Introduction

• Primary ovarian insufficiency (POI) affects 1% of women and is characterised by early loss of normal ovarian function and depletion of the ovarian reserve.
• POI is genetically mediated in up to 30% of cases.
• Many genes associated with POI have roles in early ovary development processes, including meiosis.

Methods

• Exome sequencing using an autosomal recessive model was performed in the affected patients and unaffected family members.
• RNA sequencing and quantitative reverse transcriptase PCR (qRT-PCR) were used to study the expression of key genes at critical stages of human fetal gonad development (Carnegie Stage (CS) 22/23, 9 weeks post conception (wpc), 11wpc, 15/16wpc, 19/20wpc) and in adult tissue.
• Expression data were matched to cell populations using publicly available single-cell RNAseq data.
• Immunohistochemistry was used to study protein expression.
• Bulk RNA sequencing of peripheral leukocytes from patient and controls were used to investigate the effect of variants of interest on transcript stability and splicing.
• The cellular expression and localisation of variants were studied using in vitro HeLa/HEK-293 cell systems and co-immunoprecipitation studies.
• In silico modelling and simulation studies characterised wild-type and mutant proteins.

Results

Exome sequencing revealed pathogenic variants in YTHDC2 co-segregating with the POI phenotype: in the two sisters, a homozygous variant c.2567C>G, p.P856R in the HA2 domain; in the third patient, a homozygous c.1129G>T, p.E377* variant.

The m4A modification on mRNA is mediated by "writers" and demethylases ("erasers"). "Readers" recognize the m4A modification and allow for execution of its functions. YTHDC2 is a key reader of this complex, with its known cofactors MEIOC and XRN1. YTHDC2 has been associated with meiotic progression in animal studies.

There is higher YTHDC2 expression in the late (15/16wpc) compared to early (CS22/23) fetal ovary (logFC 1.14, p.adj 2.18E-08), coincident with the female meiotic peak. Higher YTHDC2 (logFC 0.5, p.adj <0.05) expression was seen in the ovary compared to testis at 15/16wpc. MEIOC showed a similar expression pattern. YTHDC2 was highly expressed in the adult testis, corresponding with male meiosis. Human fetal single-cell RNAseq data localised YTHDC2 expression to meiotic germ cells.

Immunohistochemistry of a 20wpc human fetal ovary showed cytoplasmatic staining of YTHDC2 in meiotic cells.

Neither YTHDC2 variant affected transcript splicing nor stability. The P856R variant did not affect YTHDC2 cellular localisation nor YTHDC2/MEIOC co-localisation.

Differential gene expression analysis (ovary 15/16wpc vs testis 15/16wpc, logFC >1, p.adj <0.05) revealed RNA helicases, including YTHDC2, and piRNA biogenesis genes to be highly expressed in the fetal ovary at meiosis.

We show that the m4A reader YTHDC2 plays a key role in meiosis regulation and human gonadal development and associate pathogenic variants within YTHDC2 with POI.

In silico modelling showed that the p.P856R variant results in a more stable, less flexible protein that affects downstream conformational kinetics of the HA2 domain.

Conclusions

We investigated the genetic mechanism underlying early-onset POI in three young women presenting with absent puberty: two sisters from a consanguineous pedigree and a third unrelated proband.

We show that the m4A reader YTHDC2 plays a key role in meiosis regulation and human gonadal development and associate pathogenic variants within YTHDC2 with POI.

In silico modelling showed that the p.P856R variant results in a more stable, less flexible protein that affects downstream conformational kinetics of the HA2 domain.