Gene 647 (2018) 221–225

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short communication

Genetic contribution of *SUN5* mutations to acephalic spermatozoa in Fujian China

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ARTICLE INFO

Keywords:
Acephalic spermatozoa
SUN5
Whole-exome sequencing
ODF1

ABSTRACT

Acephalic spermatozoa is an extremely rare disease associated with primary infertility. A recent study showed that genetic alterations in the *SUN5* gene lead to this disease, and *SUN5* mutations could explain the disease in about half of the patients. Therefore, in the present study, to re-visit the genetic contribution of *SUN5* mutations to acephalic spermatozoa, we recruited 15 unrelated affected individuals and screened the *SUN5* gene for mutations by whole-exome sequencing (WES) and Sanger sequencing. Five of the 15 (33.33%) subjects were found to carry the same homozygous mutation in the *SUN5* gene c.381delA (p.V128Sfs*7). Neither homozygous nor compound heterozygous mutations in *SUN5* were found in the other 10 patients. The c.381delA mutation resulted in the truncation of the SUN5 protein and decreased the expression and altered the distribution of the outer dense fiber 1 (ODF1) protein. Thus, in our study *SUN5* mutations accounted for only one-third of the patients in our cohort, which is lower than the percentage reported previously. Thus, our study suggests that the contribution of *SUN5* mutations to acephalic spermatozoa might not be as high as described previously. These results will help in the genetic counseling of patients with acephalic spermatozoa.

1. Introduction

Infertility is becoming a major medical and sociological problem affecting human development and health worldwide. Approximately 8–18% of couples of reproductive age have fertility problems (Thonneau et al., 1991; Dunson et al., 2004). Among them, male factors, especially abnormal spermatogenesis and ejaculation disorders, account for 59% of the cases (Thonneau et al., 1991). Abnormal sperm morphology is one of the main causes of male infertility, however, the etiology is unclear in most cases, especially in rare cases of acephalic spermatozoa, for which there are few studies on the pathogenesis (Tokuhiro et al., 2009; Yang et al., 2012; Yuan et al., 2015; Zhu et al., 2016; Li et al., 2017; Sha et al., 2017a; Shang et al., 2017). Most scholars believe that acephalic spermatozoa may be related to genetic factors (Chemes et al., 1999).

SUN5 is a testis-specific gene (Frohnert et al., 2011; Jiang et al., 2011) and its expression is localized in the region of sperm head-tail junction (Yassine et al., 2015; Zhu et al., 2016; Shang et al., 2017). SUN5 belongs to the family of SUN domain proteins, which mediate the tethering of the centrosome to the nuclear membrane (McGee et al., 2006). In one previous study, homozygous or compound heterozygous mutations including p.Trp72*, p.Gly114Arg, p.Val128Serfs*7, p.Met162Lys, p.Val261Met, p.Thr275Met, p.Ser284*, p.Asn348Ile, p.Arg356Cys, and c.425 + 1G > A (splicing variant), in the *SUN5* gene, were reported as the cause of human acephalic spermatozoa syndrome in eight of 17 unrelated patients, *i.e.* in 47.06% of the affected individuals (Zhu et al., 2016).

In this study, with an aim to re-visit the genetic contribution of *SUN5* mutations to acephalic spermatozoa, we recruited 15 unrelated patients with acephalic spermatozoa over the past few years from the Fujian Province of China. Every patient was subjected to WES and screened by Sanger sequencing for the *SUN5* gene. The results of the study suggest that the genetic contribution of *SUN5* mutations to acephalic spermatozoa is not as high as described previously and that it therefore needs to be evaluated in further detail in the future.

Abbreviations: WES, whole-exome sequencing; ODF1, outer dense fiber 1; ExAC, Exome Aggregation Consortium; SNP, single nucleotide polymorphism; WB, western blot; IF, immunofluorescence

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https://doi.org/10.1016/j.gene.2018.01.035
Received 27 September 2017; Received in revised form 3 January 2018; Accepted 9 January 2018
Available online 10 January 2018

Table 1
Semen parameters of the patients harboring *SUN5* mutations.

Patients' number	Mutations	Consanguinity	Age	Volume (mL)	pH	Concentration (10^6/mL)	Motility (%)	Acephalic spermatozoa (%)	Normal spermatozoa (%)
P1	*SUN5*, homozygous, c.381delA; p.V128Sfs*7	Yes	28	2.8	7.5	57	35.4	50	2.5
P2	*SUN5*, homozygous, c.381delA; p.V128Sfs*7	Yes	29	2.1	7.4	16.2	18.5	70	1
P3	*SUN5*, homozygous, c.381delA; p.V128Sfs*7	No	31	2.8	7.4	25.2	32.1	60	3.5
P4	*SUN5*, homozygous, c.381delA; p.V128Sfs*7	No	34	2.9	7.4	37.4	29.6	55	2
P15	*SUN5*, homozygous, c.381delA; p.V128Sfs*7	Yes	31	2.3	7.2	10.8	35.2	80	0.5

2. Materials and methods

2.1. Patient

Fifteen patients and their family members were recruited from the Department of Reproductive Medicine at Xiamen Maternity and Child Care Hospital. The criterion for diagnosis of acephalic spermatozoa syndrome was 30–100% headless sperm. The chromosomal karyotype of the patients was normal 46; XY, and G-banding and real-time quantitative PCR revealed no deletion in the Y chromosome. The characteristics of the patients' semen are described in Table 1. This study was approved by the Ethics Committee of Xiamen Maternity and Child Care Hospital. Written informed consent was obtained from the study participants, and 5 mL of peripheral blood was then collected from them.

2.2. Whole-exome sequencing (WES) and Sanger sequencing validation

WES was performed as described previously (Sha et al., 2017a). Briefly, DNA library was prepared and exome was enriched using TruSeq Exome Enrichment kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol. Then high throughput sequencing was carried out by using the Illumina Hiseq 2000 sequencer. The reads were aligned against UCSC hg19 by Burrows-Wheeler Aligner (http://bio-bwa.sourceforge.net/). Variants (SNPs and indels) were called by SAMTOOLS (http://samtools.sourceforge.net/). Variants were annotated by ANNOVAR (http://www.openbioinformatics.org/annovar/) and cross-referenced against 1000 Genomes, ESP6500 and ExAC databases. Full WES data of the patients with *SUN5* mutations are available upon request. Sanger sequencing was used to validate the mutation in the *SUN5* gene in the patients and P1's family members.

2.3. Immunostaining of spermatozoa

Immunostaining of the spermatozoa was performed as described previously (Sha et al., 2017b). The specific antibodies used in this assay are listed in Supplementary Table 2.

2.4. Western blot analysis

Western blot analysis was carried out as described previously (Kee et al., 2009). The specific antibodies used in western blot analysis are listed in Supplementary Table 2.

3. Results

3.1. WES and Sanger sequencing analysis of all patients with acephalic spermatozoa

Fifteen patients with acephalic spermatozoa were recruited for this study. All the patients were subjected to WES analysis and a second round of Sanger sequencing for screening and validation. Five patients (33.33% of all affected individuals, P1, P2, P3, P4, and P15) were found to carry a common homozygous *SUN5* mutation c.381delA (rs781693813) (Fig. 1A). P1, P2 and P15 were from the consanguineous families, but P3 and P4 were not (Table 1). Sanger sequencing also confirmed that P1's unaffected father and brother harbored the heterozygous mutation (Fig. 1A). For the patients harboring the *SUN5* mutation, the percentage of sperms with acephalic spermatozoa was in the range of 50–80% (Table 1 and Fig. 1B). Furthermore, for the 10 patients not harboring the *SUN5* mutations, one patient with *BRDT* homozygous mutation (Li et al., 2017), one patient with *TSGA10* homozygous truncating mutation (Sha et al., 2017a), three patients with different *TSGA10* rare heterozygous mutations (unpublished information), the genetic causes of other patients were under investigated.

3.2. In silico analysis of the SUN5 mutation

In silico analysis using the Mutation Taster online tool revealed that the *SUN5* mutation (c.381delA; p.V128Sfs*7) is likely a disease-causing mutation (Supplementary Table 1). Moreover, this c.381delA mutation is a rare variant in the ExAC database and 1000 Genomes database (Supplementary Table 1), which is consistent with the prevalence of acephalic spermatozoa. The c.381delA mutation leads to a coding sequence frameshift and amino acid change at the V128 site and results in a gain of a stop codon after a 6-amino acid frameshift sequence, thus resulting in the formation of a truncated protein missing the C-terminal SUN domain.

3.3. SUN5's expression and ODF1's expression and distribution were altered

To determine the potential effect of the truncating mutation in SUN5, we performed immunofluorescence analysis and found that the SUN5 protein is localized to the sperm head-tail junction in normal controls (Fig. 2A). However, SUN5 showed low expression in the mid-piece in P1 (Fig. 2A). The protein distribution of the ODF1 protein, which plays a role in the correct arrangement of the mitochondrial sheath and is essential for the rigidity of the head-tail junction (Yang et al., 2012, 2014), was also altered in the sperm of P1 compared with that in the control sperm (Fig. 2B). The expression of ODF1 was also significantly reduced in P1 (Fig. 2B and C).

4. Discussion

In the present study, we screened *SUN5* mutations in 15 unrelated patients with acephalic spermatozoa using WES and Sanger sequencing analysis and found that only five of the 15 patients, *i.e.* 33.33% of all patients in our cohort, carried a *SUN5* mutation. To our surprise, all the five patients carried the same homozygous mutation c.381delA. Furthermore, we evaluated the functional impact of this mutation by immunostaining and western blot and found that the expression of