Eleven Mutations in Six Genes Causing Abnormal Sperm Morphology: A Case Series

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Case Report

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Abstract

Objective: Infertility has become a major health problem worldwide, approximately 10%–15% of male infertility cases can be attributed to genetic mutations. However, the genetic factors contributing to male infertility remain largely unknown. This study aimed to identify genetic mutations associated with morphological abnormalities in sperm.

Methods: In this study, we assessed the semen parameters of 60 patients with sperm morphological abnormalities by semen analysis, and subsequently performed targeted next-generation sequencing (NGS) to identify gene mutations.

Results: Of the 60 cases of male infertility, 8 individuals (13%) were identified as carrying genetic mutations. In brief, patient 1 carried two heterozygous mutations of *SUN5*, including c.1066C>T and c.638delC; Patient 2 and Patient 3 had a 109.73 kb homozygous deletion on chromosome 12 in *DPY19L2*; patient 4 presented two heterozygous mutations (c.1670_1671delinsTT and c.255_258del) of *DPY19L2*; patient 5 carried a homozygous variant c.1159+1G>A of *CCDC40*; patient 6 had a heterozygous mutation c.38_43del in the *SEPT12* gene; patient 7 had two heterozygous mutations in *DNAH1* (c.10060_10061insATCT and c.12278T>C), and patient 8 presented two heterozygous mutations of *CFAP43* (c.3658C>T and c.823del).

Conclusions: We identified gene mutations associated with sperm morphological abnormalities in a series of male infertility cases. Eight of sixty individuals were identified as carrying gene mutations. Genetic mutations of *SUN5*, *DPY19L2*, *CCDC40*, *SEPT12*, *DNAH1*, and *CFAP43* might be responsible for male infertility. Our study expanded the landscape of gene mutations related to sperm morphological abnormalities, which might provide new insights into the genetics underlying male infertility.

Introduction

According to the World Health Organization (WHO), infertility is defined as a failure to achieve a clinical pregnancy after 12 months or more of sexual intercourse without contraception^[1]. Infertility has become a major health problem worldwide, affecting an estimated 8–12% of couples of reproductive age^[2]. Among these, male factors may be the primary cause of infertility in approximately 50% of couples. In addition, infertility can cause serious psychological stress and social problems, which brings considerable economic burden to patients and the health care system^[3, 4]. Thus, it is necessary to facilitate early diagnoses and propose appropriate treatment strategies.

The causes of male infertility can be divided into three categories: congenital, acquired, and idiopathic. In brief, congenital factors such as chromosomal or genetic abnormalities, acquired factors such as varicocele, and idiopathic factors such as psychological stress have been proven to be associated with infertility in men^[5]. Notably, it is statistically indicated that approximately 10 %–15% of male infertility cases can be attributed to genetic mutations^[6]. Gene variations such as *CFTR*^[7], C677T, and A1298C^[8] might be responsible for male infertility. However, the genetic causes underlying male infertility are complex, with at least 2000 genes participating in spermatogenesis^[9]. Many of these genes have not been completely identified and characterized, and genetic factors contributing to male infertility remain largely unknown.

In recent years, with the development of next-generation sequencing (NGS), significant progress has been achieved in the genetic investigation of humans. NGS can be used to query a large number of genes in a single experiment at a reasonable cost, and targeted NGS methods allow sequencing of genes of interest based on the known disease. Accumulating evidence has demonstrated that NGS is a comprehensive genetic test for male infertility with excellent clinical sensitivity^[10, 11]. In this study, we used targeted NGS to analyze the genetic data of 60 patients diagnosed with abnormal sperm morphology, and eight patients carrying different gene mutations were identified.

Patients And Methods

Participants and clinical data

Sixty male patients admitted to the Shandong Provincial Hospital of Traditional Chinese Medicine and Xiyuan Hospital of China Academy of Chinese Medical Sciences between 2018 and 2020 were included in this study. These patients had experienced primary infertility for at least two years. The inclusion criteria included healthy, consensual, idiopathic male infertility, aged 20–45 years with unwanted 1-year childlessness from the same female partner, normal endocrine function, and and with the total number (or concentration) of spermatozoa, and sperm parameters lower than WHO reference limits. Exclusion criteria: testicular abnormalities, cryptorchidism, varicocele, previous genital surgery, or endocrine disorders, gonadal dysfunction test found by physical examination and clinical examination. The Ethics Committee of the Xiyuan Hospital of China Academy of Chinese Medical Sciences approved the study protocol, and all patients provided written informed consent before the examination.

Semen analyses

Semen analyses were performed to observe sperm motility in the included patients. In brief, semen samples were collected after 72 h of sexual abstinence. The smears were prepared for each sample and then sent to the clinical laboratory for sperm analysis. Sperm morphology was assessed using the WHO (5.0th edition) guidelines^[12].

Next generation sequencing (NGS)

Genomic DNA was extracted from the peripheral blood samples of patients. The Covaris method was used to generate the DNA fragments, and PCR amplification was performed to construct the whole genome library. Next, target gene fragments were captured from the whole genome library via a series of approaches, including specific DNA probe hybridization, which were utilized to construct the target gene library. The library was sequenced on an Illumina

NextSeq 550Dx instrument, and genetic variation in NGS data was determined using bioinformatics analysis. Specifically, developmental abnormalities, azoospermia, oligospermia, Y chromosome deletion, and CFTR gene-related variants were detected. The pathogenicity of these variants was predicted using the American College of Medical Genetics and Genomics (ACMG) guidelines.

Results

Of the 60 cases of male infertility, 8 individuals (13%) were identified as carrying genetic mutations. The clinical characteristics of the patients are summarized in Table 1.

Table 1
The clinical characteristics of eight patients

Patient	Age (years)	Clinical feature	Diagnosis
1	30	NA	Globozoospermia
2	29	NA	Globozoospermia
3	31	NA	Globozoospermia
4	32	Normal secondary sexual characteristics, testicular volume and vas deferens. The rate of sperm head abnormalities: 100%.	Teratozoospermia
5	31	Normal secondary sexual characteristics, testicular volume and vas deferens. Sperm concentration: 16.0 million /mL; the proportion of motile spermatozoa: 0%; the rate of normal morphology: 0%; and the rate of head deformity: 100%.	Severe asthenospermia and teratospermia
6	33	Normal secondary sexual characteristics, testicular volume and vas deferens. Total number of spermatozoa: 31.24 million; the sperm concentration: 14.2 million/mL; and the proportion of motile spermatozoa: 0.6%.	Asthenospermia
7	30	Normal secondary sex characteristic, testicular volume, and spermaduct	Oligozoospermia
8	39	NA	Severe asthenospermia
NA: Not available			

Patient 1 was a 30-year-old Chinese man diagnosed with globozoospermia. Genetic testing revealed that the patient carried two heterozygous mutations in *SUN5*. One variant was a missense mutation, c.1066C > T (p.R356C), located in exon 13 of *SUN5* (Fig. 1A), and the other was a frameshift mutation, c.638delC (p.T213fs), located in exon 10 (Fig. 1B). In addition, these two mutations were predicted to be pathogenic by ACMG.

Patient 2 was a 29 year old Chinese man with globozoospermia. NGS analysis showed a 109.73 kb homozygous deletion on chromosome 12 (No. 63952668–64062395) in *DPY19L2*. Notably, this mutation was also detected in patient 3 (31 years old, globozoospermia).

Patient 4 was a 32-year-old Chinese man. while the rate of sperm head abnormalities was 100%. Thus, he was diagnosed with teratozoospermia. Two heterozygous mutations of *DPY19L2* were detected, including c.1670_1671delinsTT (p.K557I) located in exon 18 (Fig. 1C) and c.255_258del (p.R86lfs*38) located in exon 1 (Fig. 1D). Of these, c.255_258del was predicted to be pathogenic.

Patient 5 was a 31-year-old man, the semen parameters of this patient were as follows: sperm concentration of 16.0 million /mL, the proportion of motile spermatozoa was 0%, the rate of normal morphology was 0%, and the rate of head deformity was 100%.. He was thus diagnosed with severe asthenospermia and teratozoospermia. Meanwhile, we identified one gene mutation that might be responsible for the teratospermia of this patient; a homozygous variant c.1159 + 1G > A in intron 7 of *CCDC40* (Fig. 1E), which was predicted to be pathogenic.

Patient 6 was a 33-year-old Chinese man diagnosed with severe asthenospermia. Semen analysis showed that the total number of spermatozoa was 31.24 million, the sperm concentration was 14.2 million/mL, and the proportion of motile spermatozoa was 0.6%. Next, a likely pathogenic variant in the *SEPT12* gene was detected in patient 6. This variant, c.38_43del (p.S13X), is a heterozygous mutation located in exon 2 (Fig. 1F).

Patient 7 was a 30 year old mandiagnosed with asthenospermia. NGS analysis indicated that there were two heterozygous mutations in *DNAH1*, namely, c.10060_10061insATCT (p.E3354Dfs*28) and c.12278T > C (p.L4093P), which were located in exon 64 (Fig. 1G) and exon 76 (Fig. 1H). Of these, c.10060_10061insATCT was predicted to be a likely pathogenic mutation.

Patient 8 was a 39-year-old man who was diagnosed with severe asthenospermia. Two heterozygous *CFAP43* mutations were identified in this patient. One variant was the nonsense mutation c.3658C > T (p.Q1220X) located in exon 28 (Fig. 1I), and the other was a frameshift mutation c.823del (p.H275lfs*3), located in exon 6 (Fig. 1J). In addition, these two mutations were predicted to be pathogenic.

Overall, 11 different mutations were detected in eight individuals, and detailed information on these genes is presented in Table 2.

Table 2
Overview of the gene mutations observed in the eight patients

Patient	Mutated gene	Genomic position (bp)	cDNA change	Protein change	Transcript	Location	Genotype	pathogenicity
1	SUN5	Chr20:31571674	c.1066C > T	p.R356C	NM_080675	Exon 13	Heterozygous	LP
	SUN5	Chr20:31575557	c.638delC	p.T213fs	NM_080675	Exon 10	Heterozygous	LP
2	DPY19L2	Chr12:63952668–64062395 deletion	NA	NA	NA	NA	Homozygous	NA
3	DPY19L2	Chr12:63952668–64062395 deletion	NA	NA	NA	NA	Homozygous	NA
4	DPY19L2	Chr12:63976240–63976241	c.1670_1671delinsTT	p.K557I	NM_173812	Exon 18	Heterozygous	VUS
	DPY19L2	Chr12:64061916–64061919	c.255_258del	p.R86lfs*38	NM_173812	Exon 1	Heterozygous	Pathogenic
5	CCDC40	Chr17:78024083	c.1159 + 1GA	NA	NM_017950	Intron 7	Homozygous	LP
6	SEPT12	Chr16:4837604–4837609	c.38_43del	p.S13X	NM_001154458	Exon 2	Heterozygous	LP
7	DNAH1	Chr3:52426487	c.10060_10061insATCT	p.E3354Dfs*28	NM_015512	Exon 64	Heterozygous	LP
	DNAH1	Chr3:52433054	c.12278T > C	p.L4093P	NM_015512	Exon 76	Heterozygous	VUS
8	CFAP43	Chr10:105912367	c.3658C > T	p.Q1220X	NM_025145	Exon 28	Heterozygous	LP
	CFAP43	Chr10:105967495	c.823del	p.H275lfs*3	NM_025145	Exon 6	Heterozygous	LP
LP: likely pathogenic; NA: Not available; VUS: uncertain significance.								

Discussion

Male infertility is a multifactorial disease with a significant genetic basis, and sperm abnormalities are one of the causes of infertility in males. In this study, we identified eight patients with abnormal sperm morphology carrying genetic mutations. There were 11 mutations in six genes (*SUN5*, *DPY19L2*, *CCDC40*, *SEPT12*, *DNAH1*, and *CFAP43*) caught our attention that might be involved in male infertility.

NGS analysis showed that the genes *SUN5* and *DPY19L2* were associated with patients with globozoospermia. Sperm is a polarized cell formed by the head and flagella; thus, head integrity is essential for normal sperm function. Globozoospermia is a severe and rare form of teratozoospermia that is characterized by a spherical sperm head without an acrosome^[13]. The protein encoded by *SUN5* plays a crucial role in the meiotic phase of sperm formation and is specifically expressed in the development of male germ cells. In addition, *SUN5* is specifically located at the junction of the head and tail of the sperm^[14]. A previous study revealed that *SUN5* mutations were observed in 37% of acephalic spermatozoa cases^[15], and Elkhatib *et al.*^[16] identified three homozygous deletions of *SUN5* in patients with decapitated spermatozoa. However, no direct evidence has indicated a relationship between *SUN5* mutations and globozoospermia. Thus, the role of *SUN5* in the pathogenesis of globozoospermia requires further investigation. In addition, *DPY19L2* is widely reported to be associated with Globozoospermia^[17]. A homozygous deletion of 200 kb on chromosome 12 of *DPY19L2* might result in Globozoospermia. Meanwhile, evidence suggests that chromosome 12 contains the *DPY19L2* locus, and large-scale homozygous deletion might cause the loss of *DPY19L2*, leading to rounded spermatozoa without acrosomes^[19, 20]. In this study, we found a 109.73 kb homozygous deletion on chromosome 12 in *DPY19L2* of patients with Globozoospermia, which was consistent with previous studies. In addition, we also observed two heterozygous mutations in *DPY19L2* that were associated with teratozoospermia, and semen analysis showed that the rate of sperm head abnormalities in this patient was 100%. However, these two variations have not yet been reported. Thus, we speculated that two novel detected intronic mutations, including c.1670_1671delinsTT located in exon 18 and c.255_258del located in exon 1, might be responsible for the abnormal sperm head.

Another gene, *CCDC40*, was found to be related to severe asthenospermia and teratozoospermia. The highly conserved proteins encoded by *CCDC40* are essential for motility cilia, and more than 25 pathogenic mutations in *CCDC40* have been identified to participate in primary ciliary dyskinesia (PCD)^[21]. PCD is characterized by abnormalities in the cilia structure or functional movement. In this patient, semen parameters showed that the proportion of motile spermatozoa was 0%, which may be attributed to the homozygous variant c.1159 + 1G > A in intron 7 of *CCDC40*.

Furthermore, we observed that genes such as *SEPT12* and *CFAP43* were associated with severe asthenospermia. *SEPT12* encodes the guanine-nucleotide binding protein, which is related to spermatogenic failure 10 and non-syndromic male infertility due to sperm motility disorder^[22]. A published study indicated that the expression level of *SEPT12* was lower in testicular tissue of a man with low spermatogenesis than in normal individuals, and downregulation of the *SEPT12* gene was associated with an increased number of sperm with abnormal head, neck, and tail morphology, suggesting that *SEPT12* is critical in spermatogenesis^[23]. Kuo *et al.* reported two missense mutations in *SEPT12* related to male infertility presenting as a defective sperm annulus^[24]. In this study, we also identified a novel heterozygous mutation (c.38_43del) in *SEPT12* that might cause severe asthenospermia. Proteins encoded by *CFAP43* belong to the cilia- and flagella-related protein family, and can mediate intra-manchette transport and play an important role in sperm flagella formation and

sperm head shaping^[25]. Yu *et al.*^[25] also observed that *CFAP43* mutations lead to sperm abnormalities and severe asthenospermia. Similarly, we also detected two novel heterozygous mutations in *CFAP43* (c.3658C>T and c.823del) associated with severe asthenospermia.

In the present study, two *DNAH1* mutations were identified in patients with asthenospermia. *DNAH1* encodes an inner dynein arm heavy chain that provides structural support between the radial spokes of the sperm tail and the outer double layer. Mutations in this gene have been related to PCD and various morphological abnormalities of the flagella, leading to male infertility and asthenospermia^[26]. Sha *et al.*^[27] observed 17 mutations in *DNAH1* that contributed to dysplasia of the sperm fibrous sheath and infertility in the Chinese population. In addition, two novel mutations, c.10060_10061insATCT in exon 64 and c.12278T>C in exon 76, related to asthenospermia, were found in this NGS analysis.

Although these genes have been confirmed to be involved in male infertility, we have discovered several novel genetic mutations. These variations could provide guidance to improve the genetic diagnosis of hereditary infertility. This study had some limitations. The specific mechanisms of the identified genes in male infertility have not yet been investigated. In addition, some mutations in the genes were detected in only one individual. Therefore, a larger study population and longer follow-up of clinical outcomes are needed to verify whether these mutations can be applied in clinical diagnosis.

Conclusion

Taken together, we identified 11 mutations in six genes (*SUN5*, *DPY19L2*, *CCDC40*, *SEPT12*, *DNAH1*, and *CFAP43*) in male infertility patients. These gene variations are associated with a variety of abnormal sperm morphologies, including globozoospermia, asthenospermia, and teratozoospermia. These findings broaden the genetic causes and pathogenic mechanisms of sperm morphology abnormalities and provide potential diagnostic markers for male infertility patients.

Declarations

Ethics approval and consent to participate

Ethical approval was provided by the Shandong Provincial Hospital of Traditional Chinese Medicine in advance of implementation.

Consent for publication

Written informed consent was obtained from the guardians.

Availability of data and material

The datasets used during the present study are available from the corresponding author upon reasonable request.

Competing interests

All authors have no competing interests to declare.

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Authors' contributions

Conceptualization: Zhaowang Gao Investigation: Fengrui Liu Data retrieve: Yong Wang and Zhuozhuo Shi Supervision: Chongfu Zhong and Shengjing Liu Writing original draft: Qinghong Li.

All authors read and approved the final manuscript.

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References

1. ZEGERS-HOCHSCHILD F, ADAMSON G D, DYER S, *et al.* The International Glossary on Infertility and Fertility Care, 2017 [J]. Hum Reprod. 2017;32(9):1786–801.
2. VANDER BORGHT M, WYNS C. Fertility and infertility: Definition and epidemiology [J]. Clin Biochem. 2018;62:2–10.
3. SCHISTERMAN E F, SJAARDA L A, CLEMONS T, *et al.* Effect of Folic Acid and Zinc Supplementation in Men on Semen Quality and Live Birth Among Couples Undergoing Infertility Treatment: A Randomized Clinical Trial [J]. Jama. 2020;323(1):35–48.
4. ZHANG T, WANG X, WANG Z, *et al.* A Diagnostic Model to Improve the Predictability of Natural Pregnancy Potential in Patients with Oligoasthenospermia [J]. Med Sci Monit, 2020, 26(e922316).
5. AGARWAL A, BASKARAN S, PAREKH N, *et al.* Male infertility [J]. Lancet. 2021;397(10271):319–33.

6. KRAUSZ C, RIERA-ESCAMILLA A. Genetics of male infertility [J]. *Nature reviews Urology*. 2018;15(6):369–84.
7. BIENIEK JM, LAPIN C D, JARVI K A. Genetics of CFTR and male infertility [J]. *Translational andrology urology*. 2021;10(3):1391–400.
8. HE X F HANLJ, YE X H. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and male infertility risk: An updated meta-analysis [J]. *Med (Baltim)*. 2020;99(51):e23662.
9. MITCHELL MJ, METZLER-GUILLEMAIN C, TOURE A, et al. Single gene defects leading to sperm quantitative anomalies [J]. *Clin Genet*. 2017;91(2):208–16.
10. ROBAY A, ABBASI S, AKIL A, et al. A systematic review on the genetics of male infertility in the era of next-generation sequencing [J]. *Arab journal of urology*. 2018;16(1):53–64.
11. CANNARELLA R, PRECONE V, GUERRI G, et al. Clinical Evaluation of a Custom Gene Panel as a Tool for Precision Male Infertility Diagnosis by Next-Generation Sequencing [J]. *Life (Basel, Switzerland)*, 2020, 10(10): 242.
12. MARZANO G, CHIRIACÒ MS, PRIMICERI E, et al. Sperm selection in assisted reproduction: A review of established methods and cutting-edge possibilities [J]. *Biotechnol Adv*, 2020, 40(107498).
13. FESAHAHAT F, HENKEL R. AGARWAL A. Globozoospermia syndrome: An update [J]. *Andrologia*. 2020;52(2):e13459.
14. BEUROIS J, CAZIN C, KHERRAF Z E, et al. Genetics of teratozoospermia: Back to the head [J]. *Best practice & research Clinical endocrinology & metabolism*, 2020, 34(6): p. 101473.
15. ZHU F, WANG F, YANG X, et al. Biallelic SUN5 Mutations Cause Autosomal-Recessive Acephalic Spermatozoa Syndrome [J]. *Am J Hum Genet*. 2016;99(4):942–9.
16. ELKHATIB R A, PACI M, LONGEPIED G, et al. Homozygous deletion of SUN5 in three men with decapitated spermatozoa [J]. *Human molecular genetics*. 2017;26(16):3167–71.
17. TAVALAEE M, NOMIKOS M, LAI F A, et al. Expression of sperm PLC ζ and clinical outcomes of ICSI-AOA in men affected by globozoospermia due to DPY19L2 deletion [J]. *Reprod Biomed Online*. 2018;36(3):348–55.
18. CELSE T, CAZIN C, MIETTON F, et al. Genetic analyses of a large cohort of infertile patients with globozoospermia, DPY19L2 still the main actor, GGN confirmed as a guest player [J]. *Hum Genet*. 2021;140(1):43–57.
19. ALIMOHAMMADI F, EBRAHIMI NASAB M, RAFAEE A, et al. Deletion of dpy-19 like 2 (DPY19L2) gene is associated with total but not partial globozoospermia [J]. *Reproduction, fertility, and development*, 2020, 32(8): 727–37.
20. QIU Y, YANG H, LI C, et al. Progress in Research on Sperm DNA Fragmentation [J]. *Med Sci Monit*, 2020, 26(e918746).
21. SUI W, HOU X. CHE W, et al. CCDC40 mutation as a cause of primary ciliary dyskinesia: a case report and review of literature [J]. *Clin Respir J*. 2016;10(5):614–21.
22. SHEN Y R, WANG H Y, KUO Y C, et al. SEPT12 phosphorylation results in loss of the septin ring/sperm annulus, defective sperm motility and poor male fertility [J]. *PLoS Genet*. 2017;13(3):e1006631.
23. RAFAEE A, MOHSENI MEYBODI A, YAGHMAEI P, et al. Single-nucleotide polymorphism c.474G > A in the SEPT12 gene is a predisposing factor in male infertility [J]. *Mol Reprod Dev*. 2020;87(2):251–9.
24. KUO Y C, LIN Y H, CHEN H I, et al. SEPT12 mutations cause male infertility with defective sperm annulus [J]. *Hum Mutat*. 2012;33(4):710–9.
25. YU Y, WANG J. ZHOU L, et al. CFAP43-mediated intra-manchette transport is required for sperm head shaping and flagella formation [J]. *Zygote (Cambridge England)*. 2021;29(1):75–81.
26. YANG X, ZHU D, ZHANG H, et al. Associations between DNAH1 gene polymorphisms and male infertility: A retrospective study [J]. *Med (Baltim)*. 2018;97(49):e13493.
27. LV M, LIU W. CHI W, et al. Homozygous mutations in DZIP1 can induce asthenoteratospermia with severe MMAF [J]. *J Med Genet*. 2020;57(7):445–53.

Figures

Figure 1

The analysis of gene mutation site of the eight individuals identified as carrying genetic mutations.