CXADR is constitutively expressed in Sertoli cells (SCs) and all types of germ cells (GCs). CXADR is localized at the blood-testis barrier (BTB) constituted by tight junctions and basal ectoplasmic specializations between adjacent SCs. Also, CXADR is found at the interface between SC and GC. Earlier in vitro studies indicated that CXADR plays role in BTB function and germ cell migration. However, conventional knockout of CXADR leads to embryonic lethality, making the study of CXADR in the testis impossible. We aim to generate SC-specific and GC-specific CXADR knockout (KO) mice using the Cre/loxP system to evaluate the SC-specific and GC-specific functions of CXADR on spermatogenesis in vivo. Bioinformatics analyses of RNA sequencing and Proteomics will be performed to identify and elucidate the genes and mechanisms involved in fertility impairment.

The cre excision in SC-CXADR and GC-CXADR KO mice was under the control of Amh gene and Stra8 gene respectively. The deletion of CXADR was confirmed by PCR and immunohistochemistry. Fertility assays revealed that a significant reduction (44%) in the number of litters is reported in SC-CXADR KO mice compared with the control, suggesting that SC-CXADR KO causes male subfertility. However, GC-CXADR KO mice show no apparent difference (e.g. testis morphology and fertility assays) from the control testes. Compromised BTB function and increased germ cell apoptosis has been observed in SC-CXADR KO mice. An array of BTB proteins such as ZO-1, occludin and beta-catenin was downregulated or mislocalized in SC-CXADR KO mice. Illumina sequencing revealed that SC-CXADR KO increased the expression of 360 genes and reduced the expression of 152 genes including c-kit and snai2, which have been confirmed that had important roles in male fertility in vivo studies. Functional gene ontology analysis of down-regulated genes revealed significant enrichment of genes related to cilium morphogenesis, spermatogenesis, spermatid development and differentiation, processes known for germ cell development. Taken together, our data demonstrated that CXADR in SC, but not in GC, is essential for BTB function and spermatogenesis.