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A Testis-specific Gene, *Ubqlnl*, Is Dispensable for Mouse Embryonic Development and Spermatogenesis

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Spermatogenesis is a complex process through which male germ-line stem cells proliferate and differentiate, eventually becoming spermatozoa. Successful spermatogenesis requires precise regulation of gene expression at transcriptional, post-transcriptional, translational and post-translational levels. Ubiquitination is a post-translational modification known to control protein turnover and, thus, plays a critical role in numerous biological processes, including spermatogenesis (Hou et al., 2012). The ubiquilin family consists of five ubiquitin-like proteins (*Ubqln1-4* and *Ubqlnl*), all of which contain an N-terminal UBL domain and a C-terminal ubiquitin-associated (UBA) domain (Hou et al., 2012).

We previously demonstrated that *Ubqln3* is exclusively expressed in the testis, yet ablation of *Ubqln3* causes no discernable phenotype, suggesting it has a dispensable role in mouse spermatogenesis (Yuan S, 2015). We subsequently discovered that another member of the ubiquilin gene family, *Ubqlnl*, is only ~5 kb apart from *Ubqln3* on mouse chromosome 7. Of 10 different mouse organs assessed using quantitative reverse-transcriptase PCR (qPCR), we found that, similar to *Ubqln3*, *Ubqlnl* was exclusively detected in the testis (Fig. 1A). *Ubqlnl* transcript was first detected in the testes by postnatal day 28 (P28) and plateaued in adults (P56) (Fig. 1B). The timing of *Ubqlnl* expression onset at ~P28 suggests that it is mainly expressed in elongating/elongated spermatids, which is a pattern similar to that of *Ubqln3* (Yuan S, 2015).

To study the physiological role of *Ubqlnl*, we generated *Ubqlnl* global knockout (KO) mice using cryopreserved sperm carrying a null *Ubqlnl* allele (allele information: *Ubqlnl*_A06, C57Bl/6N-Ubqlnl$^{tm1(KOMP)Vlcg}$) that is available from the Knockout Mouse Project (KOMP) repository. The *Ubqlnl*-null allele was generated using the “gene trap” strategy, in which a gene-trap cassette (ZEN-UB1) was inserted into the *Ubqlnl* locus (KOMP project ID: VG11289). Genotyping analyses demonstrated that *Ubqlnl* KO mice were homozygous for *Ubqlnl*-null alleles (Fig. 1C), and qPCR analyses confirmed the absence of *Ubqlnl* expression in the testes of KO males (Fig. 1D). Both female and male *Ubqlnl*-KO mice were

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viable, and did not exhibit discernable differences in either growth or behavior compared to their wild-type (WT) or heterozygous littermates.

A 5-month-long fecundity test using *Ubqlnl*-KO males bred with WT females of proven fertility revealed no significant difference in either litter size (7.7 ± 1.2 for WT and 7.3 ± 1.1 for KO, n=6, $P > 0.05$; *t*-test) or litter interval (22.8 ± 2.8 for WT and 23.3 ± 2.2 for KO, n=6, $P > 0.05$; *t*-test) compared to WT breeding pairs, suggesting that *Ubqlnl* KO males are fertile. Consistent with their normal fertility, testis size and weight of adult *Ubqlnl* KO males were similar to those of WT males (Fig. 1E), and *Ubqlnl* KO males displayed normal testicular histology with robust spermatogenesis (Fig. 1F) and normal sperm morphology (Fig.1G). Taken together, our data suggest that, despite its testis-exclusive expression, *Ubqlnl* is not required for spermatogenesis or fertility in male mice.

We also analyzed transcripts levels of the other four ubiquilin genes (Marin, 2014), in WT and *Ubqlnl*-KO testes using qPCR. Interestingly, levels of *Ubqln1* and *Ubqln4* mRNAs were significantly increased in *Ubqlnl*-KO compared to WT testes (Fig. 1D). This suggests that other ubiquilin family members may have compensated for the loss of *Ubqlnl*, thus maintaining a normal phenotype in *Ubqlnl*-KO males. Together, these findings demonstrate that *Ubqlnl* is dispensable for both embryonic and postnatal development and for spermatogenesis in mice.

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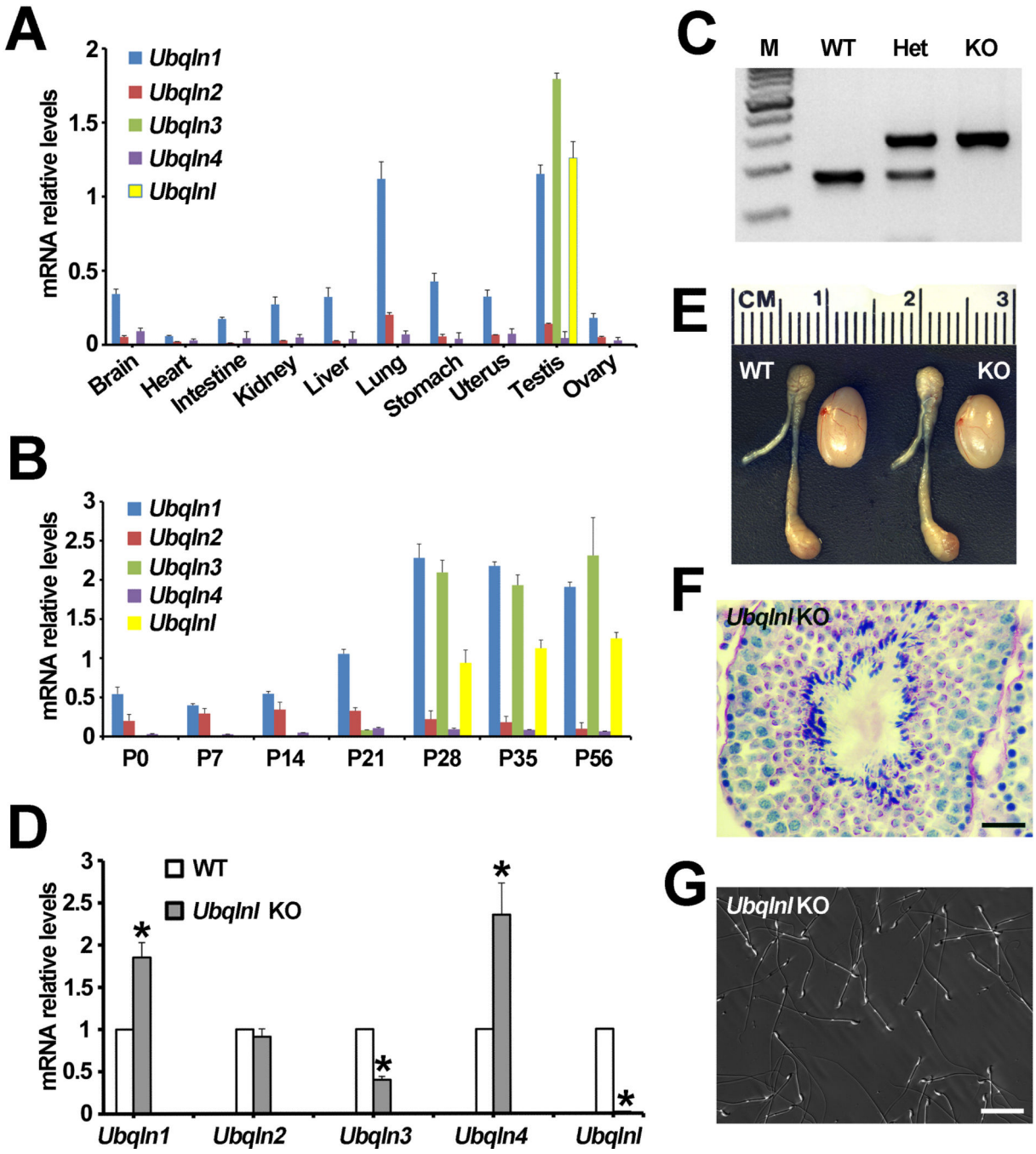

Figure 1.

Ubqlnl is a testis-specific gene dispensable for spermatogenesis. **A**: qPCR analyses of expression levels of five ubiquilin genes (*Ubqln1-4* and *Ubqlnl*) in 10 organs of adult mice. **B**: Expression levels of five ubiquilin genes in developing testes, based on qPCR analysis, from mouse testes at postnatal day 0 (P0, newborn), P7, P14, P21, P28, P35, and P56. **C**: A representative genotyping PCR result. M, MW marker; WT, wild-type; Het, heterozygous; KO, knockout. **D**: qPCR assays of mRNA levels of five ubiquilin genes in *Ubqlnl*-KO testes. *$P < 0.05$ compared to WT, n=3 (Student's *t*-test). **E**: Similar gross morphology of

WT and *Ubqlnl*-KO testes. One unit on the ruler is 1mm. **F**: A representative image of Periodic acid-Schiff-stained *Ubqlnl* KO testes section. Scale bar, 50 μm. **G**: A representative phase-contrast micrograph showing normal morphology of *Ubqlnl*-KO sperm. Scale bar, 50μm.