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P-541 Identification of novel variants and candidate genes in women with familial idiopathic premature ovarian failure using whole-exome sequencing

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Study question: Is it possible to identify a genetic cause of familial premature ovarian failure (POF) with whole-exome sequencing (WES)?

Summary answer: Whole-exome sequencing is the most efficient strategy to identify probably pathogenic mutations in different genes in pathologies of polygenic etiology such as premature ovarian failure.

What is known already: Premature ovarian failure is the loss of ovarian function before the age of 40, and it is a common cause of infertility in women. This pathology has a heterogeneous etiology. Some chromosomal and genetic alterations have been described, and could explain approximately 20% of cases. However, in most patients the origin remains unknown. Recent studies with next-generation sequencing (NGS) have identified new variants in candidate genes related with premature ovarian insufficiency (POI) or premature ovarian failure (POF). These genes are not only involved in processes such as folliculogenesis, but also with DNA damage repair, homologous recombination, and meiosis.

Study design, size, duration: Fourteen women, from 7 families, affected by idiopathic POF were included in the study from October 2019 to September 2020. Seven POF patients were recruited when they came to our clinic to

undergo assisted reproductive treatment. In the anamnesis, it was found that they had relatives with a diagnosis of POF, who were also recruited for the study. The inclusion criteria were amenorrhea before 38 years old and analytical and ultrasound signs of ovarian failure.

Participants/materials, setting, methods: WES was performed using TrusightOne (Illumina®). Sequenced data were aligned through BWA tool and GATK algorithm was used for SNVs/InDel identification. VCF files were annotated using Variant Interpreter software. Only the variants shared by each family were extracted for analysis and these criteria were followed: (1) Exonic/splicing variants in genes related with POF or involved in biological ovarian functions (2) Variants with minor allele frequency (MAF) ≤0.05 and (3) having potentially moderate/strong functional effects.

Main results and the role of chance: Seventy-nine variants possibly related with the POF phenotype were identified in the seven families. All these variants had a minor allele frequency (MAF) ≤0.05 in the gnomAD database and 1000 genomes project. Among these candidate variants, two were nonsense, six splice region, one frameshift, two inframe deletion and 68 missense. Thirty-two of the missense variants were predicted to have deleterious effects by minimum two of the four in silico algorithms used (SIFT, PolyPhen-2, MutationTaster and PROVEAN). All variants were heterozygous, and all the families carried three or more candidate variants. Altogether, 43 probably damaging genetic variants were identified in 39 genes expressed in the ovary and related with POF/POI or linked to ovarian physiology. We have described genes that have never been associated to POF pathology, however they may be involved in key biological processes for ovarian function. Moreover, some of these genes were found in two families, for example *DDX11*, *VWF*, *PIWIL3* and *HSD3B1*. *DDX11* may function at the interface of replication-coupled DNA repair and sister chromatid cohesion. *VWF* gene is suggested to be associated with follicular atresia in previous studies. *PIWIL3* functions in development and maintenance of germline stem cells, and *HSD3B1* is implicated in ovarian steroidogenesis.

Limitations, reasons for caution: Whole-exome sequencing has some limitations: does not cover noncoding regions of the genome, it also cannot detect large rearrangements, copy-number variants (large deletions/duplications), mosaic mutations, mutations in repetitive or high GC rich regions and mutations in genes with corresponding pseudogenes or other highly homologous sequences.

Wider implications of the findings: WES has previously shown to be an efficient tool to identify genes as cause of POF, and has demonstrated the polygenic etiology. Although some studies have focused on it, and many genes are identified, this study proposes new candidate genes and variants, having potentially moderate/strong functional effects, associated with POF.

Trial registration number: Not applicable

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