Genes previously linked to premature ovarian insufficiency show no pathogenicity in the general population

We investigated the presence of genetic variants for 105 genes previously associated with premature ovarian insufficiency (POI) in over 100,000 women. We found that predicted damaging variants in these genes were commonly found in the heterozygous state in women with menopause in the normal age range. This suggests that monogenic causes of POI are rare, and that POI is more likely to be a polygenic disorder.

The question

We sought to evaluate the penetrance of deleterious variants in genes previously reported to cause POI, in the general population[1]. POI affects around 1% of the female population and is characterized by menopause before the age of 40 years[2]. Menopause timing is known to be heritable and numerous single gene causes have been reported in studies analyzing women with POI, as ascertained via clinical criteria, but the penetrance of these identified genetic variants has not been evaluated in the general population. Accurate interpretation of genetic findings is essential for informing treatment for patients and their families and aiding understanding of the etiology of reproductive ageing.

The observation

We selected 105 genes previously reported to cause POI using genes from the Genomics England PanelApp or by searching for reports on single gene causes of POI in the literature. The PanelApp is a set of manually curated monogenic causes of disease used in clinical testing, where strength of evidence for association with a certain disease is colour coded: green being strong evidence; amber moderate; and red limited evidence. We grouped our selected genes according to their proposed mode of inheritance, as, dominant, recessive, or X-linked. We then detected genetic variants for 105 POI-associated genes in whole exome sequencing (WES) data from 104,733 women that were made available through the UK Biobank[3]. Menopause age was self-reported, and we included only women who had a natural menopause. We next used *in silico* tools to predict the impact of these variants on gene function and classify them as protein truncating variants (PTVs), or deleterious missense variants. Variants from each category were combined and tested in aggregate in 'burden' tests; a third burden test of all 'damaging' variants was also performed. In addition, we tested the association with individual variants that had previously been identified as POI-associated.

A total of 37 genes had a dominant mode of inheritance and had PTVs detected in the UK Biobank WES data. The mean age at menopause was 50 years in carriers of PTVs, with 99.9% of all PTVs found in women with menopause after the age of 40 (Figure 1). This implies that carriers of these damaging genetic variants generally had normal ovarian function. Moreover, we found no evidence that having a heterozygous PTV or missense variant in a gene with a previously reported autosomal

recessive or X-linked mode of inheritance was sufficient to cause POI. Although we found no evidence that any of the genes were highly penetrant monogenic causes of POI, we did identify five of the 105 genes tested that altered timing of menopause within the normal age range, changing mean menopause age by 1.3–3.5 years.

The implications

We found no evidence to support that heterozygous PTVs for any of the 105 genes that had been previously associated with POI have a highly penetrant effect on ovarian function. Most of the genes we tested are included on various gene panels for POI and we suggest that the presence of deleterious variants in the heterozygous state should be interpreted with caution. Most cases of menopause before 40 cannot be attributed to a single gene and are likely to be polygenic.

We were not able to validate our penetrance findings in an independent population-based cohort, however this is the largest study to date. We are also limited in what we can say about recessive inheritance, because we observed so few women who were homozygous for variants in these genes in UK Biobank. We can, however, infer that recessive gene variants are likely to be causes of POI only rarely. Further work is required to assess the impact of structural variants, which were not assessed in our study.

Future studies should explore the complex polygenic nature of POI in larger, clinically ascertained, cohorts and investigate the combined effect of common and rare variation on ovarian ageing. These studies will help develop new diagnostic approaches for POI, to improve diagnosis and provide appropriate genetic counselling for patients.

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EXPERT OPINION

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BEHIND THE PAPER

The Exeter and Cambridge teams have collaborated for many years on projects identifying common genetic variants associated with reproductive ageing, but the availability of sequence data at scale in hundreds of thousands of individuals enabled us to assess the penetrance of rarer coding variants on the phenotype. We expected to see reduced penetrance in a non-clinical context but were surprised to see so few robust monogenic associations with POI. As we were keen to ensure our work is applicable to clinicians looking after women with POI, we involved clinical geneticists and endocrinologists in the selection of a clinically approved gene list for testing, augmented with further genes described in the literature as POI-related. To find that PTVs in even green Panel App genes were usually found in women with normal ovarian function was a key milestone in the study. **A.M. & J.R.B.P.**

REFERENCES

1. Ke, H., *et al.* Landscape of pathogenic mutations in premature ovarian insufficiency. *Nature Medicine,* **29**, 483-492, (2023).

This paper evaluates the prevalence of predicted pathogenic variants in POI genes in a cohort of POI cases.

2. Wesevich, V., Kellen, A.N. & Pal, L. Recent advances in understanding primary ovarian insufficiency. *F1000Res* **9**, F1000 Faculty Rev-1101 (2020).

A review article about POI.

3. Backman, J.D*., et al.* Exome sequencing and analysis of 454,787 UK Biobank participants. *Nature* **599**, 628-634 (2021).

An overview of the exome sequencing data available in UK Biobank.

FIGURE

Fig 1. Age at natural menopause in women with high confidence PTVs in autosomal dominant POI genes. Age at natural menopauses (ANM) shown for all tested variants of Panel App genes (green/amber) or manually curated genes (grey). Women who did not carry an HC-PTV of these genes are shown in blue. Numbers of women with an ANM of below40 (cases) compared to an ANM of above 40 years (controls) are shown in brackets on right Y axis [cases/controls]. Boxes indicate lower quartile, median and upper quartile for ANM; whiskers the most extreme value within 1.5x the interquartile range from the lower and upper quartiles; outliers are shown as individual points.

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FROM THE EDITOR

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Gene	Count
Reference	[2,189/100,698]
NOBOX	[2/137]
POLG	[3/52]
BMPR1B	[0/4]
BUB1B	[4/65]
EIF4ENIF1	[0/17]
FIGLA	[0/6]
GDF9	[1/95]
KHDRBS1	[1/2]
LMNA	[0/16]
NANOS3	[0/16]
NOG	[0/1]
POLR2C	[1/21]
POU5F1	[1/4]
SOHLH2	[2/8]
AIRE	[4/172]
ALOX12B	[1/40]
AMH	[1/69]
ANKRD31	[7/266]
ATG7	[1/121]
ATG9A	[0/7]
BNC1	[1/2]
CDKN1B	[0/2]
CPEB1	[0/5]
ERCC6	[4/311]
FANCL	[3/62]
FMN2	[1/8]
LHX8	[0/8]
NHEJ1	[0/18]
NOTCH2	[0/18]
PRDM9	[2/88]
RAD51	[0/2]
REC8	[1/50]
SALL4	[0/4]
SMC1B	[0/42]
TP63	[0/8]
WDR62	[3/81]
WT1	[0/1]

Age at Natural Menopause (years)