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REPRODUCTIVE PHYSIOLOGY AND DISEASE

An overview of *CFTR* mutation profiles and assisted reproductive technology outcomes in Chinese patients with congenital obstructive azoospermia

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Abstract

Purpose The cystic fibrosis transmembrane conductance regulator (CFTR) is the most common causative gene attributed to congenital obstructive azoospermia (OA). The aim of this study was to conduct an epidemiological survey of congenital OA patients, to screen for *CFTR* mutations, and to follow their pregnancy outcomes in assisted reproductive technology (ART).

Methods This cohort study enrolled congenital OA patients undergoing ART and whole-exome sequencing from January 2018 to September 2023. Semen parameters, sex hormones, and seminal plasma biochemistry were evaluated. *CFTR* mutations identified in OA patients were analyzed. In addition, the laboratory outcomes, clinical outcomes, and neonatal outcomes were compared between OA patients carrying two *CFTR* mutations and the others after surgical sperm extraction-intracytoplasmic sperm injection (ICSI) treatment.

Results A total of 76 patients with congenital OA were enrolled. *CFTR* mutations were identified in 35 (46.1%) congenital OA patients. A total of 60 *CFTR* mutation sites of 27 types were identified, and 10 of them were novel. The average frequency was 1.71 (60/35) per person. The most common mutation was c.1210-11T > G (25%, 15/60). After ICSI treatment, there were no statistically significant differences in laboratory outcomes, clinical outcomes, and neonatal outcomes between OA patients carrying two *CFTR* mutations ($n = 25$) and other OA patients ($n = 51$).

Conclusion Apart from the IVS9-5T mutation, the genetic mutation pattern of *CFTR* in Chinese OA patients is heterogeneous, which is significantly different from that of Caucasians. Although carrying two *CFTR* mutations or not had no effect on the pregnancy outcomes in OA patients after ICSI, genetic counseling is still recommended for such patients.

Keywords Congenital obstructive azoospermia · Cystic fibrosis transmembrane conductance regulator · Gene mutation · Intracytoplasmic sperm injection · Surgical sperm extraction

Introduction

Obstructive azoospermia (OA) accounts for 40% of cases of azoospermia and generally exhibits normal endocrine function and spermatogenesis [1]. Due to the absence of seminal vesicle components, OA patients have a lower semen volume and pH, as well as a lack of seminal plasma fructose [2]. Congenital OA is frequently linked to congenital absence of the vas deferens (CAVD), which is characterized by the absence of the vas deferens and partial or complete absence of the epididymis [3]. CAVD has been demonstrated to be correlated with mutations in the cystic fibrosis transmembrane regulator (CFTR), which is an integral membrane protein expressed predominantly in glandular epithelial cells in the respiratory, digestive, and reproductive tracts of vertebrates [4]. The mutations in *CFTR* affect the normal function of chloride channels, which interferes with the transference of chloride and water, leading to abnormal production of viscous fluid in the cells of the male reproductive tract, and eventually resulting in congenital vasovagal agenesis [5].

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Several studies have demonstrated a high prevalence of *CFTR* mutations among patients with OA or CAVD. A previous study found that nearly 40% of OA patients without vasectomy had at least one *CFTR* mutation [6], while a meta-analysis of 38 studies reported that at least one *CFTR* mutation was present in 78% of CAVD patients [4]. Among Caucasians with CAVD, IVS9-5T, R117H, and F508del are the most frequent *CFTR* mutations identified in OA patients, while these mutations, known as "hotspot mutations" due to their frequency in this population, are not widespread in Chinese or other East Asian populations [7], in which the mutations in *CFTR* exhibit high heterogeneity [8–10]. Thus, the guidelines recommended by the European Association of Urology might not apply to the Chinese population [11], and the genetic basis of OA linked to CAVD warrants further investigation. Additionally, few studies reported the effect of *CFTR* mutations on the clinical outcomes of OA patients.

Therefore, this study aimed to analyze the genetic mutation profiles of *CFTR* in the Chinese population with congenital OA, using an epidemiological survey of patients who underwent whole-exome sequencing (WES). Furthermore, the outcomes after assisted reproductive technology (ART) treatment in these patients were followed up, yielding supportive data for diagnostic and therapeutic methods.

Materials and methods

Study design and participants

Congenital OA patients who underwent ART treatment and WES at Reproductive Medicine Center, Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology between January 2018 and September 2023 were enrolled in the study. All participants underwent two sperm collections that were performed at least 2 months apart. Diagnosis of azoospermia was confirmed when no spermatozoa were detected after centrifugation with repeated semen examinations. Patients were identified as having OA after undergoing a thorough evaluation, including a medical history-taking, physical examination, and laboratory tests, and those with congenital OA displayed a normal testicular volume, an indurated epididymis, a normal concentration of plasma follicle-stimulating hormone (FSH), and a normal karyotype without a history of reproductive tract infections. This study was approved by the Ethical Committee of Tongji Hospital and informed written consent was provided by the participants.

Semen analysis and surgical sperm extraction

The routine semen examination protocol was well described previously. After 2–7 days of abstinence, fresh ejaculated semen was obtained by masturbation and kept in sterile containers for half an hour at 37 °C for liquefaction. Azoospermia exhibited a sperm count of 0 using computer-assisted sperm analysis and no spermatozoa have been detected after centrifugation with repeated semen examinations. The surgical procedure of sperm extraction was as previously reported [12]. After local anesthesia, the epididymal head was punctured with a fine needle. Sperm were aspirated and analyzed under a light microscope.

WES and mutation analysis

The details of the WES procedure were previously described [13]. Genomic DNA was extracted from peripheral blood samples from the patients. Subsequently, exons and splice sites were captured and enriched, and sequencing was performed on an Illumina HiSeq X-TEN platform (Illumina, San Diego, USA). Raw FASTQ files were aligned to the human genome reference sequence (hg19/GRCh37) with Burrows-Wheeler Alignment. Genome Analysis Toolkit was utilized to detect single nucleotide variants and indels, which were annotated by ANNOVAR. The functional prediction was performed by Sorting Intolerant From Tolerant (SIFT), PolyPhen-2, Mutation Taster, and Mutation Assessor.

Oocyte retrieval and embryo culture

Controlled ovarian stimulation protocols were performed as previously described [14]. When 2–3 dominant follicles with a diameter of ≥ 18 mm were observed, human chorionic gonadotropin was administrated for trigger. Cumulus-oocyte complexes (COCs) were retrieved via transvaginal ultrasound 36–38 h later. After exposure to hyaluronidase, COCs were degranulated mechanically and cultured for another 1–2 h before fertilization through spermatozoon injection. Pronuclei (PN) were assessed 17–18 h after fertilization. Embryos were cultured in G1-plus medium (Vitrolife, Gothenburg, Sweden) until day 3 and were subsequently transferred to G2-plus medium (Vitrolife, Gothenburg, Sweden) until they reached the blastocyst stage on day 5. The available embryos were either cryopreserved or transferred.

Outcome assessment

Semen evaluation consisted of semen volume and pH, alongside analysis of serum concentrations of sex hormones, including FSH, luteinizing hormone, estradiol, progesterone, testosterone, and prolactin. In addition, seminal plasma biochemical analyses were performed to assess the level of neutral alpha-glucosidase, fructose, zinc, and elastase.

The laboratory outcomes comprised embryo development parameters, such as oocyte maturation rate, normal and abnormal fertilization rates, 2PN cleavage rate, blastocyst formation rate, and available blastocyst rate. Clinical

outcomes included implantation rate, biochemical and clinical pregnancy rates, and early miscarriage rate. Neonatal outcomes were observed, showing data on gestational age, birth weight, method of delivery, gender distribution, and premature birth rate, among cases of live births. Computation details were previously well described with slight modifications [15, 16].

Statistical analyses

Statistical Package for the Social Sciences (SPSS 22.0, IBM, USA) was utilized for data analysis. Continuous variables were presented as medians (first quartile-third quartile) and compared using the Mann–Whitney *U* rank-sum test. Categorical variables were presented as % (*n*/*N*) and compared using the chi-squared test. *P* value < 0.05 was considered statistically significant.

Results

A total of 76 patients with congenital OA who underwent WES and surgical sperm extraction ICSI treatment were enrolled. Among the 46 patients undergoing ultrasound, 32 (70.0%) of them showed dysplasia or absence of vas deferens. No sperm was found under the microscope after centrifugation in at least two routine semen parameter analyses. The majority of patients (61.8%, 47/76) displayed acidic semen (pH < 7.2), and nearly half (48.7%, 37/76) showed a smaller volume of semen (< 1.5 mL). Furthermore, the median values of both neutral α-glucosidase and fructose in seminal plasma fell below the standard reference range (Table S1). Since *CFTR* mutation-associated OA is an autosomal recessive disorder, we classified OA patients into two groups based on the number and type of *CFTR* mutations: the mutation group (OA patients with two *CFTR* mutations, $n = 25$) and the control group (the other OA patients, $n = 51$). Compared to OA patients in the control group, OA patients in the mutation group had lower semen volume ($P = 0.002$), pH ($P < 0.001$), and neutral α-glucosidase ($P = 0.012$, Table 1).

CFTR mutations were identified in 35 congenital OA patients, of which 10 (28.5%, 10/35) carried single heterozygous mutation, 20 (57.1%, 20/35) carried compound heterozygous mutations, and 5 (14.3%, 5/35) carried homozygous mutations (Fig. 1A). A total of 60 *CFTR* mutation sites of 27 types were identified and the average frequency was 1.71 per person. The three most frequent mutations were c.1210-11T > G (25.0%, 15/60), c.1210-12T[5]-c.1210-34TG[13] (21.7%, 13/60), and c.1210-12T[5]-c.1210-34TG[12] (10.0%, 6/60). Almost half (40.0%, 14/35) of the patients carried at least one IVS9-5T mutation, whereas IVS9-7T and IVS-9T mutations were not identified. In addition, the genetic spectrum of *CFTR* mutations in the enrolled Chinese OA patients showed high heterogeneity. Among the 27 *CFTR* mutations carried by the patients, 18 (66.7%, 18/27) were missense mutations, 1 (3.7%, 1/27) was a nonsense mutation, 4 (14.8%, 4/27) were frameshift mutations, and 4 (14.8%, 4/27) were splice mutations (Fig. 1B). Except for the R31C mutation and splice mutations, the

Table 1 Comparison of semen parameters, sex hormones, and seminal plasma biochemical

Parameters		Control group ($n = 51$)	Mutation group ($n = 25$)	*P* value
Female age (years)		29 (27–32)	28 (27–29)	0.120
Male age (years)		30 (28–33)	29 (28–32)	0.454
Semen parameters	Volume (mL)	1.8 (0.9–3.1)	1.0 (0.6–1.4)	0.002*
	pH	7.2 (6.4–7.5)	6.4 (6.4–6.4)	< 0.001*
Sex hormones	FSH (mIU/mL)	4.3 (3.2–5.0)	3.8 (2.5–5.4)	0.978
	LH (mIU/mL)	3.4 (2.6–4.9)	3.8 (2.9–4.6)	0.480
	E2 (pg/mL)	30.1 (22.8–39.2)	31.2 (23.6–42.0)	0.526
	T (ng/dL)	338 (267–423)	337 (273–530)	0.817
	PRL (ng/mL)	9.8 (7.3–13.1)	9.1 (8.0–12.1)	0.977
Seminal plasma biochemistry analysis	Neutral alpha-glucosidase (mU)	8.0 (2.2–33.3)	1.0 (0.4–7.8)	0.012*
	Fructose (μmol)	15.2 (0.6–43.0)	7.0 (2.1–13.2)	0.788
	Zinc (μmol)	7.5 (3.8–9.7)	8.8 (4.4–13.8)	0.308
	Elastase (ng/mL)	286 (131–1900)	552 (197–1327)	0.746

OA, obstructive azoospermia; *FSH*, follicle-stimulating hormone; *LH*, luteinizing hormone; *E2*, estradiol; *T*, testosterone; *PRL*, prolactin

Mutation group: OA patients carrying two CFTR mutations

Control group: the other OA patients

* *P* value <0.05 was considered statistically significant

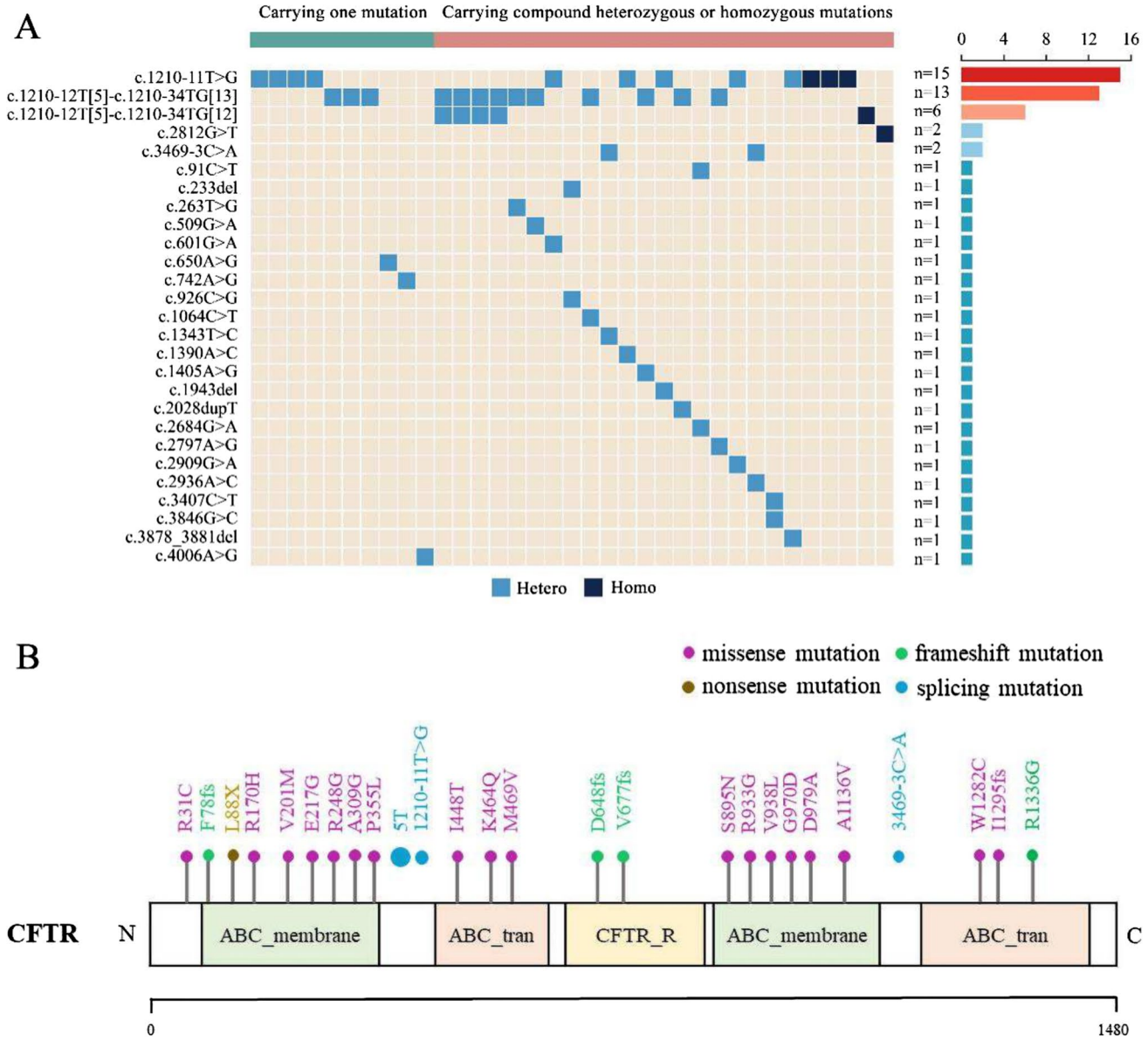

Fig. 1 Genetic mutation profiles of *CFTR* gene in congenital OA patients. **A** *CFTR* mutation carried by patients with congenital OA. A total of 27 *CFTR* mutations were identified in 35 patients. Ten patients carried a heterozygous mutation, 20 patients carried a compound heterozygous mutation, and 5 patients carried a homozygous mutation. The most frequent *CFTR* mutation was c.1210-11T > G. **B** Schematic diagram of CFTR protein domains. Of these 27 *CFTR* mutations, 18 were missense mutations, 4 were frameshift mutations, 4 were splice mutations, and 1 was a nonsense mutation. The majority of the mutations were located in the functional domains of the CFTR protein

other mutations were located in the protein structural domains of CFTR protein, thus affecting the structure and function of CFTR protein. Based on the gnomAD database, 10 of the 27 *CFTR* mutations were novel, including c.233del:p.F78fs, c.263T > G:p.L88X, c.742A > G:p.R248G, c.1390A > C:p.K464Q, c.1943del:p.D648fs, c.2797A > G:p.R933G, c.2028dupT:p.V677fs, c.2797A > G:p.R933G, c.3407C > T:p.A1136V, c.3878_3881del:p.I1295fs, and c.4006G > A:p.D1336N. According to four in silico functional prediction models (SIFT, PolyPhen-2, Mutation Taster, and Mutation Assessor), 15 of the 18 (83.8%) missense mutations were identified as deleterious mutations in two or more databases (Table 2).

The impact of the carrier status of *CFTR* mutations on ICSI outcomes after surgical sperm extraction in OA patients was analyzed. No statistically significant differences were observed in baseline characteristics and ovarian response between the patients in the mutation group and the control group (Table S2). The control group underwent a total of 60 fresh cycles of oocyte retrieval, whereas

Table 2 In silico functional prediction of *CFTR* mutations

Amino acid changes	Nucleotide changes	Frequency	In silico functional prediction			
			SIFT	PolyPhen-2	Mutation Taster	Mutation Assessor
c.91C > T	p.R31C	0.001640	Damaging	Probably damaging	Disease causing	Low
c.233del	p.F78fs	-	-	-	Disease causing	-
c.263T > G	p.L88X	-	-	-	Disease causing	-
c.509G > A	p.R170H	0.0004791	Damaging	Probably damaging	Disease causing	Low
c.601G > A	p.V201M	0.0002157	Damaging	Possibly damaging	Disease causing	Low
c.650A > G	p.E217G	0.004583	Damaging	Benign	Disease causing	Low
c.742A > G	p.R248G	-	Damaging	Possibly damaging	Disease causing	Medium
c.926C > G	p.A309G	0.00004881	Tolerable	Benign	Disease causing	Low
c.1064C > T	p.P355L	0.000007965	Damaging	Probably damaging	Disease causing	Medium
c.1210-12T[5]-c.1210-34TG[12]	-	0.02469	-	-	-	-
c.1210-12T[5]-c.1210-34TG[13]	-	0.02469	-	-	-	-
c.1210-11T > G	-	0.008495	-	-	-	-
c.1343T > C	p.I448T	0.000008208	Damaging	Benign	Disease causing	High
c.1390A > C	p.K464Q	-	Damaging	Probably damaging	Disease causing	High
c.1405A > G	p.M469V	0.00001194	Tolerable	Probably damaging	Disease causing	Low
c.1943del	p.D648fs	-	-	-	Disease causing	-
c.2028dupT	p.V677fs	-	-	-	Disease causing	-
c.2684G > A	p.S895N	0.0003255	Tolerable	Benign	Polymorphism	Neutral
c.2797A > G	p.R933G	-	Damaging	Probably damaging	Disease causing	Medium
c.2812G > T	p.V938L	0.00002031	Tolerable	Probably damaging	Disease causing	Low
c.2909G > A	p.G970D	0.00001221	Damaging	Probably damaging	Disease causing	High
c.2936A > C	p.D979A	0.00003583	Damaging	Probably damaging	Disease causing	High
c.3407C > T	p.A1136V	-	Damaging	Probably damaging	Disease causing	Medium
c.3469-3C > A	-	0.00003994	-	-	-	-
c.3846G > C	p.W1282C	0.000000407	Damaging	Probably damaging	Disease causing	Low
c.3878_3881del	p.I1295fs	-	-	-	Disease causing	-
c.4006G > A	p.D1336N	-	Tolerable	Benign	Disease causing	Low

Population frequency data from gnomAD database

SIFT, Sorting Intolerant From Tolerant

the mutation group underwent 27 cycles. After excluding sperm donation cycles, there were no significant differences in the laboratory outcomes between the two groups, including primary outcomes, such as normal fertilization rate (65.6% in the mutation group *vs.* 66.3% in the control group, $P = 0.823$) and blastocyst formation rate (64.7% in the mutation group *vs.* 59.9% in the control group, $P = 0.275$), as well as other secondary outcomes, consisting of oocyte maturation rate, abnormal fertilization rate, oocyte cleavage rate, and available blastocyst rate ($P > 0.05$, Table 3). Due to the cancellation of fresh embryo transfer, all fresh and frozen embryo transfer cycles were included in the study. Eighty-eight embryos in 80 cycles were transferred in the control group, while the mutation group received 42 embryos in 38 transfer cycles. The clinical pregnancy rate in the mutation group was 55.3% comparable to 56.3% in the control group ($P = 0.920$), and the rates of embryo implantation, biochemical pregnancy, and early abortion were similar in both groups ($P > 0.05$, Table 3). At the end of this study, the control group yielded 27 live births in 25 cycles, including two twin pregnancies. In comparison, the mutant group produced 18 live births in 17 cycles, with one twin pregnancy occurrence. There were no substantial variations related to gestational age, birth weight, mode of delivery, gender ratio, and preterm birth rate ($P > 0.05$, Table S3). Notably, no birth defects were observed in either group.

Discussion

In this study, we enrolled 76 congenital OA patients undergoing ICSI and WES. Ten heterozygous *CFTR* mutations, 20 compound heterozygous mutations, and 5 homozygous heterozygous mutations, of which 10 were novel, were identified in 35 patients. It was found that except for the IVS9-5T mutation, the genetic mutation pattern of

Table 3 Laboratory and clinical outcomes of OA couples in ART cycles

Parameters	Control group ($n=51$)	Mutation group ($n=25$)	*P* value
Laboratory outcomes			
No. of fresh cycles	60	27	
No. of non-sperm donation cycles	57	27	
No. of oocytes retrieval	798	433	
Average no. of oocytes retrieval	14 (10–19)	17 (12–20)	0.210
Oocyte maturation rate	78.6 (627/798)	80.6 (349/433)	0.402
Normal fertilization rate	66.3 (394/594)[1]	65.6 (229/349)	0.823
Abnormal fertilization rate	6.7 (40/594)	7.4 (26/349)	0.677
2PN cleavage rate	98.7 (389/394)	97.8 (224/229)	0.510
No. of extended culture day 3 embryos	319	190	
Blastocyst formation rate	59.9 (191/319)	64.7 (123/190)	0.275
Available blastocyst rate	49.8 (159/319)	54.7 (104/190)	0.285
Clinical outcomes			
No. of embryo transfer cycles	80	38	
No. of embryos transferred	88	42	
No. of gestational sac	47	22	
Implantation rate	53.4 (47/88)	52.4 (22/42)	0.174
Biochemical pregnancy rate	66.3 (53/80)	63.2 (24/38)	0.742
Clinical pregnancy rate	56.3 (45/80)	55.3 (21/38)	0.920
Early miscarriage rate	20.0 (9/45)	8.7 (2/21)	0.480
No. of live birth[2]	23 + 2	16 + 1	
No. of ongoing pregnancy	9	2	

Rates were presented as % (*n*/*N*). *OA*, obstructive azoospermia; *PN*, pronuclei

Mutation group: OA patients carrying two CFTR mutations

Control group: the other OA patients

[1]33 of 627 MII oocytes were frozen

[2]No. of live birth rate was presented as no. of singleton live births + no. of twin live births

CFTR in the Chinese OA population was different from the "hotspot mutations" pattern in the Caucasian population, which initially revealed the epidemiologic characteristics of *CFTR* mutations in the Chinese OA population. Additionally, ART outcomes, including the laboratory, clinical, and neonatal outcomes of OA patients with two *CFTR* mutations and other OA patients, were compared after ICSI treatment, and no statistically significant differences were found between the two groups.

CAVD is the most common etiology of congenital OA, while the exact mechanism of CAVD remains unclear. CFTR protein regulates the transport of chloride and bicarbonate, which affects the ion concentration, pH, and flow rate of body fluids [17]. *CFTR* mutations cause abnormal expression or structure of the CFTR proteins, leading to increased protein concentration and decreased flow rate in the reproductive tract, and ultimately resulting in atrophy, degeneration, and disappearance of the vas deferens [18]. It is supported by the existence of multiple subtypes of clinical phenotypes in CAVD patients. Although the specific mechanism of vasovagal agenesis has not been clearly clarified, there is no doubt about the close association between polymorphisms of *CFTR* mutations and OA.

CFTR mutations can be classified into six categories according to the severity of the defect in CFTR protein function [19, 20]. Classes I to III are severe mutations, usually associated with cystic fibrosis, while classes IV to VI are considered mild mutations, in which the expressed CFTR proteins still retain some of the normal protein function and are associated with CAVD. Cystic fibrosis is one of the most common autosomal recessive disorders in Caucasians and is complicated by CAVD in approximately 95% of patients. Although the prevalence of cystic fibrosis is significantly lower in non-Caucasian populations, the prevalence of CAVD does not vary significantly across regions and ethnicities [21]. Previous studies have shown that IVS9-5T polymorphism in *CFTR* is the most prevalent in CAVD [22, 23]. In our study, *CFTR* mutations were identified in nearly half of OA patients, and 40% (14/35) of OA patients with *CFTR* mutations carried at least one IVS9-5T,

which is consistent with the previous conclusion. It was previously reported that *CFTR* mutations were not found in more than half of CAVD patients after genetic screening [24, 25]. Among the 46 patients who underwent ultrasound examination in the current study, 32 (70.0%) showed the absence of at least one side of the vas deferens, and nearly one-third of them (31.3%, 10/32) did not carry any *CFTR* mutations, indicating that *CFTR* mutations are not always closely consistent with CAVD. In addition, owing to lower semen volume, pH, and neutral α-glucosidase in the patients from the mutation group, we speculate that homozygous or compound heterozygous *CFTR* mutations carried by OA patients could potentially contribute to abnormal semen parameters, aiding in the differential diagnosis of *CFTR* mutation-related OA.

Due to the low prevalence of cystic fibrosis in the Chinese population, large-scale *CFTR* mutation screening studies in this population are lacking. However, given the notable correlation between *CFTR* mutations and OA, screening studies for the *CFTR* mutation have been conducted on Chinese OA patients in recent years. Li et al. discovered 30 *CFTR* mutations, with 9 novel mutations included, in 73 Chinese CAVD patients [26]. Yang et al. identified 8 *CFTR* mutations in 19 Chinese CAVD patients, four of which were previously unknown [27]. In 66 CAVD patients, Bai et al. detected seven mutations in the promoter region of *CFTR* [28]. In a separate study of 72 patients, 28 mutations, including 5 novel ones, were identified [29]. In another study, 38 CAVD patients had 15 different *CFTR* mutations, four of which were previously unknown [8]. In addition, in a larger cohort of 276 CAVD patients, Luo et al. found 63 *CFTR* mutations, 13 of which were novel [22]. In the current study, 27 *CFTR* mutations were identified within a cohort of 76 OA patients using WES, with 10 being previously unknown. Moreover, the most common mutations were IVS9-5T and c.1210-11T > G. In summary, the genetic spectrum of *CFTR* mutations in Chinese OA patients is highly diverse, similar to previous studies in Indian patients [30, 31], whereas the high genetic heterogeneity found in Chinese patients differs from the hotspot mutation pattern in Caucasians, where several *CFTR* mutations occur at high frequencies, such as F508del, which causes damage to the synthesis and folding process of CFTR protein, and R117H, which affects the functioning of the chloride channel [32, 33]. Therefore, in Western countries, it is suggested to screen for hotspot mutations in Caucasian OA patients [34, 35]. However, due to the high heterogeneity in the Chinese OA population, WES may be a more suitable method for *CFTR* mutation screening than panels. Furthermore, while many mutations may be mild, there remains a possibility of offspring with cystic fibrosis through Mendelian inheritance patterns, and the likelihood of CAVD in the offspring is also elevated. Thus, genetic counseling before ART treatment is highly recommended.

Although *CFTR* mutations are considered the genetic cause of congenital OA, it remains unclear whether spermatogenesis and sperm function are affected. Xu et al. revealed that CFTR inhibitors and antibodies greatly reduced capacitation by inhibiting $HCO3^-$-dependent biological processes, implying that *CFTR* mutations may impair sperm fertilization capability [36]. Additionally, patients with cystic fibrosis displayed inferior sperm quality and a lower likelihood of obtaining available spermatozoa through biopsy in comparison to individuals with solely CAVD phenotype [37]. A clinical study reported that CAVD patients had a notably higher risk of miscarriage and stillbirth compared to other OA patients, along with a reduced rate of live births after ICSI [38]. In contrast, several studies reported no difference in clinical pregnancy rate after ICSI between CAVD and non-CAVD groups, in which the clinical pregnancy rate ranged from 38 to 55.2% in the CAVD group [39–41]. In this study, 76 OA patients were categorized into two subgroups depending on whether they carried bilateral *CFTR* mutations or not. It was shown that there were no statistically significant differences in laboratory, clinical, and neonatal outcomes between the two groups following ICSI. Moreover, a total of 87 fresh cycles of oocyte retrieval and 118 cycles of embryo transfer yielded a clinical pregnancy rate of 55.9% (66/118) per transfer cycle, consistent with prior studies. Overall, ICSI after sperm extraction proves to be a highly successful and feasible option for OA patients. Nevertheless, due to variances in study design, sample size, and study population compared to prior research, imperative multicenter nationwide studies with more significant sample sizes are necessary.

However, there are limitations in this study. It was a retrospective study conducted at a single center with a limited sample size and geographic scope. Therefore, a subsequent multicenter study with a more extensive sample size is necessarily urgent. Furthermore, not all OA patients involved in this research displayed CAVD phenotypes. Consequently, further investigation is necessary to assess the genetic spectrum of *CFTR* mutations in CAVD patients with strict entrance criteria.

In conclusion, 27 *CFTR* mutations were identified in 76 congenital OA patients, of which 10 mutations were novel, clarifying that *CFTR* mutations are the genetic etiology of OA. Moreover, apart from the IVS9-5T mutation, the genetic heterogeneity of Chinese patients differs from the hotspot mutation pattern in Caucasians, and the Caucasian-based *CFTR* mutation screening strategy might not apply to Chinese OA patients. Furthermore, although there was no statistically significant difference in pregnancy outcomes after ICSI despite the presence of pathogenic *CFTR* mutations, genetic counseling is recommended for these patients before ART treatment.

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Author contribution Conceptualization: LZ and LJ. Sample collection: RL, RM, XW, and LG. Experiment conduction: MW, JZ. Formal analysis: MW, JZ. Funding acquisition: LZ and LJ. Writing—original draft: MW. Writing—review and editing: LZ, YC, and LJ.

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

Declarations

Ethics approval and consent to participate This study was approved by the Ethical Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology with written informed consent provided by the participants.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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