BIALLELIC *PPP2R3C* MUTATIONS ARE ASSOCIATED WITH PARTIAL AND COMPLETE GONADAL DYSGENESIS IN 46,XY AND 46,XX INDIVIDUALS

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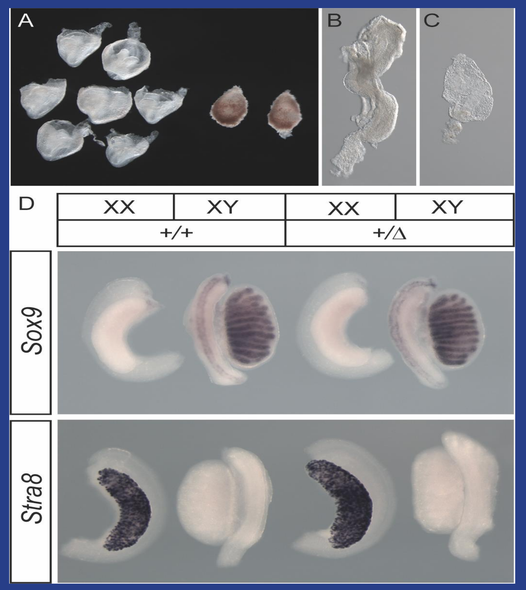
INTRODUCTION

- *PPP2R3C* encodes the B"gamma regulatory subunit of the protein phosphatase 2A (PP2A), which is a serine/threonine phosphatase involved in the phospho-regulation processes of mammalian cells.
- We have recently reported homozygous and heterozygous mutations in *PPP2R3C* in patients with syndromic 46,XY complete gonadal dysgenesis (MEGD syndrome) and impaired spermatogenesis, respectively **(1)**.
- In this study, we have further investigated the role of *PPP2R3C* in the etiology of gonadal dysgenesis.

METHOD

- We sequenced the *PPP2R3C* gene in four new patients from three unrelated families.
- The clinical, laboratory and molecular characteristics were investigated.
- We have determined the requirement for *Ppp2r3c* in mice using CRISPR/Cas9 genome editing.



RESULTS

- We have identified a homozygous c.578T>C (p.L193S) *PPP2R3C* variant in one 46,XX girl with primary gonadal insufficiency, 2 girls with 46,XY complete gonadal dysgenesis, and one undervirilized boy with 46,XY partial gonadal dysgenesis.
- The patients with complete gonadal dysgenesis had low gonadal and adrenal androgens, low AMH and high FSH and LH concentrations **(Table 1)**.
- All patients manifested characteristic features of MEGD syndrome **(Figure 1)**.
- We then generated mice (C57BL6/N) lacking functional *Ppp2r3c* by using CRISPR/Cas9 genome editing to delete an 1100 bp segment encoding a critical early exon.
- Using a published single-cell RNA sequencing (scRNAseq) dataset of XX and XY mouse gonad development, we identified expression in the majority of gonadal cell lineages, including Tcf21+ gonadal progenitors at 11.5 dpc, and Sox9+ and Fst+ supporting cells in XY and XX gonads, respectively.
- Heterozygous *Ppp2r3c* knockout mice appeared overtly normal and fertile. **(Figure 2A, D)**.
- Inspection of homozygous embryos at 14.5, 9.5 and 8.5 days post coitum revealed evidence of dead embryos **(Figure 2C)**.
- We conclude that loss of function of *Ppp2r3c* is not compatible with viability in mice and results in embryonic death from 7.5 dpc or earlier.

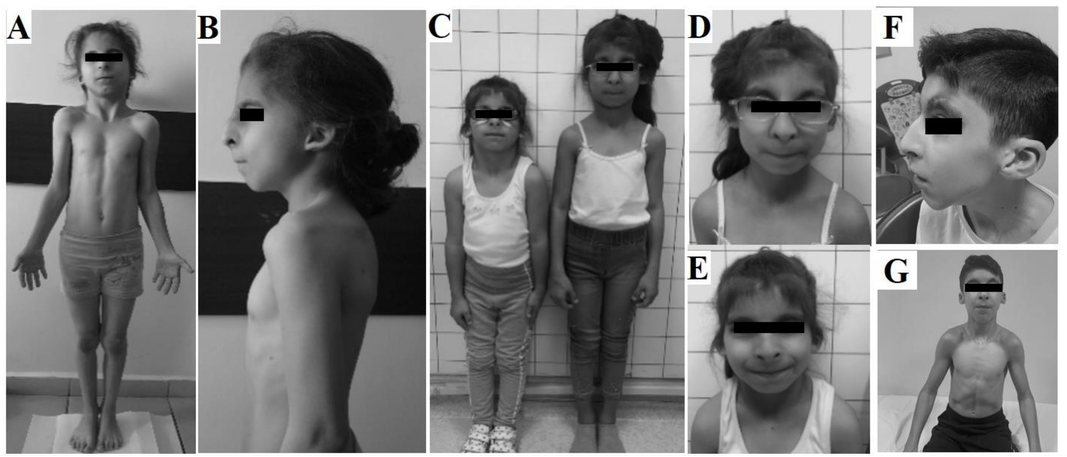


Figure 1. Clinical and molecular characteristics of patients with MEGD syndrome. Besides XY-CGD, a number of extra-gonadal syndromic features, including typical facial gestalt, low birth weight, myopathy, rod and cone dystrophy, anal atresia, omphalocele, sensorineural hearing loss, dry and scaly skin, skeletal abnormalities, renal agenesis and neuromotor delay characterize MEGD syndrome.

Figure 2. Loss of *Ppp2r3c* causes embryonic death (A,C), but heterozygous mutants have normal fetal gonads (D).

Table 1. Gonadal and adrenal function test results of patients with MEGD syndrome

Hormone	P1 ($9^{5/12}$)	P2a ($7^{11/12}$)	P2b ($6^{1/12}$)	P3 ($10^{5/12}$)
Karyotype	46, XX	46, XY	46, XY	46, XY
FSH (mIU/mL) (normal range)	41.1 (1.79-10.9)	44.59 (1.9-12.8)	43.1 (3.85-8.78)	11.8 (1.5-12.40
LH (mIU/mL) (normal range)	9.07 (1-11)	1.81 (<0.3-6.3)	1.7 (<0.3-6.3)	2.8 (1.7-8.6)
Testosterone (ng/mL) (normal range)	<0.07 (2.4-9.5)	<0.07 (2.4-9.5)	<0.07 (2.4-9.5)	1.3 (2.4-9.5)
Estradiol (pg/mL) (normal range)	<20 (27-122)	<20 (27-122)	<20 (27-122)	NA
Antimüllerian hormone (ng/mL) (normal range)	0.01 (0.00-8.8)	0.00 (0.00-8.8)	0.00 (0.00-8.8)	18.8 (38.2-332)
Cortisol (µg/dL) (normal range)	9.8 (5-21)	16.1 (5-21)	11.8 (5-21)	12.8 (5-21)
DHEAS (ng/mL) (normal range)	49 (80-560)	17.5 (35-430)	15 (35-430)	26 (80-560)
Androstenedione (ng/mL) (normal range)	0.81 (0.6-3.1)	<0.3 (0.3-2)	<0.3 (0.3-2)	NA

CONCLUSIONS

- The 4 individuals reported here illustrate the association of *PPP2R3C* variants with gonadal dysgenesis spectrum phenotypes and multiple malformations in MEGD syndrome.
- In the mouse model, studies of *Ppp2r3c* demonstrate expression in a number of developing gonadal cell lineages important for sex determination and an essential role in development.

REFERENCES

1. Guran T et al. *PPP2R3C* gene variants cause syndromic 46,XY gonadal dysgenesis and impaired spermatogenesis in humans. *Eur J Endocrinol.* 2019;180(5):291-309.

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