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Short communication

TDRD6 is associated with oligoasthenoteratozoospermia by sequencing the patient from a consanguineous family

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ABSTRACT

Oligoasthenoteratozoospermia (OAT) is characterized as low sperm count, decreased sperm motility and structural abnormalities of the sperm head in the same patient. However, very few studies reported the genetic alterations associated with OAT. Here we report a 38-year-old patient with OAT from a consanguineous family, with 2–6 million/mL sperm density, 2.1–3.8% normal sperm morphology and immotile sperm. Whole-exome sequencing (WES) identified homozygous variant c.1259A > G:p.Y420C in the *TDRD6* gene. TDRD6 is a testis-specific expressed protein that was localized to the chromatoid bodies in germ cells and played an important role in the nonsense-mediated decay pathway. This rare variant co-segregated with the OAT phenotype in this family. Bioinformatic analysis also suggested the variant a pathogenic mutation. Two intracytoplasmic sperm injection (ICSI) cycles were carried out in the patient's wife, but she did not become pregnant after embryo transfer. So the mutations in *TDRD6* may be associated with human male infertility and early embryonic lethality.

1. Introduction

Oligoasthenoteratozoospermia (OAT) is characterized as low sperm count, decreased sperm motility and structural abnormalities of the sperm head in the same patient (Jungwirth et al., 2012). Oligozoospermia was diagnosed if the spermatozoa in patient was lower than 15 million per mL, asthenozoospermia was diagnosed if the percentage of progressive motile spermatozoa was lower than 32%, teratozoospermia was diagnosed if the percentage of normal forms of spermatozoa was lower than 4%. Genetic factors have been demonstrated to contribute to oligozoospermia (Zhang et al., 2017; Li et al., 2018), asthenozoospermia (Zuccarello et al., 2008; Takasaki et al., 2014; Xu et al., 2018) and teratozoospermia (Coutton et al., 2015; Zhu et al., 2016; Sha et al., 2018; Sha et al., 2017b; Tang et al., 2017), however, very few studies reported the genetic alterations associated with OAT (O'Donnell et al., 2014).

Early embryonic developmental problems, such as early embryonic lethality or arrest, are major causes of male and female infertility. In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles failed if all of the embryos underwent the early embryonic arrest. Previous studies found that maternal genetic mutations in *TLE6* and *PADI6* caused early embryonic lethality or arrest (Alazami et al., 2015; Xu et al., 2016; Maddirevula et al., 2017). However, paternal single gene defects were rarely reported to be associated with early embryonic lethality (Li et al., 2018).

In this study, we report a patient with OAT from a consanguineous pedigree. Two ICSI cycles were carried out and failed due to the early embryonic lethality. Whole-exome sequencing (WES) and pedigree analyses suggested homozygous sequence variant in *TDRD6* may be associated with OAT and early embryonic lethality. These data suggested that *TDRD6* might be a novel gene associated with OAT and early embryonic lethality.

2. Materials and methods

2.1. Patient and his family's information

A consanguineous Chinese family comprising two brothers, one infertile,

Abbreviations: OAT, oligoasthenoteratozoospermia; WES, whole-exome sequencing; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; ExAC, Exome Aggregation Consortium; gnomAD, genome Aggregation Database; dbSNP, Short Genetic Variations database; 1000G, 1000 Genomes; SNP, single nucleotide polymorphism

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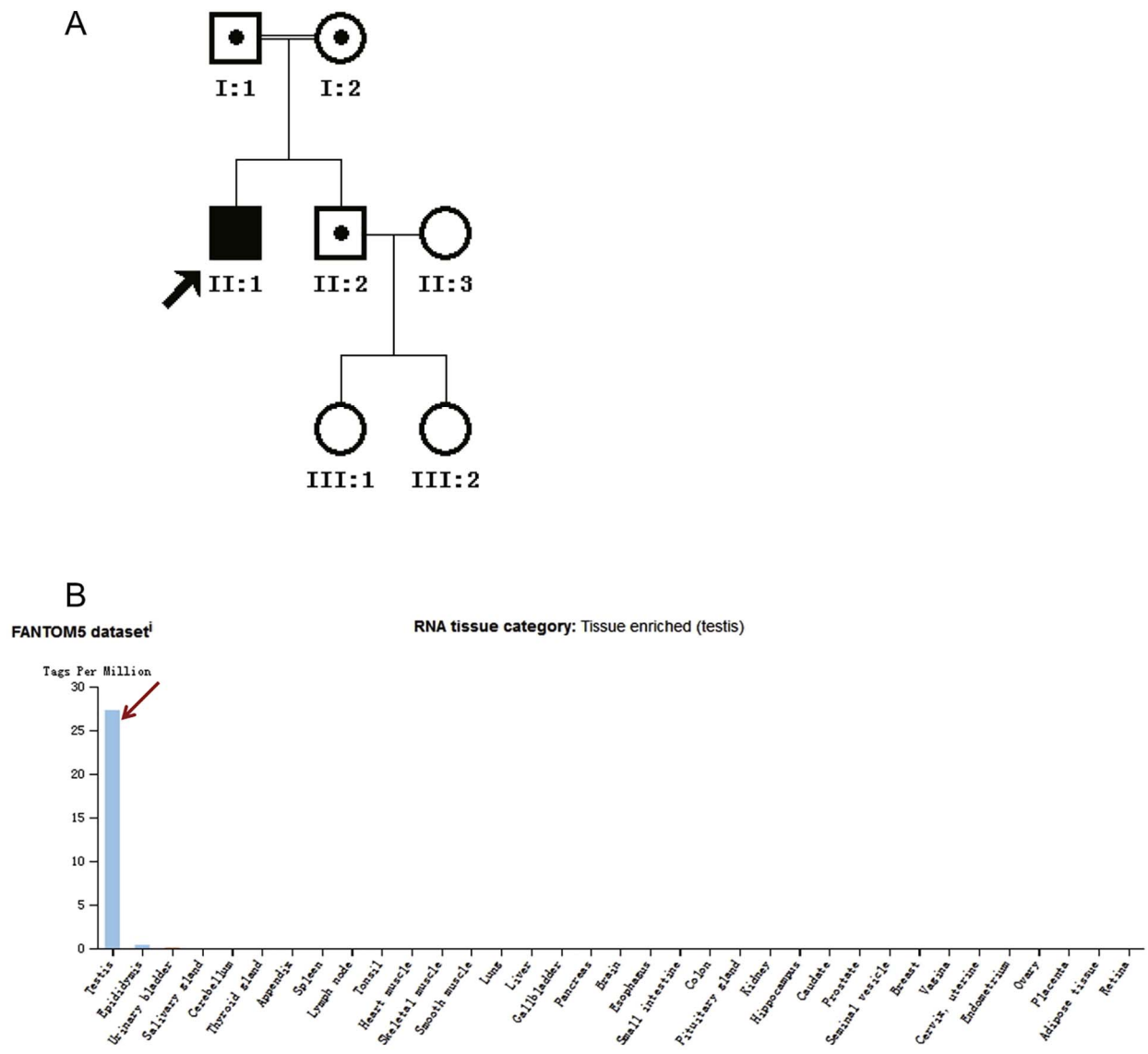

Fig. 1. The consanguineous pedigree and *TDRD6* expression. (A) The family tree showed a patient with OAT from a consanguineous family. (B) The *TDRD6* is specifically expressed in human testis. The data was from the online database, https://www.proteinatlas.org/ENSG00000180113-TDRD6/tissue. The red arrows point to the expression level of the *TDRD6* in testis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

one fertile, was recruited in this study. The infertile man was a patient (38 years old, married in 2012) with oligoasthenoteratozoospermia (II:1, Fig. 1A). He exhibited normal erection, ejaculation, and sexual activity (two to three times per week). However, his wife had not become pregnant, despite a lack of contraception. His wife was 32 years old, and was normal upon clinical examination. The patient also showed no abnormities on routine examination. The patient was from a consanguineous family (Fig. 1A). He had no history of exposure to a hazardous environment or poor habits. Physical examination results were as follows: height, 170 cm; weight, 68 kg; external genital development, normal; and bilateral testicular size (12 mL) and bilateral spermatic vein, normal. There were no abnormalities in the peripheral blood chromosomes or no Y chromosome micro-deletions. Semen examination results of several times at our hospital or other hospitals were as follows: semen volume, 2.8–3.5 mL; sperm density, 2–6 million/mL. Sperm morphology, as determined by Papanicolaou staining, showed 2.1–3.8% normal form. There was no motile sperm. Therefore, the patient was diagnosed as oligoasthenoteratozoospermia.

This project has been approved by the Ethics Committee of Xiamen Maternity and Child Care Hospital. A written consent has been obtained for all participants before samples were collected and then 5 mL of peripheral blood was collected from each participant.

2.2. Whole-exome sequencing and Sanger sequencing validation

Whole-exome sequencing (WES) was applied in the patient and was carried out as previously described (Sha et al., 2017b). Briefly, WES was carried out in the HiSeq 2500 sequencer (Illumina, USA). Raw reads were aligned to the reference genome Hg19 by using Burrows-Wheeler Aligner. Variants including single nucleotide polymorphism and indels were called using SAMtools and annotated using the ANNOVAR software.

Sanger sequencing was used to validate the mutation of the *TDRD6* gene in the patient, his parents and brother. Briefly, we first amplified the PCR products with 732 bp harboring the *TDRD6* mutation site using specific primers (the forward primer is 5′-GATGGACATTGGTACAGAG CACTGT-3′, and the reverse primer is 5′-ATCCACACTCTTGTCATCCA ATTTG-3′). The PCR products were sequenced using above forward primer, and the sequencing reactions were run on an ABI 3730 automated sequencer (Applied Biosystems, Forster City, California, USA).

2.3. Protein sequence alignment

Protein sequence alignment was conducted using CLC Genomic