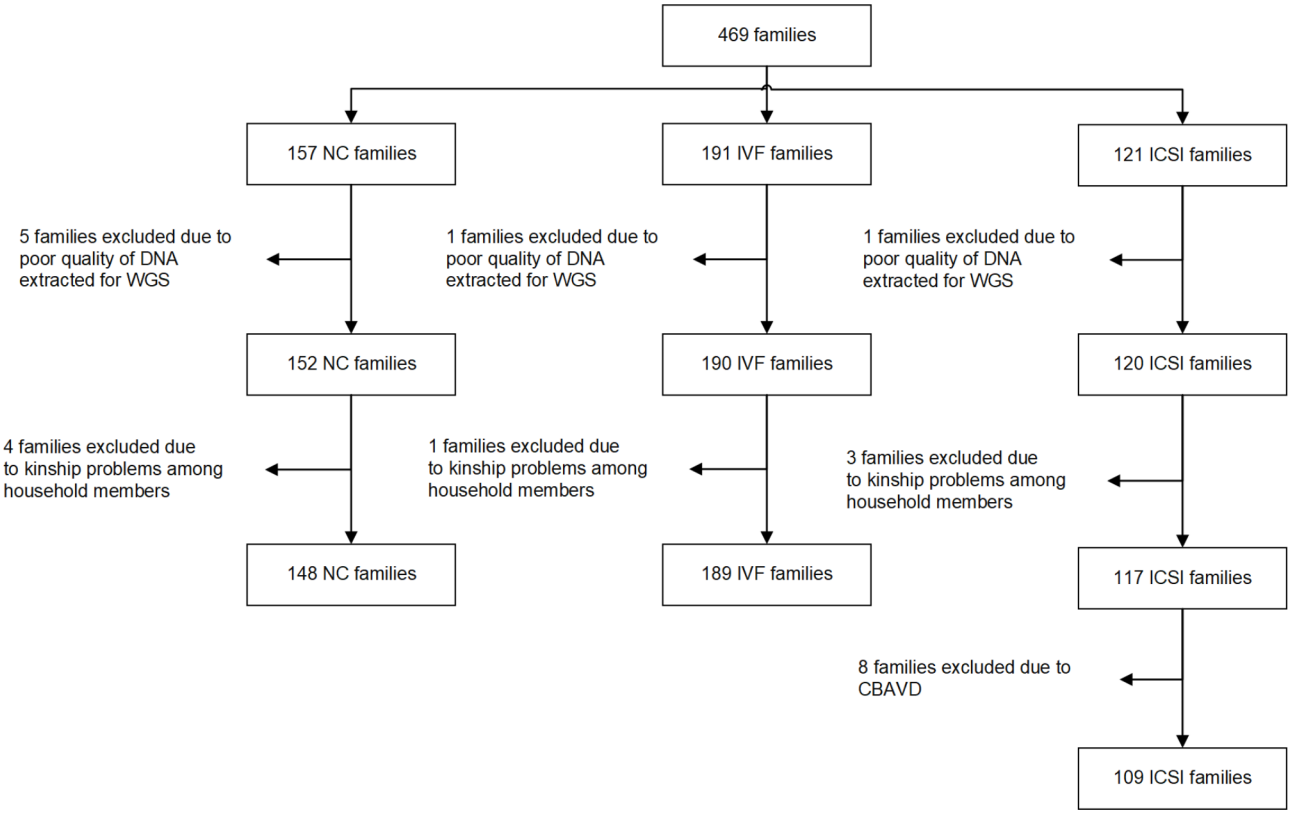

Fig. 1 Flow chart of the cohort study

The clinical characteristics of the families in the NC, IVF, and ICSI groups are presented in Table 1. The 892 unrelated parents were predominantly Han Chinese (99.6%, 888/892), followed by Miao (0.2%, 2/892), She (0.1%, 1/892), and Zhuang (0.1%, 1/892). The birthplaces of the parents were mainly Zhejiang Province (71.3%, 636/892), followed by Anhui Province (5.9%, 53/892), and Jiangxi Province (3.4%, 30/892). Paternal age, maternal age, and maternal BMI were not significantly different among the NC, IVF, and ICSI groups ($p > 0.050$; Table 1). The proportion of male participants with oligospermia and azoospermia was significantly higher in the ICSI group than in the IVF group ($p < 0.001$; Table 1). The proportions of female parents with POI and PCOS were not significantly different between the IVF and ICSI groups ($p > 0.050$; Table 1). The proportions of participants with fallopian tube obstruction and endometriosis were significantly higher in families that underwent IVF than in families that underwent ICSI ($p < 0.001$, Table 1). COS protocols were not significantly different between the IVF and ICSI cohorts ($p = 0.408$; Table 1). The incidences of gestational diabetes, hypertension, cholestasis during pregnancy, placenta previa, placenta abruption, placenta accreta, premature rupture of fetal membranes, and PTB were not significantly different among the NC, IVF, and ICSI groups ($p > 0.050$; Table 1). A total of 349 couples had a single child, whereas 97 couples had twins. The ratio of parents to children was 1.64 (446×2 / 543). The mean number of offspring was significantly higher in the ART group than in the NC group ($p < 0.001$; Table 1).

Frequency of pathogenic *CFTR* variants in Chinese families without CF

In total, 2340 variants were identified using WGS. A total of 12 pathogenic/likely pathogenic variants were identified across the entire cohort. The frequencies of pathogenic/likely pathogenic CFTR variants in the male and female parents were 6.2% (55/892) and 2.2% (20/892), respectively. The most common pathogenic CFTR variant in unrelated male and female Chinese parents was K464N (c. 1392G > T, allele frequency: 2.9%, 52/1784). The 12 pathogenic/likely pathogenic variants and their prevalence rates are shown in Supplementary Table 1. The CFTR F508del mutation, the most common mutation causing CF in Western populations, was not detected. There were no significant differences in *CFTR* variants in male and female parents and their offspring among the NC, IVF, and ICSI cohorts ($p > 0.05$). Given the lack of significant differences in pathogenic *CFTR* variants among the NC, IVF, and ICSI cohorts ($p > 0.050$) and the small sample size of individuals carrying pathogenic/likely pathogenic CFTR variants, we combined

Table 1 Clinical characteristics of the 148 couples who conceived naturally and the 298 couples who conceived through assisted reproductive technology treatment

Variables	NC	IVF	ICSI	*p*
Number of couples (%)	148	189	109	-
Paternal age (y)	34.05 ± 5.41	34.29 ± 5.27	33.42 ± 3.96	0.779
Maternal age (y)	32.15 ± 4.62	32.40 ± 3.91	32.07 ± 3.57	0.831
Maternal BMI	21.94 ± 3.33	21.44 ± 2.70	21.70 ± 2.69	0.449
Sperm count				< 0.001
Normal	-	184 (97.4)	77 (70.6)	
Oligospermia	-	5 (2.6)	20 (18.3)	
Azoospermia	-	0 (0.00)	12 (11.0)	
Premature ovarian insufficiency	-	31 (16.4)	14 (12.8)	0.409
Polycystic ovary syndrome	-	26 (13.8)	10 (9.2)	0.242
Fallopian tube obstruction	-	118 (62.4)	32 (29.4)	< 0.001
Endometriosis	-	42 (22.2)	11 (10.1)	0.008
COS protocols				0.408
GnRH agonist long protocol	-	82 (43.4)	60 (55.0)	
GnRH antagonist protocol	-	66 (34.9)	30 (27.5)	
Flare-up GnRH agonist protocol	-	12 (6.3)	6 (5.5)	
Luteal-phase stimulation protocol	-	18 (9.5)	9 (8.3)	
Minimal stimulation protocol	-	11 (5.8)	4 (3.7)	
Gestational diabetes mellitus	39 (26.4)	41 (21.7)	22 (20.2)	0.447
Gestational hypertension	13 (8.8)	18 (9.5)	17 (15.6)	0.169
Cholestasis during pregnancy	7 (4.7)	10 (5.3)	7 (6.4)	0.836
Placenta previa	5 (3.4)	5 (2.6)	3 (2.8)	0.918
Placental abruption	0 (0.00)	5 (2.6)	1 (0.9)	0.097
Placenta accreta	9 (6.1)	14 (7.4)	11 (10.1)	0.483
Premature rupture of fetal membranes	13 (8.8)	28 (14.8)	9 (8.3)	0.117
Preterm delivery	23 (15.5)	44 (23.3)	24 (22.0)	0.193
Offspring				< 0.001
Singleton	137 (92.6)	138 (73.0)	74 (67.9)	
Twin	11 (7.4)	51 (27.0)	35 (32.1)	

NC: natural conception; IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection. COS protocols: controlled ovarian stimulation protocols

Data are shown as the mean ± standard deviation or No (%)

these variants into a single group for a more comprehensive analysis.

CFTR variants and preterm birth

Univariate analysis revealed several factors significantly associated with preterm birth, including gestational hypertension, gestational diabetes, placenta previa, placenta accreta, twin pregnancy, and the presence of pathogenic or likely pathogenic CFTR variants in offspring ($p < 0.050$, Table 2). Notably, maternal CFTR variant status did not significantly influence PTB incidence, with carrier and non-carrier rates at 15.0% (3/20) and 20.7% (88/426), respectively ($p = 0.742$). However, a striking difference emerged when considering fetal CFTR variants: pregnancies with fetuses carrying any CFTR variant exhibited a significantly higher PTB rate (43.1%, 22/51) compared to those without fetal CFTR variants (17.5%, 69/395; $p < 0.001$). In fetuses carrying pathogenic/likely pathogenic CFTR variants, the risk of preterm birth was 3.58-fold higher (95% CI: 1.94–6.61, $p = 0.001$) compared to those without CFTR variants.

Because the number of newborns per pregnancy is an important factor leading to PTB, we conducted a subgroup analysis to further analyze the relationship between offspring CFTR variants and PTB. Singletons without CFTR variants had a PTB rate of 9.3% (30/321), whereas those with CFTR variants exhibited a slightly higher rate of 10.7% (3/28). However, the statistical analysis indicated no significant difference between the two singleton groups ($p = 1.000$). In contrast, the data revealed a more pronounced difference when considering twins: twins without pathogenic CFTR variants had a PTB rate of 52.7% (39/74), whereas in cases where at least one fetus carried a pathogenic/likely pathogenic CFTR variant, the PTB rate increased to 82.6% (19/23), which was a statistically significant difference ($p = 0.011$). Multivariate logistic regression analysis indicated that gestational hypertension, placenta accreta, anterior placenta, twin pregnancy, premature rupture of fetal membranes,

Table 2 Univariate analysis of factors associated with preterm delivery

Variables	Preterm birth		Significance
	Yes	No	*p*
Female age	31.84 ± 4.32	32.34 ± 4.00	0.293
Male age	33.58 ± 4.62	34.10 ± 5.14	0.599
Female BMI	21.88 ± 2.86	21.62 ± 2.94	0.396
Pregnancy			0.193
NC	23 (15.5)	125 (84.5)	
IVF	44 (23.3)	145 (76.7)	
ICSI	24 (22.0)	85 (78.0)	
PCOS			0.049
NC	23 (15.5)	125 (84.5)	
No	56 (21.4)	206 (78.6)	
Yes	12 (33.3)	24 (66.7)	
POI			0.198
NC	23 (15.5)	125 (84.5)	
No	58 (22.9)	195 (77.1)	
Yes	10 (22.2)	35 (77.8)	
Gestational diabetes mellitus			0.044
Yes	28 (27.5)	74 (72.5)	
No	63 (18.3)	281 (81.7)	
Gestational hypertension			0.002
Yes	18 (37.5)	30 (62.5)	
No	73 (18.3)	325 (81.7)	
Anterior placenta			< 0.001
Yes	9 (69.2)	4 (30.8)	
No	82 (18.9)	351 (81.1)	
Placental abruption			0.778
Yes	2 (33.3)	4 (66.7)	
No	89 (20.2)	351 (79.8)	
Placenta accreta			< 0.001
Yes	16 (47.1)	18 (52.9)	
No	75 (18.2)	337 (81.8)	
Premature rupture of fetal membranes			0.297
Yes	13 (26.0)	37 (74.0)	
No	78 (19.7)	318 (80.3)	
Number of newborns			< 0.001
Singleton	33 (9.5)	316 (90.5)	
Twin	58 (59.8)	39 (40.2)	
Female parents with pathogenic/likely pathogenic CFTR variants			0.742
Yes	3 (15.0)	17 (85.0)	
No	88 (20.7)	338 (79.3)	
Fetus with pathogenic/likely pathogenic CFTR variants*			< 0.001
Yes	22(43.1)	29 (56.9)	
No	69 (17.5)	326 (82.5)	

NC: natural conception; IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; PCOS: polycystic ovary syndrome; POI: primary ovarian insufficiency. NC: natural conception; IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection. The data are shown as the mean ± standard deviation or no (%); Fetus with pathogenic/ likely pathogenic CFTR variants* indicates carrier status in singleton pregnancies or at least one carrier in twin pregnancies

and offspring with a pathogenic/likely pathogenic CFTR variant were significantly associated with PTB ($p < 0.050$; Table 3).

Since the CFTR K464N variant was the most common mutation in the offspring, we analyzed the relationship between the K464N variant and PTB. Among the singleton pregnancies, the incidence of PTB was slightly higher in offspring with the K464N variant (10.0%, 2/20) than in those without the variant (9.4%, 31/329). However, among the twins, the offspring with the K464N variant had a significantly higher rate of PTB (90.0%, 18/20) than those without the variant (51.9%, 37/72, $p = 0.002$). Multivariable logistic regression analysis showed that fetuses with the CFTR K464N variant had a 3.39-fold increased

Table 3 Multivariate analysis of factors associated with preterm birth

Variables	Crude OR (95% CI)	Crude *p*	Adjusted OR (95% CI) *	Adjusted *p*
Gestational hypertension	2.67 (1.43–5.05)	0.003	5.25 (2.39–11.52)	< 0.001
Anterior placenta	9.63 (2.90-32.04)	< 0.001	14.19 (3.19–63.20)	< 0.001
Placenta accreta	4.17 (1.99–8.75)	< 0.001	4.47 (1.75–11.46)	0.002
Premature rupture of fetal membranes	1.43 (0.73–2.82)	0.299	3.73 (1.59–8.82)	0.003
Twin pregnancy	14.24(8.29–24.48)	< 0.001	17.39 (9.34–33.50)	< 0.001
Fetus with pathogenic/likely pathogenic CFTR variants	3.58(1.94–6.61)	< 0.001	2.83(1.26–6.33)	0.011

*Adjusted OR of each variable was adjusted for all other variables

Table 4 The CFTR K464N variant in fetuses is an independent risk factor for preterm birth

Variables	Crude OR (95% CI)	Crude *p*	Adjusted OR (95% CI) *	Adjusted *p*
Gestational hypertension	2.67 (1.43–5.05)	0.003	5.05(2.30-11.08)	< 0.001
Anterior placenta	9.63 (2.90-32.04)	< 0.001	13.98 (3.15–62.15)	< 0.001
Placenta accreta	4.17 (1.99–8.75)	< 0.001	4.36 (1.69–11.20)	0.002
Premature rupture of fetal membranes	1.43 (0.73–2.82)	0.361	3.76 (1.59–8.90)	0.003
Twin pregnancy	14.24(8.29–24.48)	< 0.001	17.46 (9.08–33.58)	< 0.001
Fetus with CFTR 464 N variant	4.72(2.41–9.23)	< 0.001	3.39 (1.39–8.23)	0.007

* Adjusted OR of each variable was adjusted for all other variables

risk of PTB compared to those without the K464N variant (95% confidence interval (CI): 1.39–8.23, $p = 0.007$). Twin pregnancy, gestational hypertension, anterior placenta, placenta accreta, and premature rupture of fetal membranes were identified as independent risk factors for PTB ($p < 0.050$; Table 4).

Pathogenic CFTR variants and pregnancy-related complications

Univariate analysis demonstrated no significant correlation between the presence of pathogenic or likely pathogenic CFTR variants in mothers and the incidence of various pregnancy-related complications, such as gestational hypertension, cholestasis during pregnancy, placenta previa, placenta abruption, placenta accreta, or premature rupture of fetal membranes ($p > 0.050$, Supplemental Table 2). Similarly, analyzing the offspring revealed no significant association between the presence of pathogenic or likely pathogenic CFTR variants and pregnancy-related complications, with the notable exception of gestational diabetes (Supplemental Table 2). Mothers carrying fetuses with pathogenic/likely pathogenic CFTR variants exhibited a significantly higher incidence of gestational diabetes at 39.2% (20/51), compared to 20.8% (82/395) in mothers whose fetuses without CFTR variants ($p = 0.003$, OR 2.46, 95% CI: 1.33–4.54).

Pathogenic CFTR variants and neonatal diseases

Pathogenic or likely pathogenic CFTR variants in the offspring were not associated with neonatal weight or Apgar scores at 10 min ($p > 0.050$; Supplemental Table 3). There was no statistically significant difference in neonatal respiratory distress between offspring with and without pathogenic CFTR variants ($p = 0.274$). The pathogenic/likely pathogenic CFTR variants were not associated with neonatal hyperbilirubinemia ($p = 0.232$).

Discussion

The *CFTR* gene, known for its role in CF, has a diverse range of mutations that differ across ethnic groups. In the Chinese population, the CFTR G970D mutation (c.2909G > A) is prevalent and has been identified in 12.1% of patients with CF [26]. Conversely, Japanese patients with CF often present with the Δ(G970-T1122) CFTR mutation [27], while the F508del mutation is predominantly observed in Europeans and their descendants, identified in approximately 70% of patients with CF [28]. Recently, Shen et al. (2023) underscored the notable geographic differences in the distribution of CFTR mutations among patients with CF across China [26]. Specifically, the G970D (c.2909G > A) variant is predominantly found in the northern and eastern regions, whereas the c.1766 + 5G > T and R553*(c.1657 C > T) variants are more commonly observed in the southern and eastern coastal areas [26]. In CBAVD, a condition linked to *CFTR*, the 5T variant exhibits considerable importance. In China, this variant is found in 46.58% of patients with CBAVD [29], whereas it is found in approximately 30% of the patients in Japan [30]. These investigations revealed a notable diversity in CFTR variants, which are significantly influenced by factors such as nationality, ethnicity, and CFTR-related diseases; this variability is evident even within different regions of China. However, the prevalence of pathogenic or likely pathogenic *CFTR* variants in the general Chinese population unaffected by CF or CFTR-RD has not been fully elucidated. Our findings indicate that 6.2% of unrelated Chinese men and 2.2% of unrelated Chinese women carried pathogenic/likely

pathogenic CFTR variants, with the K464N (c.1392G > T) variant being the most frequent.

The CFTR K464N variant (c.1392G > T) is rare in the Chinese population. The gnomAD database showed extremely low occurrence in European (non-Finnish) groups. This finding contrasts with that of Central Italy, where Lucarelli et al. (2017) reported a 0.2% frequency of this variant in patients with CF. However, this variant was not detected in northern Italian patients with CF [31]. Additionally, while the CFTR K464N variant is not listed in the CFTR2 database (https://cftr2.org/, accessed 2023 -1-17), it is listed in the CFTR France database as a heterozygous variant in one patient with CF [32]. The NCBI ClinVar database categorizes this variant as either pathogenic or likely pathogenic (https://www.ncbi.nlm.nih.gov/clinvar/variation/53240, accessed 2024-1-17). Considering the higher prevalence of this variant in the Chinese population without CF or CFTR-RD, further assessment is required to determine its pathogenic potential.

In terms of pregnancy outcomes, most women with CF have normal pregnancies; however, the risk of premature birth is significant, occurring in up to 24% of cases [34, 35]. Reduced lung function, CF-related diabetes, and immune changes are common risk factors for preterm birth [36]. Ramos et al. (2017) reported that CF in infants was also associated with an increased relative risk (95% CI) of 6.8 (1.7–26.5) for preterm birth [37]. To the best of our knowledge, there are currently no reports on the relationship between maternal or fetal *CFTR* genotype variants and preterm birth. Our study revealed that the CFTR K464N variant in infants, but not in mothers, was an independent risk factor for PTB after adjusting for twin pregnancy, gestational hypertension, anterior placenta, and placenta accreta (adjusted OR 3.39, $p = 0.007$).

While our study demonstrates a statistical association between fetal CFTR K464N variant and preterm birth, the underlying molecular mechanisms warrant further investigation. Recent studies show CFTR plays critical roles in placental function through multiple pathways. CFTR regulates aquaporins (particularly AQP9) that mediate placental fluid exchange, as evidenced by altered AQP9 functionality when CFTR expression decreases in preeclamptic placentas [38, 39]. Additionally, CFTR in the syncytiotrophoblast apical membrane facilitates ion transport and may influence nutrient exchange between mother and fetus [17].

The CFTR K464N variant affects both splicing mechanisms and protein function through its location in exon 10 [33]. The substitution of lysine with asparagine at position 464 occurs within the first nucleotide-binding domain, a critical region for channel function. This mutation may alter protein folding and trafficking to the cell membrane, leading to defective protein maturation and reduced channel activity [33]. These molecular alterations could disrupt ion transport and fluid homeostasis in placental tissues.

Notably, we found that mothers carrying fetuses with pathogenic/likely pathogenic CFTR variants had a significantly higher incidence of gestational diabetes (39.2%, 20/51) compared to mothers whose fetuses did not carry CFTR variants (20.8%, 82/395; $p = 0.003$). This association suggests that fetal CFTR variants may influence maternal glucose metabolism during pregnancy through altered placental function. Further studies are needed to determine whether CFTR variants directly affect glucose transport or indirectly influence maternal metabolism through other pathways.

Our findings contribute to the growing evidence that genetic variants play important roles in preterm birth susceptibility through multiple pathways. While previous studies have identified variants in inflammatory mediators (IL-6, IL-10, TNFα) and factors affecting progesterone receptor function and placental protein expression that contribute to preterm birth risk [40–43], the role of ion channels like CFTR has been underexplored. Understanding these mechanisms, particularly how K464N affects CFTR-mediated placental processes, could reveal new therapeutic targets for preventing adverse pregnancy outcomes in carriers of CFTR variants. Future research combining clinical data with functional genomics and placental studies would help elucidate these pathways, though investigating these mechanisms presents challenges due to limited access to fetal tissues and species differences in CFTR function.

Several studies have reported that the CFTR variants affect the incidence of neonatal diseases. Infants with CF often exhibit various symptoms and complications, including lung infections and inflammation [44], digestive problems [45], meconium ileus [46], failure to thrive [47], and cholestasis [48]. Carriers of CF have also been reported to have a high risk of respiratory failure, feeding difficulties, meconium obstruction, and jaundice [49]. Therefore, we investigated the association between pathogenic CFTR variants and neonatal weight, Apgar score at 10 min, respiratory distress, and hyperbilirubinemia. In our study, pathogenic/likely pathogenic *CFTR* variants in fetuses did not significantly correlate with newborn weight, Apgar score at 10 min, neonatal respiratory distress, or neonatal hyperbilirubinemia.

Our study has several limitations that should be considered when interpreting the results. First, the study cohort was predominantly composed of a Han Chinese population from the Zhejiang Province. Given the unique CFTR mutation patterns identified in Chinese and Caucasian populations and the evident regional variations in CFTR mutations among Chinese individuals, there is a potential constraint on the global applicability of our conclusions. Second, our sample size was relatively small, potentially

leading to statistical bias. Third, there may be issues with the sequencing accuracy for mutations in exon 9 of CFTR [50]. Due to the lack of clinical samples, we could not verify the K464N mutation using Sanger sequencing. In previous studies, we used Sanger sequencing to validate 416 single-nucleotide variants detected by WGS, which confirmed an impressive accuracy of 98% for these variants. Considering our study's regional focus, sample size, and possible sequencing errors, further research with a more diverse population, a larger sample size, and improved validation methods is essential to confirm our results and ensure their relevance and precision across various populations and genetic differences.

Conclusion

In conclusion, we have described the frequency of pathogenic/likely pathogenic CFTR variants in unrelated Chinese individuals without CF or CFTR-RD. Our findings suggest that the CFTR K464N variant in fetuses may be associated with an increased risk of preterm birth in this population. These observations provide insights into potential relationships between pathogenic CFTR variants and perinatal outcomes. Further research with larger and more diverse populations is needed to validate these findings and investigate the underlying mechanisms that may link fetal CFTR variants to preterm birth risk. This work highlights the potential importance of considering CFTR variants in understanding pregnancy outcomes in Chinese populations.

Supplementary Information

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Supplementary Material 1

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Author contributions

J.L., L.Z., and F.X. wrote the main manuscript text. C.L. and Q.Z. conducted the investigation. Q.Z. and W.C. performed formal analysis. S.Z. reviewed and edited the manuscript. F.J. conceptualized the study and supervised the project. All authors reviewed the manuscript.

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Data availability

The CFTR variation data presented in this paper has been deposited in the publicly accessible Genome Variation Map (accession number GVM000635) maintained by the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences. Clinical data are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

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