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FLACC1 is Testis-Specific but Dispensable for Fertility in Mice

Jessica Y. Chotiner[1,2], **N. Adrian Leu**[1], **P. Jeremy Wang**[1,2]

[1]Department of Biomedical Sciences, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104, USA.

[2]Cell and Molecular Biology Graduate Program, University of Pennsylvania, Philadelphia, PA 19104, USA.

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Spermatogenesis occurs sequentially in mitotic, meiotic, and post-meiotic phases. Many genes regulating these phases are germ cell-specific (Chen et al., 2018). Previously, *Flacc1* (Flagellum Associated Containing Coiled-Coil Domains 1) (also known as *Als2cr12*) was identified as a testis-specific gene (Choi & Cho, 2011). It was reported that FLACC1 localizes in the principal piece of the sperm flagellum and is associated with the fibrous sheath (Choi & Cho, 2011). Here we examined whether FLACC1 plays a role in fertility in mice.

To study the functional requirement of *Flacc1*, we disrupted *Flacc1* in mice using the CRISPR/Cas9-mediated genome editing approach (Figure 1a) (Yang, Wang, & Jaenisch, 2014). Exon 2 contains the start codon. The resulting allele lacked parts of the exon 2 and intron 2 and thus was expected to be null (Figure 1a). We generated rabbit polyclonal antibodies against the short isoform of FLACC1 (245 residues; AK016678). The short isoform overlaps with the N-terminal region of the FLACC1 long isoform (383 residues; NP_780579). Therefore, our knockout strategy should delete both isoforms.

The antibody was used to assess the expression of FLACC1 during the first wave of spermatogenesis by Western blotting. The first wave of spermatogenesis occurs synchronously. Spermatogonia, pachytene spermatocytes, and sperm first appear at postnatal day 6 (P6), 14, and 35 respectively. After P35, spermatogenesis occurs asynchronously. Western blot analysis of testicular protein lysates revealed that FLACC1 (~50 kD; the long isoform) was absent at P6, detectable at P14, abundant at P35, and most abundant at P56 (adult) (Figure 1b). The increase in protein abundance at P35 and P56 is consistent with its expression in sperm. The absence of the FLACC1 protein in *Flacc1*$^{-/-}$ adult testis showed that the mutant allele was null (Figure 1c). Contrary to previous findings (Choi & Cho,

Correspondence P. Jeremy Wang, Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. pwang@vet.upenn.edu.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

2011), we did not detect the short isoform of FLACC1 (~35 kD) in wild type testis or sperm by Western blot. In addition, the previous study reported the localization of FLACC1 to sperm flagella (Choi & Cho, 2011). We also detected a similar signal in the principal piece with the FLACC1 antibody (Figure 1g). However, the immunofluorescence signal was still present in sperm from *Flacc1*$^{-/-}$ males, demonstrating that this signal is non-specific due to cross reactivity of the antibody (Figure 1g). This result highlights the importance of using knockout cells to control for the specificity of immunolocalization signals. Both *Flacc1*$^{-/-}$ males and females were viable and fertile. Histological analysis of adult *Flacc1*$^{-/-}$ testes revealed apparently normal spermatogenesis (Figure 1d). The testis/body weight ratios were comparable between *Flacc1*$^{-/-}$ and control mice (Figure 1e). Furthermore, loss of FLACC1 did not affect the number of sperm in *Flacc1*$^{-/-}$ epididymis as compared to controls (Figure 1f). Mating tests showed that the litter size sired by *Flacc1*$^{-/-}$ males (7.7 ± 3.7; n = 5 males) was similar to that by *Flacc1*$^{+/-}$ males (6.9 ± 3.1; n = 5 males). These data demonstrate that FLACC1 is dispensable for viability and fertility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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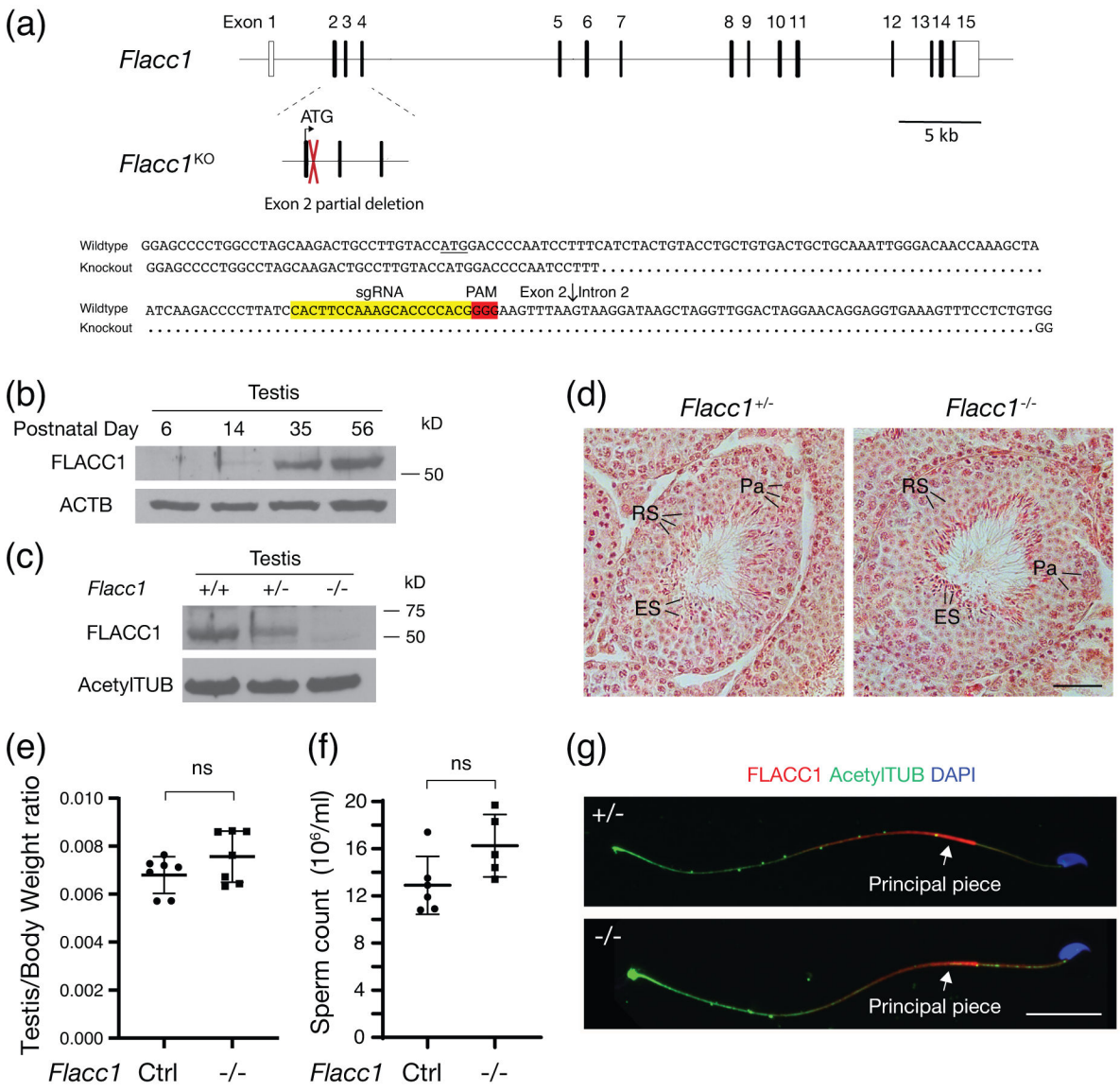

Figure 1.

(a) Diagram of the *Flacc1* gene structure and knockout strategy. sgRNA and PAM site are highlighted. The start codon is underlined. The 147-base deletion removes the exon 2/intron 2 splicing donor site and parts of exon 2 and intron 2. Solid bars, coding exons; Open boxes, 5' and 3' UTRs. The short isoform was described previously (Fig. 1 in Choi & Cho, 2011). (b) Western blot analysis of FLACC1 in wild-type testes at P6, P14, P35, and P56. ACTB (β-actin) serves as a loading control. (c) Western blot analysis of FLACC1 in adult (8-week-old) $Flacc1^{+/+}$, $Flacc1^{+/-}$, and $Flacc1^{-/-}$ testes. AcetylTUB (acetylated tubulin) serves as a loading control. (d) Histological analysis of adult $Flacc1^{+/-}$ and $Flacc1^{-/-}$ testes. Pa, pachytene spermatocytes; RS, round spermatids; ES, elongating spermatids. Scale bar, 50 μm. (e) Comparison of testis/body weight ratios between adult control ($Flacc1^{+/+}$ and $Flacc1^{+/-}$; n=7) and $Flacc1^{-/-}$ (n=7) mice. (f) Comparison of sperm count between adult control ($Flacc1^{+/+}$ and $Flacc1^{+/-}$; n=6) and $Flacc1^{-/-}$ (n=5) epididymis. ns, non-significant.

(g) Immunofluorescence of sperm from *Flacc1*$^{+/+}$ and *Flacc1*$^{-/-}$ mice with anti-FLACC1 and anti-AcetylTUB antibodies. DNA was stained with DAPI. Scale bar, 25 μm.