The NR5A1 mutation was identification in male infertility

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Abstract

Background: Recent surveys report one in seven couples with infertility worldwide of which male infertility accounts for 30-50%. Azoospermia is one of the most serious forms of male infertility in men, the reason is that no sperm in the semen and genetic basis remains largely unknown. However, the cause of the disease and the genetic mechanism are still unclear. The aim of this study was to investigate the association between Chinese infertile men and mutation of NR5A1 (Nuclear receptor subfamily 5 group A member 1) gene.

Methods: In order to study the mutations related to spermatogenesis genes in male with azoospermia, we sequenced the entire coding region of 52 genes related to spermatogenesis in 200 infertile Chinese men. Screening for genetic variation and SNPs throughout the coding region by targeted exome sequencing. One previously described missense p.G197E was detected in exon 5 while no mutations were found in other exons. We performed Sanger sequencing and confirmed heterozygous missense mutation in the patient.

Results: This patient has a normal karyotype with 46,XY and no abnormality exist in Y chromosome and hormone levels are changed. (eg:FSH, LH, E2, T). FSH is higher than normal, LH is higher than normal, E is lower than normal, PRL is within the normal range, T is lower than normal. Combining the results of this experiment, NR5A1 gene mutation may be associated with male infertility but to confirm this hypothesis more in-depth research is required.

Conclusion: In a word, our research reveals a mutation in c.G590A (p.G197E) in NR5A1 which extended the mutation of NR5A1 in Chinese infertile men and increased awareness of infertility caused by azoospermia.

Background

According to statistics, one in seven couples in the country has infertility [1]. Male infertility accounts for almost half of all infertility cases [2]. The root cause is complex, including environmental, physiological, genetic and other factors. In recent years, more and more people are beginning to worry about reproductive health. This trend is growing due to poor treatment of infertility. The disease may occur due to genetic factors, including genetic defects, Y chromosomes and karyotypes but the specific etiology and genetic mechanism are still unclear. In some Western countries, as many as 8% of children are born due to reproductive technology [1]. The number of sperm in several European countries are declining. In Denmark, 20% of healthy young adult males have lower sperm concentrations than the World Health Organization's reference level of 20×10^6 sperm/ml. But in the vast majority of cases, the cause of male infertility is still unclear.

NR5A1 (MIM184757), is a transcription factor of the nuclear receptor family, also known as Steroidogenic factor (SF-1), located on chromosome 9 (9q33.3) containing six functional exons 8 [3–7]. The full length gene spans 33185 bp with 7 exons and 3095 bp long mRNA, encoding 461 amino acids [8]. The protein

The protein is comprised of a DNA-binding domain (DBD), two C4 zinc finger structures (Zn I and Zn II), N-terminal variable domain, flexible hinge domain, C-terminal ligand binding domain (LBD) and main activation domain (AF2) [4, 6, 9]. NR5A1 gene was expressed in Sry-box 9 and AMH [7, 10].

NR5A1 may play a decisive role in sex differentiation, regulation of testosterone and MIS synthesis to mediate male-specific gene expression, and expression of estrogen specific repressor gene [11]. NR5A1 mutation in humans were first identified in two cases of 46,XY karyotype female gonadal dysplasia with adrenal insufficiency, and 46,XX karyotype with isolated adrenal insufficiency [12]. Subsequently, mutation of NR5A1 gene was spotted in individuals with 46,XY karyotypes female gonadal dysplasia and extensive genital phenotype abnormalities but normal adrenal function [13, 14].

In our present study, we reported a SNP variation c.G590A (p.G197E) in exon 5 of NR5A1 that was firstly screened out by targeted next-generation sequencing. Our study indicated that the variation might be associated with azoospermia, resulting in infertility. Our case report of c.G590A (p.G197E) in NR5A1 is important for the clinical diagnosis and assessment of azoospermia.

Methods

The study was approved by The Ethics Committee of the First Hospital of Jilin University. The patient was Han nationality. We got informed consent from patient and selected the patient who had primary infertility. Through the inspections including a detailed clinical information (including drug addiction, penis development, male feature, testicular touch, etc), hormone profiles, Y-chromosome microdeletion and semen analysis, the patient was diagnosed as having azoospermia. If the patient was checked to have a Y chromosome deletion, no further research was conducted. Finally, the sequencing result of the spermatogenesis associated gene was analyzed.

Genomic DNA was collected by QIAamp® DNA Blood Mini Kit. Exome capture of genomic DNA using an internal targeting genome. (Peking Medriv Academy of Genetics and Reproduction, Peking) followed by next-generation sequencing on the Illumina MiSeq sequencing platform including 52 spermatogenesis associated genes. Remove duplicate readings from library and PCR preparation by using Picard tools. After comparison with the human reference genome (GRCh37/hg19), non-harmful variants are most likely to be filtered as described [15]. Databases such as dbSNP, 1000 Genomes Project, Exome Aggregation Consortium, Exome Variant Server are excluded because they are less likely to be harmful. Using polyphen-2 (<http://genetics.bwh.harvard.edu/pph2>) and SIFT (<http://sift.jcvi.org>), we predicted the pathogenicity by protein function. The NR5A1 sequences were utilized to identify homologous peptides through BLASTP search (e-value cut off of 1.0). Variant classification based on American Medical Genetics and Genomics (ACMG) standards [16].

Mutation analysis of NR5A1 gene was performed by ABI and Sanger Sequencing (ABI 3730XL, BGI Genomics, Beijing Genomics Institute-Shenzhen, Shenzhen) with primer 5'-GCTTGGCTTGCAGCATTT-3' (Forward) and 5'-ACTGTGGTTGGGTGGTTGAT-3' (Reverse) of mutation p.G197E in NR5A1.

Results

Targeted exome were sequenced in 200 individuals with azoospermia and evaluated for mutations in the 52 spermatogenesis associated genes, we examined whether there is a genetic defect in NR5A1 associated with azoospermia. As a result, variation in patient was heterozygous missense mutation c.G590A (p.G197E) in exon 5 of NR5A1. This mutation a novel mutation that may be associated with azoospermia. The novel mutation, which was located in the exon 5 of NR5A1, heterozygous and categorized as pathogenic by using polyphen-2 and SIFT. The mutation was determined by PCR and Sanger sequencing (Fig. 1a). The amino acid sequence of NR5A1 to its orthologs 10 different homolog species. Comparison of its highly conserved domains using DNAMAN, as shown in Fig. 1b. According to the patient's scrotum color Doppler ultrasonography showed that the patient's testicular volume was 10 mL left and 10 mL right and no lesions were found. This patient has a normal karyotype with 46,XY. Subsequently, the Y chromosome results were analyzed and it was found that all of the sites existed, and no Y chromosome deletion was spotted. Specific hormone levels are shown in Table. 1. FSH is higher than normal, LH is higher than normal, E is lower than normal, PRL is within the normal range, T is lower than normal.

Discussion

In this study, we explored the relationship of NR5A1 gene mutations associated with infertility in healthy man. To date, the NR5A1 mutation has only been associated with more severe forms of gonadal dysplasia or significant genital abnormalities such as undescended testes, penoscrotal hypospadias, or anorchia [17]. Although male infertility can be used as a primary manifestation of medical evaluation in some cases, our data suggest that men with severe azoospermia may also have endocrine dysfunction and testosterone failure with age. It is well known that about 12%-15% of men with spermatogenic disorders have decreased serum testosterone levels, and the level of LH is elevated [18]. Some people have suggested that these problems may be related to mild testicular hypoplasia in men [18]. Combined with our result, individuals with NR5A1 mutations may represent part of the group.

The report indicated that NR5A1 gene mutations are associated with 46 XY dysplasia and 46 XX primary ovarian insufficiency. It further illustrated that NR5A1 gene has research value in infertility. Bashamboo and his colleagues found missense mutations of p.G146A in the NR5A1 gene in seven of 315 infertile patients with severe spermatogenesis disorders but the cause is not clear. However, it is clear that mutations seriously affect transcriptional activity. In the control group, no mutation type was found. NR5A1 gene was found mutated in infertile patients with spermatogenic disorder and about 4% of the reasons are unknown [17]. The man who carries NR5A1 change may bring more serious diseases(eg. severe oligozoos permia or azoospermia), and accompanied by elevated testosterone and gonadotropin. In this study, we found mutation site of c.G590A(p.G197E) in NR5A1. The research showed people who carried NR5A1 changes and accompanied by FSH, LH rises, E and T decrease. Our results are similar to those reported in the literature, and the hormone results have changed.

In addition, there are some mechanisms which can explain the relationship between spermatogenesis disorder and NR5A1 gene mutation. Cell-specific NR5A1 gene were knocked out in mice resulting in

incomplete testis and the reason is that spermatogonia never led to sperm [19]. The expression of Cyp11a and StAR key genes were reduced in testosterone synthesis. In patient with azoospermia, expression of NR5A1 in gonads was proportional to testosterone concentration, suggesting correlation between these two factors [20]. Lack of NR5A1 gene in mice reduced LH and FSH. In mice, the gonad function, testicular volume and Leyding cells decreased and mature sperm cells disappeared, resulting in infertility [21]. In our study, Hormone levels have also changed, unlike the literature reports that both LH and FSH are elevated.

Conclusion

Although we did not give a clear conclusion of mutation c.G590A (p.G197E) in NR5A1, what it is clear that mutations can lead to functional changes. In short, our report reveals a type of mutation c.G590A (p.G197E) in NR5A1 in Chinese azoospermia and found that NR5A1 mutation is important in the diagnosis of azoospermia in male infertility.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all of the adult participants and the guardians of participants under 18 years old. The present study was approved by the Ethics Committee of the First Hospital of Jilin University (China).

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

The datasets generated and analysed during the current study are available in polyphen-2 (<http://genetics.bwh.harvard.edu/pph2>) and SIFT (<http://sift.jcvi.org>).

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Authors'contributions

YL primarily analyzed the outcomes of whole-exome sequencing and Sanger sequencing, and was a major contributor in writing the manuscript. HZ, YY, LL, LL, JH collected the clinical information and contributed to the analysis of sequencing outcomes. RL conceived and designed the study and experimental methods. All authors read and approved the final manuscript.

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Abbreviations

NR5A1: Nuclear receptor subfamily 5 group A member 1

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Table

Table 1 Clinical and hormone profile of patient with azoospermia with novel *NR5A1* stopgain mutation

Patients ID	FSH mIU/ml	LH mIU/ml	Estradiol pg/ml	PRL uIU/ml	Testosterone
2017A097	26.1	16	24.09	236	6

FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin
FSH:1.5-12.4mIU/ml, E2::25.8-60.7pg/ml, LH:1.7-8.6mIU/ml, PRL:86-324uIU/ml and T:8.64-29.

Figures

Figure 1

The NR5A1 mutation in patient was identified by Sanger sequencing. The direction of the arrow is the mutation point (A). Multiple sequence alignment in NR5A1 from other species reveals that position 333, where the mutation site (p.G197E), is a conserved domain after the red letter (B).