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Androgen receptor gene mutations are associated with male infertility in Northeast China: Clinical features and identification of two novel mutations

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Abstract

The purpose of this study was to investigate the prevalence of androgen receptor gene mutations in idiopathic male infertility in north-east China and to analyse the relationship between genotype and phenotype. Cohorts of 400 male patients with idiopathic infertility and 200 fertile controls were recruited. Clinical investigations were carried out by experienced andrologists. Targeted exome sequencing was used to detect androgen receptor gene mutations, and reproductive hormone levels were measured. We found four mutations in 8/400 patients (2%) and no mutations in controls. In addition, two recurrent mutations (p.S176R and p.A403V) were found in four patients and two patients respectively. Of four mutations, two (p.S233F and p.H715N) represented novel mutations. Almost all of the men with androgen receptor gene mutations showed smaller testicular volumes. However, the reproductive hormone levels were normal or lightly higher in seven men with mutation, apart from one man (P24) who had high follicle-stimulating hormone and luteinising hormone levels but a low testosterone level. In north-east China, there was a higher androgen receptor gene mutation rate among these infertile men, and the mutation of p.S176R deserved more attention particularly. Hormone levels and clinical phenotypes did not help in screening patients at risk of mutations.

KEYWORDS
androgen receptor, male infertility, novel mutation, recurrent mutation

1 | INTRODUCTION

Infertility affects 10%–15% of couples of childbearing age and has become a worldwide health problem (Krausz et al., 2006). Male infertility is a multifactorial disease with complex causes. Genetic abnormalities leading to the disease are responsible for 15%–30% of cases (Tahmasbpour, Balasubramanian, & Agarwal, 2014). Increasing numbers of genetic factors leading to male infertility have been reported with the most common causes being chromosomal abnormalities and Y chromosome microdeletions. Gene mutations including mutation of cystic fibrosis transmembrane receptor gene (CFTR), mitochondrial DNA (mtDNA) mutations and endocrine disorders caused by genetic factors have been reported recently attracting researcher's attention. Many genes are closely linked with spermatogenesis and the male reproductive system, and their mutations and polymorphisms can lead to male infertility (Tüttelmann & Simoni, 2008).

The human androgen receptor (AR) is a ligand-activated transcription factor. Encoded by a single copy gene located on Xq11–12, it is composed of eight exons, encoding a protein of 920 amino acids (Wang, Gong, Wang, & Qin, 2017). Like other nuclear receptors, the AR protein contains four major domains, the *N*-terminal

transactivation domain (TAD, exon 1), the DNA-binding domain (DBD, exons 2 and 3), the hinge region and the ligand or androgen-binding domain (LBD, exon 4–8; Brinkmann, 2001). AR gene mutations can lead to androgen insensitivity syndrome (AIS), which has different types that can cause various phenotypes in men with an XY genotype. In complete AIS (CAIS), the individuals have a female phenotype and show female external genitalia. The patients with partial AIS (PAIS) show genital ambiguity while males with mild AIS (MAIS) have normal male phenotype with or without normal spermatogenesis (Ferlin et al., 2006).

Exon 1 in the AR gene, as the longest coding region, plays a significant role in regulating AR activity and encodes roughly 58% of the protein (Philibert et al., 2010). According to the AR gene mutations database (https://www.androgendb.mcgill.ca, last updated 10 September 2014), approximately 20.7% of all mutations have been reported in exon 1, among which only 9.6% (mainly point mutations) result in the MAIS phenotype.

Previously, various mutations and polymorphisms in AR gene have been identified to cause impaired spermatogenesis and are associated with male infertility (Lund et al., 2003; Massin et al., 2012; Mou & Gui, 2016). However, a few studies have shown that AR variants have nothing to do with male infertility in some ethnic population (Badran et al., 2009; Singh et al., 2006). To identify the mutations of AR gene in infertile men in north-east China, we collected 400 patients diagnosed with male infertility and sequenced all exons of the *AR* gene for them. Our results may be helpful in diagnosing the aetiology of idiopathic male infertility and useful for genetic counselling.

2 | MATERIALS AND METHODS

2.1 | Patients and controls

Four hundred patients aged 21–41 years were referred between January 2014 and December 2016, from the Centre for Reproductive Medicine (the First Hospital of Jilin University, Changchun, Jilin province, P. R. China) for molecular analysis. All patients had experienced at least 1 year of infertility. Experienced andrologists first performed basic exclusionary aetiological examinations on the patients. These excluded causes including overt virilisation disorders (e.g., hypospadias and gynecomastia) and other genetic (e.g., chromosomal aberrations and AZF microdeletions), endocrine (e.g., hypogonadotropic hypogonadism and prolactinoma), infectious or obstructive causes (e.g., congenital bilateral aplasia of the vasa deferentia). Questionnaires about family history and lifestyle habits regarding libido and shaving frequency were also collected.

Routine testing of circulating reproductive hormone levels in all patients included measures of luteinising hormone (LH), follicle-stimulating hormone (FSH), testosterone (T) and oestradiol (E_2) by electrochemiluminescent immunoassays using a Elecsys® 2010 Serum Chemistry Analyzer (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Normal ranges of hormone levels were as follows: FSH, 1.5–12.4 IU/L; LH, 1.7–8.6 IU/L; T, 9.9–27.8 nmol L^{-1}; and E_2, 27.96–155.92 pmol L^{-1}. Semen analyses were performed on two different occasions, according to the guidelines of the World Health Organization (WHO, 2010). Two hundred of the patients showed azoospermia (no spermatozoa in the ejaculate even after centrifugation), and 200 showed oligozoospermia (sperm count $<15 \times 10^6$ ml^{-1}).

Two hundred caucasian males served as controls. They had no adverse reproductive history and had given birth to at least one healthy child. These controls, as well as the subjects, underwent routine hormone testing and genetic testing. Informed consent to participate in the study was signed in advance by both patients and controls, and this study was approved by the Chinese Association of Humanitarianism and Ethics.

2.2 | Targeted exome sequencing

Targeted next-generation sequencing was carried on 600 patients and controls, using an Illumina MiSeq platform and an in-house targeted gene panel (Peking Medriv Academy of Genetics and Reproduction, Peking), which includes 25 NOA-associated genes (AR gene included). Genomic DNA was extracted from peripheral blood using the Tiangen blood DNA extraction mini kits (Beijing Tiangen Biotech Co., Ltd., China). Capture probes were also prepared based on those NOA-associated genes. Fragments with 3′–5′ linkers and short fragments of low quality were removed using Cutadapt (https://pypi.python.org/pypi/cutadapt) and FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The Burrow-Wheeler Aligner was used to align pre-processed clean reads and the hg19 human reference sequence. Duplicated reads from library and PCR preparation were removed with Picard tools. The SNVs and Indel variations in the pre-processed sequence were analysed using the Genome Analysis Tool Kit (https://www.broadinstitute.org/gatk). Quality inspection was performed on the obtained sequence data through detection indexes including the alignment rate, duplication rate and coverage rate with at least 20× sequencing depth. For further analysis of SNVs, ANNOVA (annovar.openbioinformatics.org/en/latest/) algorithms were used to predict the degree of damage of genetic variants to protein function. Variants in intronic regions, regulatory regions and nonregulatory intergenic regions were removed.

In the study of variants in exonic regions, synonymous mutations were first filtered out, and then some variants with unknown clinical significance were removed. At the same time, variants of the population reported in public databases including the 1,000 Genomes Project, Exome Variant Server, Exome Aggregation Consortium and the dbSNP database with a population frequency greater than 1% were also excluded. As our research objective, the remaining variants were also considered for correlation with each patient's clinical phenotype. The 100% alignment rates were >95%. The coverage rates with at least 20× sequencing depth were 92%–99.9% and 100% duplication rates were <20%. In the target regions, the mean coverage was >80×.

TABLE 1 Androgen receptor mutations identified in patients with idiopathic male infertility

Variant no.	Exon	Sequence variant	Amino acid change	Patients, *n*	Reported	rs Number	Sample ID
1	1	c.C528A	p.S176R	4	Yes	rs777131133	P15,P24,P47,P61
2	1	c.C698T	p.S233F	1	No	/	P66
3	1	c.C1208T	p.A403V	2	Yes	rs772490323	P39,P52
4	4	c.C2143A	p.H715N	1	No	/	P58

Note. *n*: number.

2.3 | AR gene mutation predictions

In this study, our results mainly focussed on the relationship between AR gene mutations and idiopathic infertility. The mutations in the other 57 genes have not been studied in this paper. According to the NCBI reference sequence (NM_000044.3), the latest version of the AR gene mutation database (https://androgendb.mcgill.ca) adapted to naming genomic and proteomic factors was used. The prediction of the effects of the identified mutations on protein function was based on two softwares, PolyPhen-2 (https://genetics.bwh.harvard.edu/pph2/) and SIFT (https://sift.jcvi.org/). PolyPhen-2 software was used to predict the effect of missense mutations on protein function according to the score. Thus, a score of >0.85 indicates that the mutation is probably damaging, a score of 0.15–0.85 is considered to be possibly damaging, and a score <0.15 is considered a benign mutation. SIFT software is used to predict the effect of a single amino acid substitution in a protein sequence on disease. The mutation with a SIFT score <0.05 is considered to be pathogenic, and those with a score ≥0.05 are considered to be benign.

3 | RESULTS

To study the relationship between AR gene mutations and idiopathic male infertility, this study focused on genetic variants in the exonic region of the AR occurred in 400 patients with idiopathic infertility and 200 controls of normal fertility. Among the 400 patients, eight (2%) were detected as carrying missense variants. There were no variants in the control cases. Four different AR gene mutations were identified in the eight cases with variants, including two that were novel (Table 1). The p.S176R missense mutation has been reported in the ExAC database previously; the Allele Frequency (AF) was 0.001128. This was detected in two patients with azoospermia and in two with oligozoospermia. Another variant of p.A403V was found in two patients: one with azoospermia and other with oligozoospermia. It has also been described in the ExAC database; the AF was 4.844e−05. The two patient-specific missense variants (p.S233F and p.H715N) were not found in the dbSNP135 database, 1,000 Genome Project database or ExAC database. The two novel variants were found in two patients with oligozoospermia. According to SIFT and PolyPhen-2.0 software, the two variants (p.S176R and p.S233F) were predicted to be damaging to the protein's function. The p.A403V and p.H715N missense mutations were predicted to be damaging according to PolyPhen-2.0 analysis, while they could be tolerated according to SIFT analysis (Table 2).

Two separate semen analyses in the patients with AR gene mutations showed complete azoospermia in three of them and oligozoospermia in five. Physical examination by the andrologists showed normal male characteristics in terms of penile anatomy and pubic hair in the eight patients carrying AR gene variants. Scrotal colour Doppler ultrasonography of the seven patients (P15, P24, P47, P61, P39, P52 and P58) with missense mutations revealed small testes in the scrotal sac, while P66 harbouring mutation of p.S233F showed normal testicular volumes (Left, 15 ml and Right, 16 ml). The hormonal profile of one patient (P24) carrying the p.S176R mutation was obviously abnormal. Compared with the other seven patients, he exhibited high FSH and LH levels and a low T level. Reproductive hormone levels were normal or slightly higher than normal in the remaining seven patients with AR gene mutations (Table 3).

4 | DISCUSSION

AR has been reported to be a steroid receptor in the nuclear receptor superfamily and is mainly localised in the nucleus of the following cells: sertoli cells, peritubular myoid cells and periarterial cells,

TABLE 2 List of missense variants predicted to be functionally significant by SIFT and PolyPhen 2.0 programs

			SIFT		PolyPhen-2.0	
Variant no.	Nucleotide change	Amino acid change	Score	Prediction	Score	Prediction
1	c.C528A	p.S176R	0.01	Deleterious	0.997	Probably damaging
2	c.C698T	p.S233F	0	Deleterious	0.998	Probably damaging
3	c.C1208T	p.A403V	0.06	Tolerated	0.997	Probably damaging
4	c.C2143A	p.H715N	0.31	Tolerated	0.842	Possibly damaging

TABLE 3 Clinical and hormone profile of patients with male infertility with androgen receptor missense mutations

Sample ID	Age (years)	Sperm count (mill./ml)	FSH (IU/L)	LH (IU/L)	*T* (nmol L^{-1})	E_2 (pmol L^{-1})	Left testicular volume (ml)	Right testicular volume (ml)
P15	32	0	4.7	4.8	22.7	156.78↑	8	8
P24	33	0	22.9↑	10.8↑	4.9↓	108.71	9	9
P47	36	0.08	13.61↑	6.39	17.21	65.33	12	12
P61	25	0.58	3.9	8.3	15.4	147.86	5	5
P66	29	0.75	8.3	8.2	20.8	117.04	15	16
P39	23	0	13.99↑	7.65	21.66	140.93	12	12
P52	29	2.35	8.6	3.8	12.6	101.51	10	10
P58	26	8.76	3.4	5.8	18.8	160.67↑	12	12

Note. E_2: estradiol (27.96–155.92 pmol L^{-1}); FSH: Follicle-stimulating hormone (1.5–12.4 IU/L); LH: luteinising hormone (1.7–8.6 IU/L); T: testosterone (9.9–27.8 nmol L^{-1}); ↑: elevated; ↓: decreased.

Leydig cells and fibroblasts (Gur & Timurkaan, 2012; Suárez-Quian, Martínez-García, Nistal, & Regadera, 1999; Timurkaan, Gur, & Karan, 2012; Van Roijen et al., 1995). Studies using mouse *AR* gene knockout models found that *AR* gene defects can cause insufficient androgen action, leading to spermatogenic dysfunction by blocking the meiosis process of spermatogenesis (Chang et al., 2004; De Gendt et al., 2004). AR gene mutation is X-linked recessive inheritance, the incidence rate is 1:60,000 in newborns, and can be as high as 2% in infertile men according to the previous reports (Hiort et al., 2000). This study collected 400 cases of idiopathic infertile men for sequencing of all coding regions of the AR gene. It showed a prevalence of 2% in men with idiopathic infertility in north-east China, which similar to previous studies. This confirms that some forms of male infertility with unknown aetiology might be caused by mutations in the AR gene.

We found four different AR gene mutations (p.S176R, S233F, A403V and H715N) among the eight cases with variants, including two (S233F, H715N) that were novel. Of the four mutations, three (75%) were localised in exon 1 (TAD), and one (25%) in exon 4 (LBD). AR gene point mutations were the most important pathogenic mechanism in all patients in this study, and these could lead to substitutions in amino acid sequences and affect the function of the corresponding receptor protein.

The most frequent mutation in the study was p.S176R that recurring in four cases. The other recurrent mutation was p.A403V occurring in two patients. In the previous studies, most of the pathogenic mutations detected in male infertility were sporadic. To different these, our study rarely reported the above two recurrent missense mutations in the patients. It was more indicative of the pathogenicity of the mutations.

Interestingly, the recurrent mutation of p.S176R was identified in two patients (P15, P24) diagnosed with azoospermia and in two with severe oligozoospermia (P47, P61). This observation differed from previous reports in which three patients with the same mutation showed clinical manifestations of PAIS. Similarly, the patients of P39 and P52 in this study were shown to harbour a p.A403V mutation and were diagnosed with azoospermia and oligozoospermia, respectively, whereas a previous study found this mutation to be associated with PAIS (Wang et al., 2017). There have been many reports about the identical mutations in the AR gene but with the carriers showing different clinical phenotypes. Some reports have found that different members carrying the same mutation can have different clinical manifestations even within the same family. At present, 45 different AR gene mutation types have been included in the existing database, and these can lead to a variety of clinical phenotypes (Gottlieb, Beitel, Nadarajah, Paliouras, & Trifiro, 2012). This might be caused by somatic mosaicism (Gottlieb, Beitel, & Trifiro, 2001), the combination of some genes, and the complex causes of AIS involving auxiliary regulatory factors (Jääskeläinen, 2012).

The androgen sensitivity index (ASI) is derived by multiplying LH and T levels. Hiort et al believed that use of the ASI might help in identifying high-risk populations carrying AR gene mutations from a number of patients with androgen insensitivity syndrome. They found that patients with no significant increase in ASI levels were unlikely to carry AR gene mutations (Hiort et al., 2000). However, our results did not support this view. In our study, only P24 harbouring the p.S176R mutation exhibited high FSH and LH levels and a low T. The ASI of this patient was 52.92 IU nmol L^{-2} (LH 10.8 IU L^{-1} × T 4.9 nmol L^{-1}) below the prescribed cut-off limit (170 IU nmol L^{-2}). Reproductive hormone levels were normal or slightly higher in the remaining seven patients with AR gene mutations and these men lacked an elevated ASI. Therefore, our study believes that patients with normal LH and T hormone levels should also actively detect AR mutations.

In summary, the in vivo mechanisms of high-frequency phenotypic heterogeneity caused by AR gene mutations, as well as their impact on spermatogenesis, are still worthy of further study. Clinically, the effective evaluation of AR gene mutations has important implications: AR gene defects can be transmitted from the father to the offspring through the reproductive process and might cause more severe clinical phenotypes in their offspring. Therefore, infertile men should undergo appropriate genetic testing and counselling before attempting to reproduce, especially those who are looking for help by means of assisted reproductive technology (Dowsing et al.,

1999). The detection of abnormal molecular variations in AR gene in normally virilised patients showing only spermatogenic dysfunction will provide new insights into the different receptor mechanisms of the AR gene, and alternatively mediated androgen actions and the associations with human disease.

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