Asian Journal of Andrology (2023) 25, 754–755
www.asiaandro.com; https://journals.lww.com/ajandrology

Open Access

LETTER TO THE EDITOR

Male Infertility

The role of homozygous LOF variant of the *PNLDC1* gene in oligo-astheno-teratozoospermia (OAT) and male infertility

Zi-Xin Cheng, Li Du, Zuping He

Asian Journal of Andrology (2023) **25**, 754–755; doi: 10.4103/aja202322; published online: 02 June 2023

Dear Editor,

An interesting article from Zhao *et al*.[1] recently published in *Asian Journal of Andrology*, has shown the association of a novel loss-of-function (LOF) variant in PARN-like ribonuclease domain-containing exonuclease 1 (*PNLDC1*) and male infertility, and we would like to contribute a commentary on this article.

Infertility has become a major health problem, and notably, it has recently been estimated that 17.5% of people are infertile worldwide.[2] Among the infertile couples, male factor accounts for approximately 50% of cases.[3] Infertility causes psychological stress, social distress, and economic burden on patients and health-care systems. Early diagnosis and proper treatment can alleviate these problems.[4] Male infertility might be largely resulted from idiopathic azoospermia, severe oligozoospermia, and teratozoospermia.[5]

Mutations in *PNLDC1* have been shown to cause astheno-teratozoospermia and oligo-astheno-teratozoospermia (OAT) in 2021.[6] PNLDC1 is required for PIWI-interacting RNA (piRNA) biogenesis, transposon silencing, and spermatogenesis.[7] Deficiency in piRNA processing on meiosis of spermatogenesis leads to male infertility,[6,8] highlighting the importance of *PNLDC1* and piRNA processing in regulating spermatogenesis and male fertility. Certain severe male infertility can be treated by the assisted reproductive technologies, e.g., *in vitro* fertilization and embryo transfer (IVF-ET), particularly intracytoplasmic sperm injection (ICSI).[9] However, the etiology of male infertility remains largely unknown, and thus, it is of great significance to uncover the cause of idiopathic male infertility.

In light of implications of piRNA pathway factors, including RNA helicase Mov10 like RISC complex RNA helicase 1 (*MOV10L1*) and exonuclease *PNLDC1* in human infertile patients, the piRNA pathway genes emerge as promising genetic factors accounting for infertility in men.[6,7,10] This can be illustrated from an interesting study of Zhao *et al*.[1] demonstrating the association of a novel loss-of-function variant in *PNLDC1* and oligo-astheno-teratozoospermia (OAT).[1] A homozygous LOF variant (NM_173516.2, c.142C>T, p.Gln48Ter) of the *PNLDC1* gene has been found to cause OAT of the patient from a nonconsanguineous Chinese Han family.[1] Whole-exome sequencing (WES) and targeted Sanger sequencing were employed to identify the homozygous LOF variant.[1] The spermatozoa of the OAT patient showed the whole or partial deletion of the acrosome, and they displayed an abnormal head phenotype, including microcephaly, head tapering, and globozoospermia.[1] In addition, the spermatozoa of this patient showed higher disomy rates of chromosomes X/Y and chromosome 18 than those of the fertile control.[1] ICSI might not be an effective option for patients with deleterious homozygous LOF variant of *PNLDC1* gene, since spermatid aneuploidy results in the poor outcome of ICSI cycles using spermatozoa from patients carrying *PNLDC1* variants.[1] *PNLDC1* gene could serve as a novel target to develop small-molecule inhibitors for human male contraception.[7] Significantly, homozygous LOF variant of *PNLDC1* gene has been shown to cause OAT disease. As such, this study sheds a new insight into the genetic basis of male infertility and it opens potential avenues for male infertility diagnostics and therapeutics.

On the other hand, we have some suggestions for the conclusion of this study to be strengthened. First, large samples with hundreds of OAT patients might be included to further demonstrate the association between the homozygous LOF variant of *PNLDC1* gene and OAT disease. Second, the transcription and protein levels of *PNLDC1* could be compared in the testicular tissues of OAT patients and fertile men by real-time polymerase chain reaction (PCR), RNA sequencing, spatial transcriptomics, western blot, immunohistochemistry, tissue arrays, and spatial proteomics, which might determine whether abnormal expression levels of PNLDC1 are associated with the OAT. Finally, the *PNLDC1* mutant or conditioned-knockout animal models, using *PNLDC1*$^{flox/flox}$ and *Vasa* promoter-driven Cre recombinase (*Vasa*-Cre), *Stra8* promoter-driven Cre recombinase (*Stra8*-Cre), and/or anti-Müllerian hormone promoter-driven Cre recombinase (*AMH*-Cre), can be employed to examine whether *PNLDC1* causes the deficiencies in the development of male germ cells and somatic cells and three main stages of spermatogenesis. Further studies to identify more variants for *PNLDC1* could provide novel genetic regulator mechanisms underlying spermatogenesis disorder and offer new targets for gene therapy of male infertility.

The Key Laboratory of Model Animals and Stem Cell Biology in Hunan Province, School of Medicine, Hunan Normal University; The Research Center of Reproduction and Translational Medicine of Hunan Province; The Manufacture-Based Learning and Research Demonstration Center for Human Reproductive Health New Technology of Hunan Normal University, Changsha 410013, China.
Correspondence: Dr. Z He (zupinghe@hunnu.edu.cn)
Received: 09 April 2023; Accepted: 18 April 2023

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

This work was funded by the grant from the National Natural Science Foundation of China (No. 32170862).

REFERENCES

1 Zhao SY, Meng LL, Du ZL, Tan YQ, He WB, *et al*. A novel loss-of-function variant in *PNLDC1* inducing oligo-astheno-teratozoospermia and male infertility. *Asian J Androl* 2023. Doi: 10.4103/aja20233. [Online ahead of print].
2 Cox CM, Thoma ME, Tchangalova N, Mburu G, Bornstein MJ, *et al*. Infertility prevalence and the methods of estimation from 1990 to 2021: a systematic review and meta-analysis. *Hum Reprod Open* 2022; 2022: hoac051.
3 Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol* 2015; 13: 37.
4 Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, *et al*. Male infertility. *Lancet* 2021; 397: 319–33.
5 Huynh T, Mollard R, Trounson A. Selected genetic factors associated with male infertility. *Hum Reprod Update* 2002; 8: 183–98.
6 Nagirnaja L, Morup N, Nielsen JE, Stakaitis R, Golubickaite I, *et al*. Variant *PNLDC1*, defective piRNA processing, and azoospermia. *N Engl J Med* 2021; 385: 707–19.
7 Ding D, Liu J, Dong K, Midic U, Hess RA, *et al*. PNLDC1 is essential for piRNA 3' end trimming and transposon silencing during spermatogenesis in mice. *Nat Commun* 2017; 8: 819.
8 Izumi N, Shoji K, Suzuki Y, Katsuma S, Tomari Y. Zucchini consensus motifs determine the mechanism of pre-piRNA production. *Nature* 2020; 578: 311–6.
9 Esteves SC, Roque M, Bedoschi G, Haahr T, Humaidan P. Intracytoplasmic sperm injection for male infertility and consequences for offspring. *Nat Rev Urol* 2018; 15: 535–62.
10 Li L, Tan YQ, Lu LY. Defective piRNA processing and azoospermia. *N Engl J Med* 2022; 386: 1675–6.