



Published in final edited form as:

Mol Reprod Dev. 2015 April ; 82(4): 266–267. doi:10.1002/mrd.22475.

***Ubqln3*, a Testis-Specific Gene, is Dispensable for Embryonic Development and Spermatogenesis in Mice**

SHUIQIAO YUAN¹, WEIBING QIN², CONNOR R. RIORDAN¹, HAYDEN MCSWIGGIN¹, HUILI ZHENG¹, and WEI YAN^{1,*}

¹Department of Physiology and Cell Biology, University of Nevada Reno School of Medicine, 89557, Reno, NV, USA

²Family Planning Research Institute of Guangdong, Guangzhou 510600, China

Increasing lines of evidence suggest that the ubiquitin-proteasome pathway (UPP) plays a key role in spermatogenesis (Hou and Yang 2013). Ubiquilins are Ubiquitin-like proteins, all of which contain an amino-terminal UBL domain and a carboxy-terminal ubiquitin-associated (UBA) domain in its structure. Among all Ubiquilin proteins identified so far, Ubiquilin 3 is the only one that has been reported to be testis-specific (Conklin et al. 2000). By examining the expression of *Ubqln3* mRNAs and protein in 8 different mouse organs using quantitative PCR (qPCR) and Western blot, we found that *Ubqln3* was indeed expressed exclusively in the testis, with both transcript and protein detectable in the testes starting at postnatal day 28 (P28); the highest levels were detected in adult testes (Fig. 1A–D).

The onset of testicular *Ubqln3* expression at P28 coincides with the elongation steps during late spermiogenesis, suggesting that *Ubqln3* is mainly expressed in elongating/elongated spermatids. To define its physiological role in the testis, we generated *Ubqln3* global knockout mice (on the C57BL/6J background) using a targeted embryonic stem cell line (cell-line number 11472C-G12) obtained from the Knockout Mouse Project repository; in this case, a gene-trap cassette (LacZ-pA) had recombined with exon 2 of *Ubqln3*, leading to a “gene trap” allele (Fig. 1E). Neither *Ubqln3* mRNAs (Fig. 1F) nor UBQLN3 protein (Fig. 1B) was detected in homozygous knockout mice, suggesting that these animals are truly *Ubqln3*-null (herein called *Ubqln3* KO mice). Both male and female *Ubqln3* KO mice were viable and displayed normal development, with no discernable differences in growth compared to wild-type (WT) mice. Together, these data indicate that *Ubqln3* is dispensable for embryonic and postnatal development in mice.

To determine the fertility of *Ubqln3* KO males, we performed a fecundity test using *Ubqln3* KO males bred with fertility-proven adult, WT females. Our breeding data showed that no significant difference in either litter size or litter interval compared to those WT breeding pairs over a 6-month period (data not shown), suggesting that *Ubqln3* KO males are fertile. Consistent with their normal fertility, both sperm count and sperm motility are comparable

*Corresponding author: Department of Physiology and Cell Biology, University of Nevada Reno School of Medicine, Reno, NV 89557., wyan@medicine.nevada.edu.

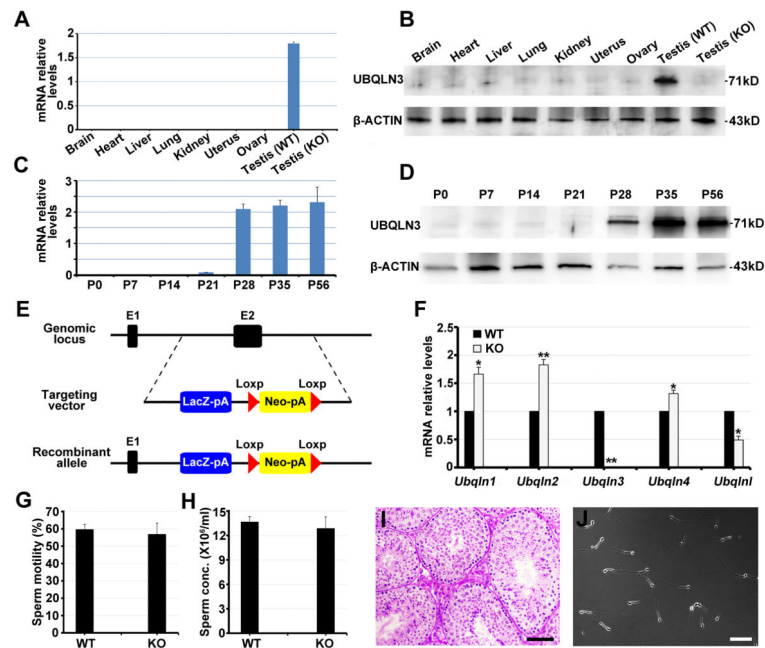
between gametes isolated from *Ubqln3* KO and WT males (Fig. 1G–H) and histological analyses of *Ubqln3* KO testes and epididymides demonstrated normal spermatogenesis and sperm morphology (Fig. 1I–J). Taken together, these data indicate that *Ubqln3* is not required for male germ-cell development or spermatogenesis in mice. Interestingly, *Ubqln1*, *Ubqln2*, and *Ubqln4* transcript abundance was increased significantly in *Ubqln3* KO compared to WT testes (Fig. 1F), suggesting that other member of the Ubiquilin family may compensate for the loss of *Ubqln3*. Therefore, the testis-specific expression of *Ubqln3* is dispensable for both embryonic development and spermatogenesis in mice.

Acknowledgments

This work was supported, in part, by National Institutes of Health (NIH) Grants HD060858, HD071736, and HD074573 (to W.Y.).

References

- Conklin D, Holderman S, Whitmore TE, Maurer M, Feldhaus AL. Molecular cloning, chromosome mapping and characterization of UBQLN3 a testis-specific gene that contains an ubiquitin-like domain. *Gene*. 2000; 249(1–2):91–98. [PubMed: 10831842]
- Hou CC, Yang WX. New insights to the ubiquitin-proteasome pathway (UPP) mechanism during spermatogenesis. *Molecular biology reports*. 2013; 40(4):3213–3230. [PubMed: 23268313]

**Figure 1.**

Ubqln3 is a testis-specific gene dispensable for spermatogenesis. **A:** qPCR analyses of *Ubqln3* mRNA levels in 8 organs of adult mice. **B:** A representative Western blot showing that UBQLN3 is exclusively detected in wild-type (WT) but not in *Ubqln3* KO testes. (The rabbit polyclonal UBQLN3 antibody (1:1000) was purchased from ProteinTech Group, Inc. (Chicago, IL.), catalog number 13568-1AP.) **C:** qPCR analyses of *Ubqln3* mRNA levels in developing testes. Testes at postnatal day 0 (P0, newborn), P7, P14, P21, P28, P35, and P56 were analyzed. **D:** A representative Western blot showing UBQLN3 expression in developing mouse testes. **E:** Schematic illustration of the targeting strategy for generating a *Ubqln3*-null allele in mouse embryonic stem cells. **F:** qPCR assays showing mRNA levels of five Ubiquilin family genes in *Ubqln3* KO testes. * $P < 0.05$, ** $P < 0.01$ compared to WT (n=3, Student's *t*-test). **G** and **H:** Normal sperm concentration and sperm motility in *Ubqln3* KO males. **I:** Normal histology in *Ubqln3* KO testes and epididymides. **J:** Representative phase-contrast micrograph showing *Ubqln3* KO sperm. Scale bars, 100 μ m (I) or 50 μ m (J).