Explaining the unexplained: new genetic mutations in unexplained premature ovarian insufficiency

Premature ovarian insufficiency (POI) or hypogonadotropic hypogonadism results from abnormal and declining ovarian function in women younger than 40 years and can lead to infertility and a range of long-term adverse health outcomes, including an increased risk of heart disease and osteoporosis (1). The cause of POI is unknown for most women who have not been exposed to gonadotoxic agents, though there are documented associations with environmental exposures, karyotype abnormalities such as Turner syndrome, and autoimmune and metabolic disorders. Additionally, there are well-defined genetic diseases associated with POI, such as the fragile X premutation and classic galactosemia (2). Numerous additional rare mutations are likely associated with the development of POI that have not yet been identified.

In recent years, there have been tremendous technological advances in genetic research with the development and widespread use of next-generation sequencing (NGS). This has allowed for the discovery of common variants associated with disease, which has provided a starting point for investigations of disease etiology. Though widely used, this method is not powered to detect rare variants that are present at a very low population frequency or are even private to an individual or family. Such rare variants must thus be evaluated individually and assessed to determine the probability that they cause disease, allowing us to better understand the underlying causes of disease. With each rare variant that is discovered, we learn more about the gene and which regions are most crucial for proper downstream functioning.

Sassi and colleagues (3) take this approach in their evaluation of a proband with POI and her family. Rare variant testing was indicated for this proband who had developed a severe form of POI at a young age, which often signals genetic underpinnings. Additionally, POI had been diagnosed in her sister as well, which made a Mendelian inheritance pattern where a single point mutation causes the disease a significant possibility. Sequencing of a gene panel of 31 genes believed to be related to POI revealed two point mutations (a single nucleotide change) in the follicle-stimulating hormone receptor (*FSHR*) gene. One recessive mutation was inherited from the proband's father and one from the proband's mother, resulting in the proband and her sister each having two defective copies of the *FSHR* gene. The authors then pursued multiple lines of evidence to evaluate the potential causality of each mutation.

The authors first validated the mutations using Sanger sequencing, which was performed using chain termination with four fluorescent dyes. Validation with another sequencing method is vital because NGS is not without sequencing error. The Sanger sequencing confirmed the maternal inheritance of a c.646G>A, G216R mutation and paternal inheritance of a c.1313C>T,T438I mutation. These mutations altered the amino acid sequence of the resulting protein, which may impair its function.

There are five levels of classification of variants recommended by the American College of Medical Genetics: benign, likely benign, uncertain significance, likely pathogenic, and pathogenic. These categories are used in reporting variants to patients based on the likelihood that the variant causes disease. Variants of uncertain significance are particularly challenging because there is not enough evidence to draw conclusions about the pathogenicity of the variants. In this study, the authors further explored pathogenic and likely pathogenic variants and variants of uncertain significance called by six different variant classification methods. Using multiple methods allowed the authors to have greater confidence in the classification of each variant and pointed to the most deleterious variants for further testing.

One of the strengths of this study is that the authors went on to further characterize the potential role of the two identified variants in POI. They characterized these mutations using a cell culture model transfected with plasmids containing a common *FSHR* allele, each mutation, both mutations together, or a vector control. By comparing the cell surface expression of *FSHR* between the common allele, each mutant, and the combined mutants, the authors concluded that there was a marked reduction of up to 93% of cell surface expression in the mutants compared with the common allele. This would substantially reduce the ability of the cell to respond to FSH and lends support to the idea that mutations in the *FSHR* gene results in FSH resistance. This is additionally supported by the high levels of FSH measured in the proband and her sister despite normal anti-müllerian hormone levels, suggesting that FSH cannot stimulate small antral follicles to grow and mature.

Finally, the authors performed an in vitro functional study to determine how cyclic adenosine $3':5'$ monophosphate (cAMP) production was impacted by both mutations. Intracellular cAMP, a measure of FSH signal transduction, demonstrates the downstream activation of the cascade after FSH/FSHR interaction. The authors demonstrated that at a saturating concentration of FSH, cAMP production was approximately 50% lower in cells with either or both variants, indicating that FSH-induced activity was reduced and these mutations impact the downstream functionality of the cell so that it cannot effectively signal internally to initiate folliculogenesis.

In this case report, the authors present a convincing argument for the pathogenicity of two variants in the *FSHR* gene in development of POI. They use multiple lines of evidence to validate the gene sequencing results and the impact of each mutation on cellular function. As sequencing technology continues to become more widely available and accessible, case reports such as this will become more commonplace. This is important because common variants have not been sufficient to predict the development of POI. Rare variants often have more severe consequences than common variants, and thus may explain some POI cases where a cause cannot be identified. This would allow for the advancement of

personalized medicine by being able to interrogate rare, potentially disease-causing mutations in patients. This study suggests that targeting the faulty FSH receptors may allow for the development of novel treatment options for POI in women with *FSHR* mutations. This case study provides an exceptional example of how rare variants should be identified and tested for pathogenic effects.

There is significant importance of this work to the field of POI research. As many causes of POI are unknown, further pursing rare genetic variants may allow for the discovery of additional underlying etiologies as multiple rare mutations are discovered in particular genes. In this study, the authors note that several other pathogenic mutations have been identified in the *FSHR* gene, which further supports the key role that *FSHR* plays in POI. In addition to providing a target for novel treatments, this knowledge can also improve patient counseling for women with previously unexplained POI. The impact of rare variant research will continue to grow as investigators move into whole-genome sequencing rather than using panels, though panels are simpler and more cost effective. Whole-genome sequencing would allow for the identification of novel genes and variants that may be involved in POI, and the potential positive impact of this on research and patient care is enormous.

Anna K. Knight, Ph.D.
Department of Gynecology and Obstetrics, Emory University
School of Medicine, Atlanta, Georgia

https://doi.org/10.1016/j.xfre.2020.09.001

You can discuss this article with its authors and other readers at
https://www.fertstertdialog.com/posts/xfre-d-20-00184

REFERENCES

1. Tsiligiannis S, Panay N, Stevenson JC. Premature ovarian insufficiency and long-term health consequences. Curr Vasc Pharmacol 2019;17:604–9.
2. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. Hum Reprod Update 2015;21:787–808.
3. Sassi A, Desir J, Janssens V, Marangoni M, Daneels D, Gheldof A, et al. Novel inactivating follicle-stimulating hormone receptor (FSHR) mutations in a patient with premature ovarian insufficiency identified by next generation sequencing gene panel analysis. F S Rep 2020;1:193–201.