The heterozygous mutation of c.346-1G>A in *SOHLH1* gene is irrelevant to nonobstructive azoospermia

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Abstract

Nonobstructive azoospermia (NOA) is an important cause of male infertility, and the genetic pathogenesis is still incompletely understood. The previous study reported that heterozygous mutation of c.346-1G>A in *SOHLH1* gene was identified in two NOA patients. However, in our research, this heterozygous mutation was confirmed in a Chinese infertile patient who was suffered from teratozoospermia, and intriguingly, a homozygous mutation of c.346-1G>A in *SOHLH1* gene was detected in another patient with severe oligozoospermia. Additionally, we correlated the good prognosis of intracytoplasmic sperm injection (ICSI) in the patient carrying the heterozygous mutation of c.346-1G>A in *SOHLH1* gene. Thus, we suggested that the heterozygous mutation of c.346-1G>A in *SOHLH1* may not be the direct genetic cause for NOA, and this homozygous mutation might impair spermatogenesis and further lead to the reduced sperm count and abnormal sperm morphology, eventually causing male infertility.

Key words: *SOHLH1*, heterozygous mutation, homozygous mutation, male infertility, variant screening, ICSI

Infertility is one of the major issues affecting human health, and the pathogenesis is not completely veiled (Ji G et al., 2012). As whole-exome sequencing (WES) is being widely used, many genetic variations that are responsible for male infertility have been discovered. In 2010, Choi Y et al. reported the heterozygous mutation in *SOHLH1* gene (NG_033784.1: c.346-1G>A) caused the original splicing site disruption and partial deletion within exon 4, thus generated a truncated bHLH domain and resulted in nonobstructive azoospermia (NOA) in two Korean patients (Choi et al., 2010). The authors also performed hematoxylin-eosin staining (H&E) to exam patient's testis and the result showed a severe dysfunction in spermatogenesis characterized by no spermatogonia, spermatocyte, spermatids, or mature spermatozoa. Therefore, the author assumed this heterozygous mutation in *SOHLH1* resulted in NOA due to the absence of normal spermatogenesis.

Interestingly, we found this heterozygous mutation in a Chinese interfile male (patient A) by WES (Figure 1a). This patient was not affected to NOA, whose sperm concentration and sperm count were normal, while almost all the sperm were immotile (Table 1). Particularly, most of the sperm from patient A had amorphous head and the anomies in flagella compared to the normal control by Papanicolaou staining (Figure 1b). Moreover, scanning electron microscopy (SEM) further

confirmed that the sperm of patient A had aberrant head including round-head, double-head, tapered-head, pyriform head and/or irregular head with short, bent, coiled and flagella (Figure 1c). Additionally, we analyzed the ultrastructure of the spermatozoa by transmission electron microscopy (TEM). TEM analysis represented that normal spermatozoa had proper ratio of head's length to width, and the 9+2 structure (nine peripheral microtubule doublets and two central microtubules, surrounded by outer dense fiber and fibrous sheath) was integrated and regularly arranged. For patient A, we observed that sperm plasma membrane was swollen and damaged, cell nucleus contained vacuoles, and mitochondria was empty foamed, combined with an atypical 9+0 arrangement of axonemal microtubules in the sperm flagella (Figure 1d). According to these findings, we speculated that patient A was suffered from teratozoospermia but not NOA.

Remarkably, we detected a homozygous mutation of c.346-1G>A in *SOHLH1* gene in another Chinese patient B (Figure 1a). For this patient, his sperm count was extremely low. In addition, we found his sperm morphology was also abnormal. Though Papanicolaou staining, we observed deformities of head and flagella (Figure 1b). SEM and TEM further identified abnormality of sperm morphology and defective sperm ultrastructure (Figure 1c and d). Thus, patient B was affected to severe oligozoospermia with aberrant sperm morphology. Although c.346-1G>A in *SOHIL1* gene is a deleterious mutation which disrupted splice site, we have provided evidence that heterozygous c.346-1G>A in *SOHIL1* gene was not responsible for NOA, and homozygous mutation led to severe oligozoospermia.

Notably, due to the patient A showed the normal sperm count, ICSI would be a useful technology for conception. To date, no study has determined whether teratozoospermia patients with mutation in *SOHLH1* have a good prognosis following ICSI. The patient A, his wife had normal basal endocrine assessment and regular menstrual cycle. She underwent a long gonadotrophin-releasing hormone (GnRH) agonist protocol (Table 2). The couple was following one ICSI cycle, and 16 oocytes were retrieved after GnRH treatment. Then, 14 mature oocytes (metaphase II, M II) were successfully microinjected, and finally, 6 oocytes were normally fertilized (2PN/ injected oocytes = 42.85%). Following the extend culture, we obtained 3 day-3 good-quality embryo (8-cell, GradeII) and 3 day-5 blastocysts (4BB,4BC,4BC). Subsequently, two 8-cell blastocysts were transferred into the uterus with no obvious complications (Table 2). This study first presented that teratozoospermia patient associated with *SOHLH* heterozygous mutation could be rescued by ICSI.

SOHLH1 is a transcription factor which specifically expresses in germ cells, and plays an important role in the development of spermatogenic cells through transcriptional regulation of downstream genes such as *SYCP1* (Ballow D et al., 2006; Li et al., 2019). Some researchers have focused on the relationship between *SOHLH1* and male infertility. *Sohlh1* knockout male mice (*Sohlh1*$^{-/-}$) had a large number of apoptotic spermatocytes in the meiotic stage, resulting in failure of spermatogenesis and led to male infertility (Li et al., 2019). Similarly, our patient B carrying homozygous *SOHLH1* mutation showed the severe reduction in sperm number. Considering the low homology of *SOHLH1* gene in humans and mice

(https://www.ncbi.nlm.nih.gov/homologene/), it is reasonable that there are some differences in phenotypes between humans and mice with homozygous variation of *SOHLH1*, but both could contribute to a decrease in the number of spermatozoa. However, Li et al. didn't mention any sperm parameter about heterozygous male mice, and we speculate that heterozygous male mice had normal fertility thus could produce the homozygous mice. Moreover, our patient A with the heterozygous mutation showed the normal sperm number. All the observations might be explained that another normal allele of *SOHLH1* could totally translated into the intact protein and further performed normal biology functions, while the homozygous mutation led to the complete absence of SOHLH1 expression. Moreover, Song B et al. performed single nucleotide polymorphism (SNP) linkage analysis of *SOHLH1*, and discovered that there was no relationship between the rs558113 in *SOHLH1* gene and susceptibility to NOA in Chinese population, suggesting that heterozygous mutation in *SOHLH1* may not associated to NOA (Song et al., 2015). In addition, no evidence suggests the *SOHLH1* gene has haploinsufficiency (https://clinicalgenome.org/). More prominently, patient A in our study followed ICSI, and his wife became pregnant and had successful delivery, suggesting that patients with heterozygous c.346-1G>A mutation could provide sperm to complete fertilization. Actually, we didn't deny that heterozygous mutation of *SOHLH1* has a certain degree of contribution to male infertility, and it may combine with other gene mutations or factors to drive the occurrence and development of NOA, or epigenetic modifications affect the gene transcriptional expression level.

According to our evidences, the heterozygous mutation of c.346-1G>A in *SOHIL1* was not the directly genetic cause for NOA. Due to the complexity of the spermatogenesis process, and the etiological factors of male infertility are intricate, we should be more cautious to identify the genetic cause on infertile patients and performed more functional experiments to constitute the relationship between genotype and phenotype, so that we can provide more strong and accurate evidence for clinical diagnosis.

Figure Legend

Figure 1 The morphology and ultrastructure of spermatozoa from two patients. (a) Sequence chromatograms of two patients. Sanger sequencing confirmed the *SOHLH1* mutation in the patients. The red arrow denotes the mutation site (NG_033784.1: c.346-1G>A). (b) Papanicolaou staining of patients' sperm, most of which were amorphous head and irregular flagella. (c) The abnormal sperm phenotypes were observed in the patients by SEM. (d) TEM showed the abnormal ultrastructures of the head and flagella from the patients' spermatozoa.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

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

WEB RESOURCE

NCBI: https://www.ncbi.nlm.nih.gov/homologene

ClinGen: https://clinicalgenome.org

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