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Prps1l1, a Testis-specific Gene, Is Dispensable for Mouse Spermatogenesis

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Phosphoribosyl pyrophosphate synthetase (PRPS) plays an important role in nucleotide biosynthesis by transferring pyrophosphate groups from adenosine triphosphate (ATP) to ribose-5-phophate to form 5-phosphoribosyl-1-pyrophosphate (PRPP)(Cunningham et al. 2014; de Brouwer et al. 2010). The PRPS family contains three members encoded by *Prps1*, *Prps2* and *Prps1l1* (de Brouwer et al. 2010; Taira et al. 1989). *Prps1* and *Prps2* are X-linked genes expressed in a wide variety of organs/tissues, whereas *Prps1l1* on chromosome 12 is exclusively expressed in the testis (Taira et al. 1989). Despite its testis-specific expression, the function of *Prps1l1* remains unknown.

To explore the physiological role of *Prps1l1*, we examined its expression profile and generated a global *Prps1l1* knockout (KO) mouse line. *Prps1l1* and *Prps1*, but not *Prps2*, were abundantly expressed in the testis, and unlike *Prps1* which was also expressed in other organs, *Prps1l1* expression appeared to be restricted to the testis (Fig. 1A). The onset of *Prps1l1* mRNA expression was at postnatal day 21 (P21) and its levels remained high thereafter (Fig. 1B), suggesting that *Prps1l1* is mainly expressed in haploid male germ cells, i.e., round and elongating/elongated spermatids, in adult murine testes. In contrast, levels of *Prps1* mRNAs were lower prior to P7 and became much higher thereafter (Fig. 1B), suggesting that it is expressed in all types of spermatogenic cells in adult murine testes. The cellular origin of *Prps1* and *Prps1l1* mRNA expression was further confirmed by qPCR analyses using pachytene spermatocytes, round and elongating spermatids purified from the adult murine testes (Fig. 1C). Next, we generated *Prps1l1* global knockout (KO) mice using

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Please see Supplemental Materials for methods and primer sequences used in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

the CRISPR/Cas9-based genome editing technology. A PCR-based genotyping method was developed such that presence of external and absence of internal PCR products would indicate homozygous *Prps1l1* KO (Fig. 1D and Supplemental Table S1). In addition to genotyping assays, the complete lack of *Prps1l1* mRNAs in the testes of *Prps1l1* KO males was further confirmed using qPCR analyses (Fig. 1E).

A 3-month-long fertility test showed that *Prps1l1* KO males had normal fertility (data not shown). Testicular weight and histology of adult *Prps1l1* KO males were comparable to those of WT males (Fig. 1F and Fig. 1G). *Prps1l1* KO males also displayed normal sperm counts and motility, as well as sperm morphology (Fig.1H and Fig. 1I). Levels of *Prps1* and *Prps2* mRNAs were slightly, although not significantly, increased in *Prps1l1* KO compared to WT testes (Fig. 1E). No significant upregulation of either *Ppat* or *Ahcy*, two genes known to encode proteins that can bypass PRPP (de Brouwer et al. 2010), was observed either (Fig. 1E). Taken together, our data suggest that, despite its testis-exclusive expression, *Prps1l1* is dispensable for spermatogenesis in mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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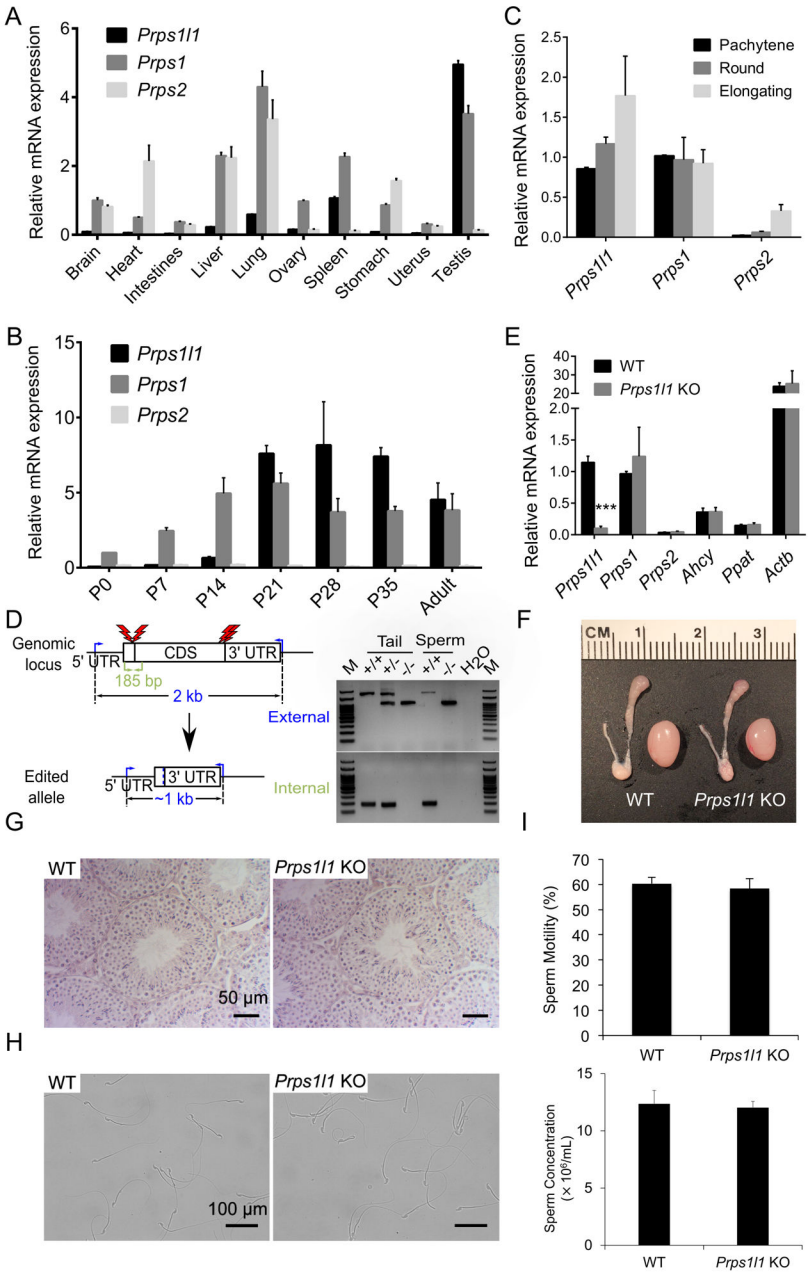

Figure 1.

Prps1l1 is dispensable for spermatogenesis despite its high expression in the testis. A. qPCR analyses of mRNA expression levels of three PRPS genes (*Prps1*, *Prps2* and *Prps1l1*) in ten organs of adult mice. Expression levels were normalized to *Gapdh*. Data are presented as means±SEM, n=3. B. qPCR analyses of mRNA expression levels of three PRPS genes (*Prps1*, *Prps2* and *Prps1l1*) in developing murine testes at postnatal day 0 (P0), P7, P14, P21, P28, P35 and adulthood. Expression levels were normalized to *Gapdh*. Data are presented as means±SEM, n=3. C. qPCR analyses of *Prps1l1* mRNA levels in pachytene spermatocytes, round and elongating spermatids purified from WT adult murine testes. Expression levels were normalized to *Gapdh*. Data are presented as means±SEM, n=3. D. Schematic

illustration of the generation of *Prps1l1* KO mouse and a representative genotyping result of *Prps1l1* heterozygous (+/−), homozygous (−/−) and wild type (+/+). M, marker. The red lightning bolt represents gRNAs used, and its right and left orientations indicate the gRNAs targeting the reverse and forward strands of the genomic DNA, respectively. Blue arrows show the position of external primers, while light green arrows indicate that of internal primers. The expected size of PCR products is indicated in the same color. E. qPCR analyses of mRNA expression levels of the three PRPS genes (*Prps1*, *Prps2* and *Prps1l1*) and two related genes (*Ppat* and *Ahcy*) in *Prps1l1* KO testes. Expression levels were normalized to *Gapdh*. Data are presented as means±SEM, n=3, ***p<0.001. F. Normal morphology of WT and *Prps1l1* KO testes. One unit on the ruler is 1mm. G. A representative image of Haematoxylin and Eosin (HE)-stained WT and *Prps1l1* KO testes section. Scale bar=50 μm. H. A representative phase-contrast micrograph showing normal morphology of WT and *Prps1l1* KO sperm. Scale bar=100 μm. I. Sperm motility and concentration assays on adult *Prps1l1* KO males.