

SHORT COMMUNICATION

Open Access

Ovol2, a zinc finger transcription factor, is dispensable for spermatogenesis in mice



Jin Zhang^{1,2†}, Juan Dong^{1†}, Weibing Qin^{3†}, Congcong Cao¹, Yujiao Wen¹, Yunge Tang^{3*} and Shuiqiao Yuan^{1,4*} 

Abstract

Ovol2, a mouse homolog of *Drosophila ovo*, was identified as a zinc finger transcription factor predominantly expressed in testis. However, the function of *Ovol2* in postnatal male germ cell development remains enigmatic. Here, we firstly examined the mRNA and protein levels of *Ovol2* in developing mouse testes by RT-qPCR and western blot and found that both mRNA and protein of *Ovol2* are continually expressed in postnatal developing testes from postnatal day 0 (P0) testes to adult testes (P56) and exhibits its higher level at adult testis. Further testicular immuno-staining revealed that *OVOL2* is highly expressed in the spermatogonia, spermatocytes and round spermatids. Interestingly, our conditional *ovol2* knockout mouse model show that loss of *ovol2* in embryonic germ cells does not affect fecundity in mice. Our data also show that *Ovol1* may have compensated for the loss of *Ovol2* functions in germ cells. Overall, our data indicate that *ovol2* is dispensable for germ cell development and spermatogenesis.

Keywords: *Ovol2*, Spermatogenesis, Fertility, Knockout mice

Main text

Ovol2, a mouse homolog of *Drosophila ovo*, was identified as a zinc finger transcription factor predominantly expressed in testis [1]. Previous studies revealed that *Ovol2* exhibits its functions in keratinocyte transient proliferation and differentiation [2], mouse embryonic stem cells differentiation [3] and primordial germ cell development [4]. However, the function of *Ovol2* in postnatal male germ cell development remains enigmatic. Thus, we firstly examined the mRNA and protein levels of *Ovol2* in multiple adult mouse tissues by RT-qPCR and western blot. We found that both the mRNA and protein of *Ovol2* are highly expressed in testis and lung (Fig. 1a-c). We then examined the expression levels of *Ovol2* in postnatal developing testes and found that both mRNA and protein of *Ovol2* are continually expressed in postnatal developing testes from postnatal day 0 (P0) testes to adult testes (P56) and exhibits its

highest level at adult testis (Fig. 1d-f). Further testicular immuno-staining revealed that *OVOL2* is highly expressed in the germ cells (spermatogonia, spermatocytes and round spermatids) (Fig. 1g and Additional file 1: Figure S1A-D). Thus we hypothesized that *Ovol2* could play an important role in postnatal germ cell development and spermatogenesis.

Due to *Ovol2* conventional mutant mice displayed an embryonic mortality [4], we tried to specific knockout of *Ovol2* in mouse germ cells to determine the physiological roles of *Ovol2* in germ cell development. We then generated *Ovol2* conditional knockout mouse model by crossing *Ovol2*-floxed mice with *Vasa-cre* (Cre was specifically activated at embryonic day 15.5) to inactivate *Ovol2* gene in testicular germ cells (Fig. 2a). The genotype of *Ovol2* germ cell-specific knockout mice (*Vasa-Cre; Ovol2^{flx/flx}*, hereafter called *Vasa-cKO*) was confirmed by PCR-based genotyping analyses (Fig. 2b). In addition to genotyping analyses, both mRNA and proteins of *Ovol2* were appeared to be significantly reduced in *Vasa-cKO* testes compared with that of WT controls by RT-qPCR, Western blot and Immunofluorescence analyses (Fig. 2c-e and Additional file 1: Figure S1E). Therefore, these data suggest that *Ovol2* was specifically inactivated in testes efficiently.

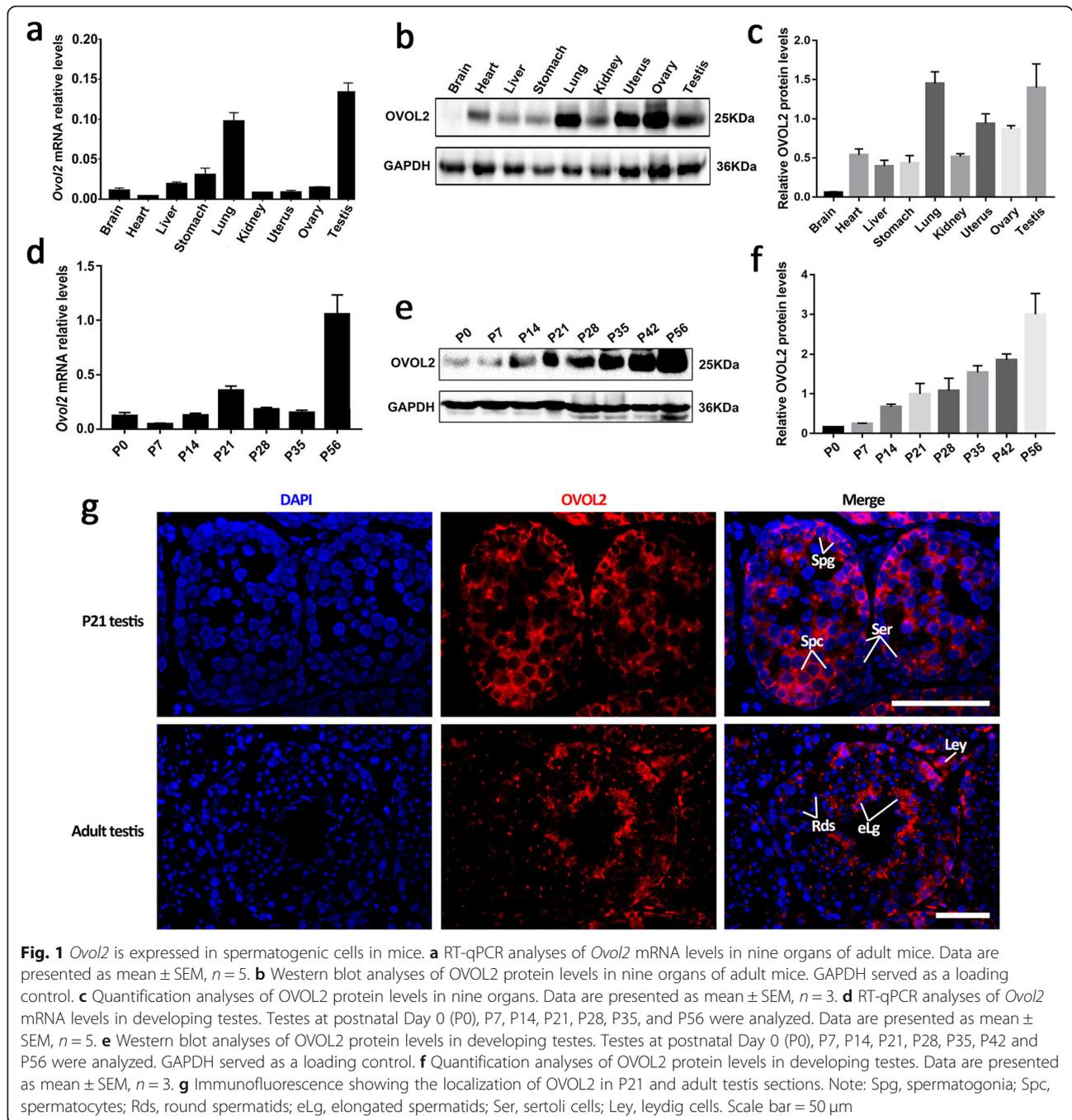
* Correspondence: tyg813@126.com; shuiqiaoyuan@hust.edu.cn

[†]Jin Zhang, Juan Dong and Weibing Qin contributed equally to this work.

³NHC Key Laboratory of Male Reproduction and Genetics, Family Planning Research Institute of Guangdong Province, Guangzhou, People's Republic of China

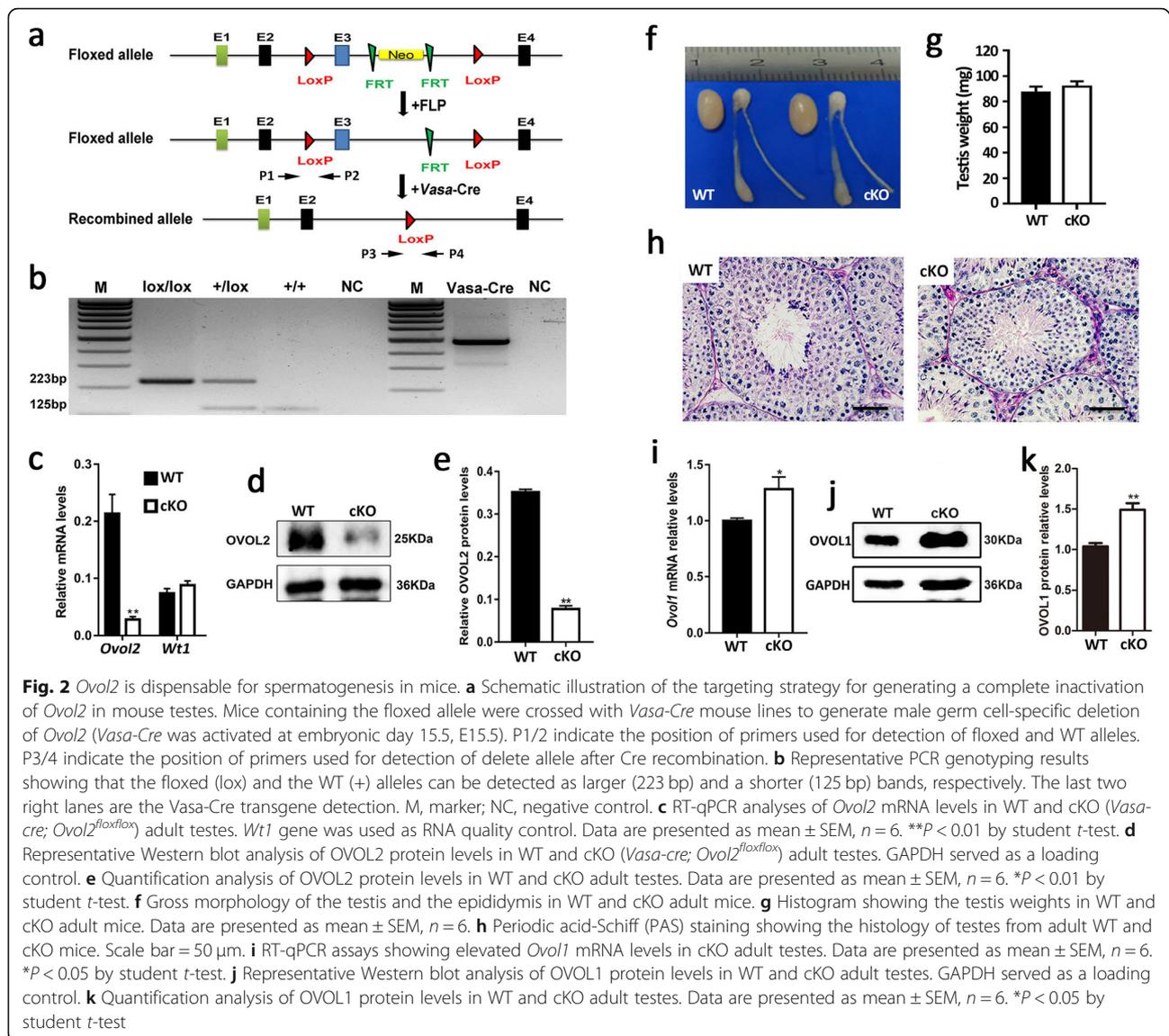
¹Institute of Reproductive Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China
Full list of author information is available at the end of the article





To investigate the fertility of *Vasa*-cKO mice, we bred the *Vasa*-cKO males with fertility-proven WT females for at least 5 months. Unexpectedly, *Vasa*-cKO breeding pairs can produce comparable litter size to WT breeding pairs (data not shown), which indicated that *Vasa*-cKO male mice are completely fertile. Consistent with this fertile phenotype, testis gross morphology and weights are comparable between *Vasa*-cKO and WT control mice (Fig. 2f-g). Histological analyses further revealed that *Vasa*-cKO

testes display normal spermatogenesis (Fig. 2h). To further confirm the *Vasa*-Cre recombined deletion efficiency in DNA level, we detected the delete allele of *Ovov2* in the offspring derived from *Vasa*-cKO male breeding pairs by PCR-based DNA analyses. As we expected, all of pups are contained *Ovov2* delete allele (Additional file 1: Figure S2). Together, these data indicate that *Ovov2* is not essential for spermatogenesis and male germ cell development in mice despite its high expression in testis.



Ovov1, another *Drosophila ovo* mouse homologue, was confirmed to express in overlapping tissues with *Ovov2*, such as skin, kidney and testis. Ablation of *Ovov1* in mice led to abnormal male germ cell development and male infertility, and *Ovov1* is essential for spermatogenesis [5]. Thus, we analyzed the expression levels of *Ovov1* in adult WT and *Vasa-cKO* testes by RT-qPCR. Interestingly, both *Ovov1* mRNA and protein levels were significantly increased in *Vasa-cKO* testes compared with those of WT testes (Fig. 2i-k). Therefore, these data suggest that *Ovov1* may have compensated for the loss of *Ovov2* functions in germ cells, which leads to normal phenotype in *Vasa-Cre;Ovov2^{lox/lox}* mice. However, it is worthwhile pointing out that other transcription factors need to be elucidated in future, which may contribute to the compensation of OVOL2 loss-of-function in male germ

cells. Overall, in this study, we report that *Ovov2* is dispensable for testicular germ cell development and spermatogenesis in mice, and provide a molecular therapeutic clue for human male infertility caused by genetic mutation.

Conclusion

Both mRNA and protein of *Ovov2* are continually expressed in postnatal developing testes from postnatal day 0 (P0) testes to adult testes (P56) and exhibits its highest level at adult testis. *Ovov2* is dispensable for testicular germ cell development and spermatogenesis in mice. *Ovov1* may have compensated for the loss of *Ovov2* functions in germ cells, which leads to normal phenotype in *Ovov2* conditional mutation mice.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12958-019-0542-3>.

Additional file 1: Figure S1: The localization of OVOL2 in mouse testicular sections was revealed by immunofluorescence. **(A)** Co-immunofluorescent staining for OVOL2 and WT1 (a Sertoli cell marker) antibodies on P21 WT testicular section showing OVOL2 expressed in Sertoli cells. Nuclei were stained with DAPI. **(B)** Co-immunofluorescent staining for OVOL2 and γ -H2AX antibodies on P21 testicular section showing OVOL2 expressed in pachytene spermatocytes. Nuclei were stained with DAPI. **(C)** Co-immunofluorescent staining for OVOL2 and γ -H2AX antibodies on P56 testicular section showing OVOL2 expressed in round spermatids. Nuclei were stained with DAPI. **(D)** Co-immunofluorescent staining for OVOL2 and γ -H2AX antibodies on P56 testicular section showing OVOL2 expressed in elongating spermatids. Nuclei were stained with DAPI. **(E)** Co-immunofluorescent staining for OVOL2 and γ -H2AX antibodies on WT and Vasa-cKO (Vasa-cre; *Ovol2lox/lox*) testis sections at adulthood. Nuclei were stained with DAPI. Arrowheads indicate Sertoli cell; Arrows indicate Leydig cells. Scale bar = 50 μ m. **Figure S2.** PCR based genotyping of the offspring derived from the Vasa-cKO (Vasa-cre; *Ovol2lox/del*) male breeding pairs. All pups contain delete allele.

Acknowledgments

Not applicable.

Available of data and materials

The data and materials for supporting the conclusion of this short communication are included within the article.

Ethics and approval

All the animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Tongji Medical College, Huazhong University of Science and Technology, and the mice were housed in the specific pathogen-free facility of Huazhong University of Science and Technology. All experiments with mice were conducted ethically according to the Guide for the Care and Use of Laboratory Animal guidelines.

Authors' contributions

SY conceived and designed the study. JZ, JD, WQ, CC, and YW performed all bench experiments. JD and SY wrote the manuscript. SY and YT supervised the project. All authors read and approved the final manuscript.

Funding

This work supported by grants from National Natural Science Foundation of China (31671551 to S.Y.), the Science Technology and Innovation Commission of Shenzhen Municipality (JCYJ20170244 to S.Y.), Natural Science Foundation of Hubei Province (2017CFA069 to S.Y.).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Institute of Reproductive Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China. ²College of Animal Science and Technology, Northwest A&F University, Yangling, People's Republic of China. ³NHC Key Laboratory of Male Reproduction and Genetics, Family Planning Research Institute of Guangdong Province, Guangzhou, People's Republic of China. ⁴Shenzhen Huazhong University of Science and Technology Research Institute, Shenzhen, People's Republic of China.

Received: 8 August 2019 Accepted: 12 November 2019

Published online: 23 November 2019

References

- Masu Y, Ikeda S, Okuda-Ashitaka E, Sato E, Ito S. Expression of murine novel zinc finger proteins highly homologous to Drosophila ovo gene product in testis. *FEBS Lett.* 1998;421:224–8.
- Wells J, Lee B, Cai AQ, Karapetyan A, Lee WJ, Rugg E, Sinha S, Nie Q, Dai X. Ovol2 suppresses cell cycling and terminal differentiation of keratinocytes by directly repressing c-Myc and Notch1. *J Biol Chem.* 2009;284:29125–35.
- Zhang T, Zhu Q, Xie Z, Chen Y, Qiao Y, Li L, Jing N. The zinc finger transcription factor Ovol2 acts downstream of the bone morphogenetic protein pathway to regulate the cell fate decision between neuroectoderm and mesendoderm. *J Biol Chem.* 2013;288:6166–77.
- Hayashi M, Shinozuka Y, Shigenobu S, Sato M, Sugimoto M, Ito S, Abe K, Kobayashi S. Conserved role of Ovo in germline development in mouse and Drosophila. *Sci Rep.* 2017;7:40056.
- Li B, Nair M, Mackay DR, Bilanchone V, Hu M, Fallahi M, Song H, Dai Q, Cohen PE, Dai X. Ovol1 regulates meiotic pachytene progression during spermatogenesis by repressing Id2 expression. *Development.* 2005;132:1463–73.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

