Zebrafish *gsdf* mutant provides a disease model for human polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is the most frequent problem when a couple comes to a fertility clinic. PCOS in women includes the accumulation of premature oocytes in ovaries. We discovered that zebrafish lacking activity of the *gsdf* gene (gonad soma derived factor) due to gene editing (TALENS) mimic the pathology of PCOS. Homozygous *gsdf* mutants have delayed differentiation of the bi-potential gonad to ovary or testes during gonad development accumulation of young oocytes, elevated androgen, premature loss of fertility, obesity, and inappropriate expression of genes for lipid metabolism and insulin. We conclude that the *gsdf* mutant in zebrafish provides a disease model for human PCOS and suggests pathways for potential therapies.

Introduction

- As a vertebrate animal, the genome of zebrafish is similar to humans. Based on transparent embryos and larvae, zebrafish allow easy visualization of developmental processes.
- zebrafish could produces a larger number of offspring in each generation and its embryos are able to absorb chemicals added to their water, and it is possible to make mutations which resemble human clinical disorders
- Knock out zebrafish *gsdf* by TALEN-mediated mutagenesis could cause mutant of zebrafish and in female, it shows as similar symptom to human PCOS
- PCOS involve members of the transforming growth factor-β(TGFB) family, mutations on *gsdf* gene in zebrafish affect the expression level of *amh*, *gata4* and *cyp19a1a*.

Results

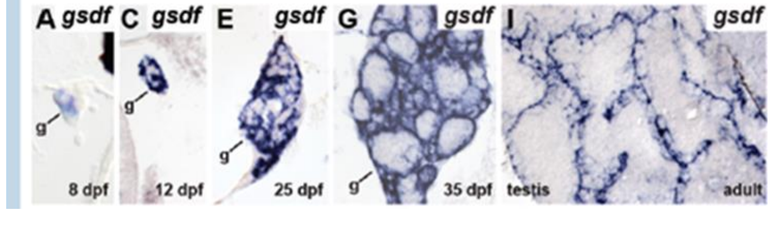


Fig.1 In situ hybridization shows that *gsdf* is expressed by 8 dpf (days post fertilization), and that expression continues until gonads tissue mature. The blue color identifies cells expressing *gsdf*

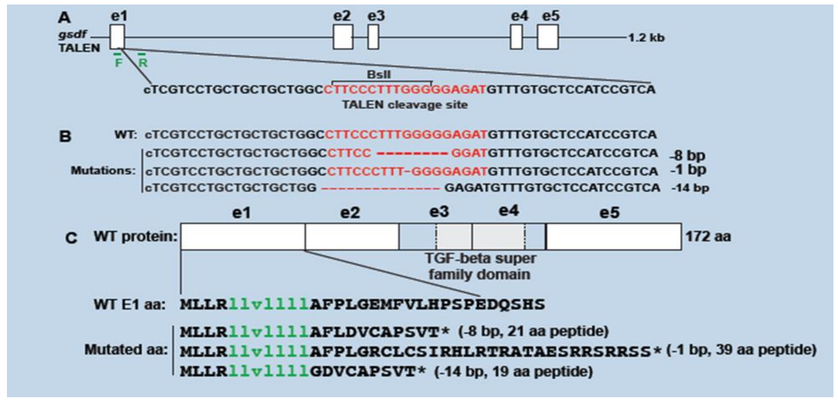


Fig.2 The diagram in part A shows the gsdf gene. Base pairs in red are the TALEN targeted region in exon1. Three types of mutants, with deletions of -8, -1 and -14 base pairs were produced (part B), which interrupt the restriction site of Bs1I. All three types of mutations in gsdf predict frameshifts during gsdf translation, resulting in lost gsdf function.

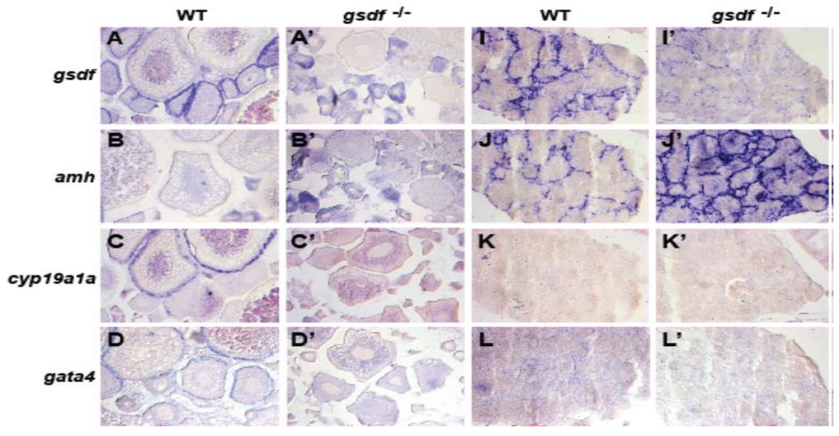
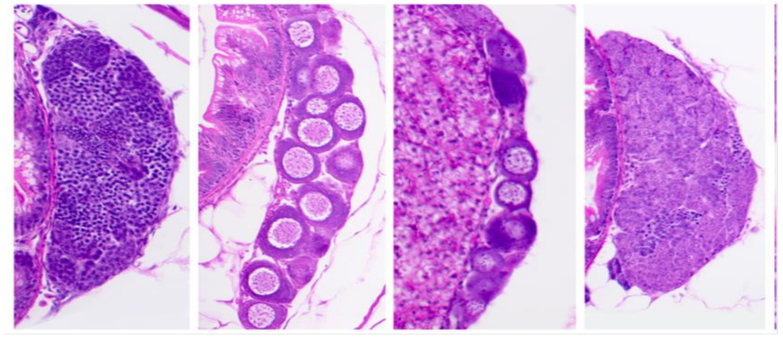


Fig.3 In situ hybridization shows the gene expression patterns. The expression of *gsdf* is down in *gsdf* mutants compared to wild types (Part A, A'I, I'). Expression of *amh* is higher in mutant testis compared to wild types (Fig.3 J, J'). Expression analysis also shows decreasing *cyp19a1a* (Fig.3 C, C') and *gata4* (Fig.3C D, D') expression compared to wild types.



A. WT testis B. WT ovary C. mutant ovary D. mutant testis
Fig.4 Histology of gonads at 35 dpf, A. Wild-type testis, B, Wild-type ovary. C. The right side of gonad in one mutant fish formed an ovary and D. left side formed testis. Normally, after 35 dpf, both sides of gonad in one fish should be the same type. However, some gsdf mutant fish formed both testis and ovary, which means that gsdf normally helps speed fate change.

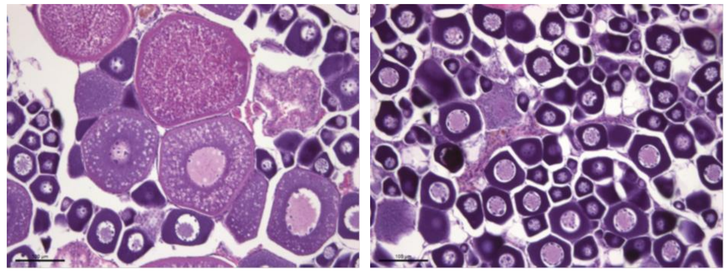


Fig.5 Histology of ovaries of wild types and mutant. Wild types have premature and mature oocytes in the ovary (left side). In contrast, mutant ovaries only accumulate immature follicles (Fig.5), which is similar to the phenotype of human PCOS patients (Fig.6).

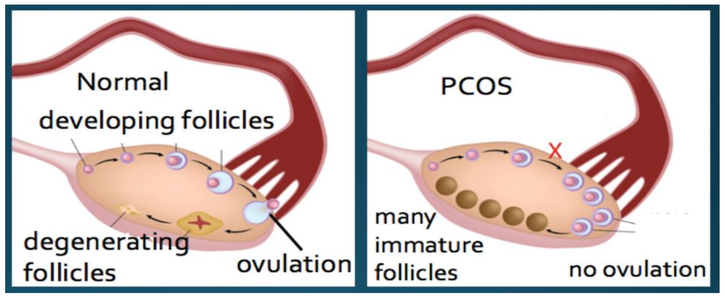


Fig.6 PCOS in humans. Normally, developing follicles release from the ovary after they mature. For PCOS patients, however, follicles don't develop to a mature state, so there is no ovulation. Many immature follicles just accumulate in the ovary.

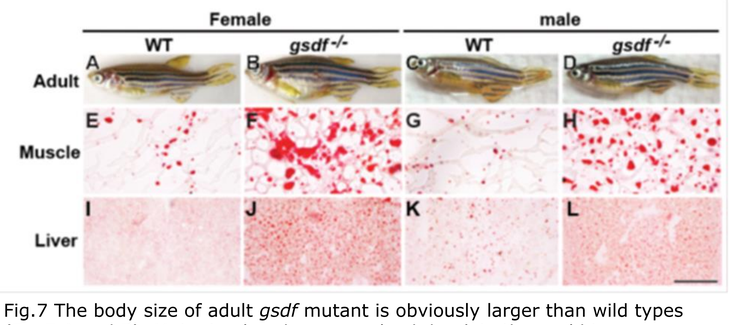


Fig.7 The body size of adult *gsdf* mutant is obviously larger than wild types (Fig.7 A and B). Mutants also show more lipid droplets than wild types (fig.7E-L).

Conclusions

In zebrafish, *gsdf* mutant females present a phenotype that mimics polycystic ovarian syndrome (PCOS) in humans. Phenotypes include the accumulation of immature ovarian follicles, obesity, insulin abnormalities, and female sterility. Because *gsdf* does not exist in the human genome, another TGFB signaling molecule is likely involved in the etiology of PCOS, and the zebrafish mutant provides a model to search for therapies.