REPROD ACTION

RQR
Réseau Québécois en reproduction

ENGLISH / JANUARY 2019

New mutation of the FSH Receptor

*A collaborative project between **Dr. Daniel Bernard**, McGill University, and **Dr. Pierre Miron**, president and founder of FERTILYS, an industrial partner of the RQR.*

Successful in vitro maturation of oocytes in a woman with gonadotropin-resistant ovary syndrome associated with a novel combination of FSH receptor gene variants: a case report. C. Flageole, C. Toufaily, D. J. Bernard, S. Ates, V. Blais, S. Chénier, M. Benkhalifa & P. Miron. J Assist Reprod Genet. 2019 Jan 5

Gonadotropin-Resistant Ovary Syndrome (GROS) is a rare cause of hypergonadotropic hypogonadism. When this syndrome is accompanied by infertility, this represents a major challenge both diagnostically and therapeutically.

History

A couple consulted the FERTILYS Reproductive Health Center for a second opinion. The patient was 31 years old and her husband was 36 years old. Their medical history indicated primary infertility for 2 years. The patient had a progressive lengthening of her menstrual cycle, up to 45 days. The patient was previously diagnosed with hypothyroidism that was well-controlled with Synthroid. Based on repeated high FSH dosing, a diagnosis of premature menopause

Gonadotropin-Resistant Ovary Syndrome (GROS) is a rare type of hypergonadotropic hypogonadism. Patients have prolonged or absent menstrual cycles, elevated FSH levels but have normal anti-Mullerian hormone levels, as well as normal antral follicle counts.

was made and the couple was offered the only possible alternative: egg donation.

New analyses, new hope

Further analyses were then carried out at FERTILYS and although they did confirm the presence of elevated levels of FSH (62, 43 and 55 IU / L), a normal level of AMH (3.14 ng/ml) and a normal ultrasound count of antral follicles (n = 13) was discordantly observed. Other analyses came back as normal: karyotype, genotyping of the FRAXA locus (Fragile-X), and autoimmune tests.

Anti-Mullerian Hormone (AMH) was first described for its role in the regression of Müllerian ducts during embryogenesis in the male fetus. Later, it was shown that this hormone has a very important inhibitory action on folliculogenesis. Its serum level is proportional to the number of follicles growing in the ovary making this hormone a very good marker for the ovarian reserve. The dosing of this hormone allows to adjust the required doses of exogenous gonadotropins for ovarian stimulation treatment.

The clinical examination of the patient was atypical. She had completely disordered ovulatory function with the presence of large follicles at the beginning of the cycle, chronic anovulation, and no response to maximal doses of exogenous gonadotropins (aka ovarian stimulation treatment). *In vitro* maturation (IVM) of oocytes was therefore suggested to the couple. Six oocytes were collected by follicle puncture and then matured in the laboratory for up to 48 hours. Once the metaphase II stage was reached, oocytes were fertilized by intracytoplasmic sperm injection (ICSI). A grade 1 eight-cell embryo (denoting good quality) was transferred on the 3rd post-ovulatory day. After a pregnancy without any difficulties, the patient was finally able to give birth to a healthy boy.

In Canada, 17% of couples are infertile. The annual incidence of premature ovarian failure in women between 30 and 39 years old is 76:100 000. ORS is even more rare.

A new mutation discovery

More sophisticated genetic tests (genetic panel for premature ovarian failure) highlighted the presence of heterozygous mutations in the FSH receptor gene (FSHR); FSH is the hormone that stimulates follicle growth. The first mutation, called I160T, is a pathological variant already described in the literature. In contrast, the second mutation, N558H, had never been reported previously. The I160T mutation is clearly associated with GROS when present on both FSHR alleles.

The effect of the second mutation is still unknown, but its position in the receptor is highly conserved between species. *In silico* analyses revealed that this alteration might be deleterious and that the structure and/or

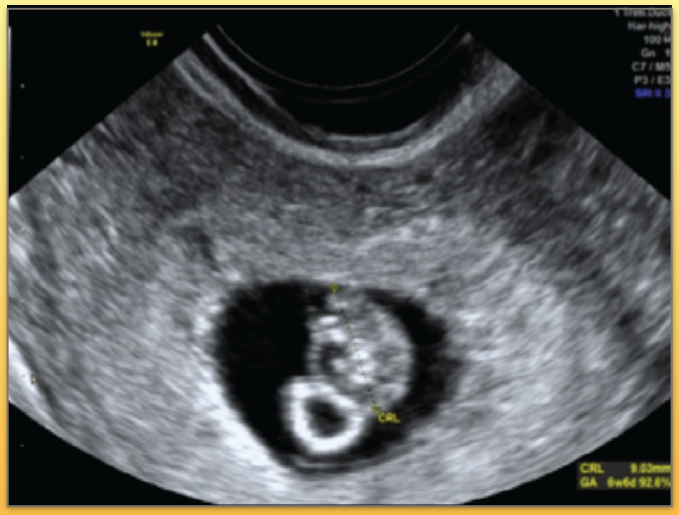


Figure 1: Vaginal ultrasonography performed at 6 weeks and 6 days showing a normal uterine pregnancy.

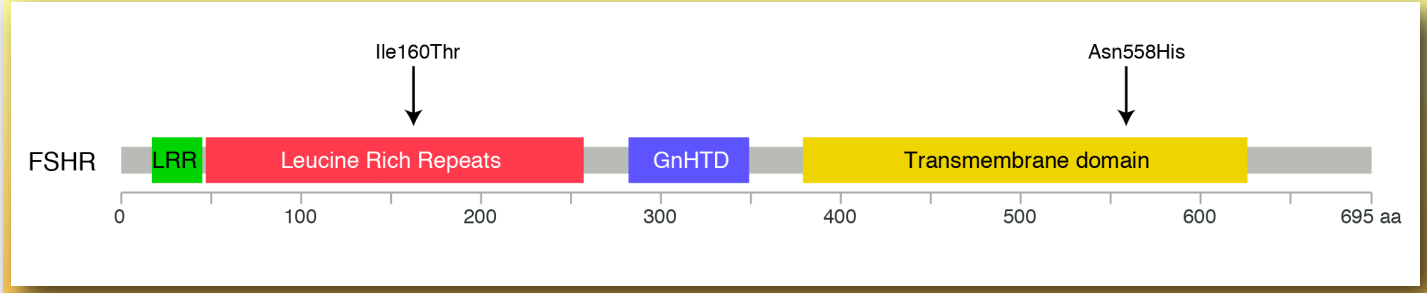


Figure 2: FSH receptor gene, located on the short arm of the chromosome 2 where both mutations are described. The Ile160Thr mutations is located in exon 6 and the Asn558His mutation is in exon 10. GnHTD=gonadotropin hormone transmembrane domain.

function of the FSH receptor might be impaired. More precisely, the mutation occurs in the 3rd intracellular loop of the receptor, which is involved in receptor binding to G proteins and consequent signal transduction.

In silico analysis is performed using computer models. These analyses are mainly used in the fields of genomics and bioinformatics.

A new collaboration with the Bernard Lab from McGill University

As mentioned above, unlike the I160T mutation, the N558H mutation had never before been identified. The laboratories of Dr. Pierre Miron and Dr. Daniel Bernard have collaborated to study the effect of this new mutation at a functional level. Dr. Bernard's team introduced both mutations individually into expression vectors (biological tools that allow the expression of a specific protein) for FSHR. The wild-type (without mutation) FSHR is localized at the cell membrane and its activation by FSH induces cascades of different signaling molecules leading to an increase in the production of a second messenger called cyclic AMP (cAMP) and to the activation of the CREB protein, whose role is to activate specific genes. The effects of the new mutation on these signaling cascades were compared to the wild-type receptor as well as to the I160T mutation already described. The results showed that in the presence of the I160T mutation, FSH cannot activate the cAMP / CREB system. However, in the presence of the novel mutation, N558H, the activation of the cAMP / CREB system as well as FSHR localization on the cell membrane look comparable to the wild-type receptor. Though these results might suggest that the N558H mutation does not affect the FSH receptor, it is possible that it affects receptor function in ways that were not assessed.

Mutagenesis *is the appearance of a mutation that can be natural or artificially-induced. In nature, this process can be the root cause of cancer, hereditary diseases or evolutionary innovations and is primarily responsible for species biodiversity.*

Site-directed mutagenesis *is used to understand the function of genes. This technique consists of the voluntary introduction of mutations using chemical or physical agents in a DNA sequence in order to deduce information on the role of genes. In the laboratory, the modification of the amino acid sequence of a specific protein allows us to evaluate the importance of targeted amino acids in the function of the specific protein.*

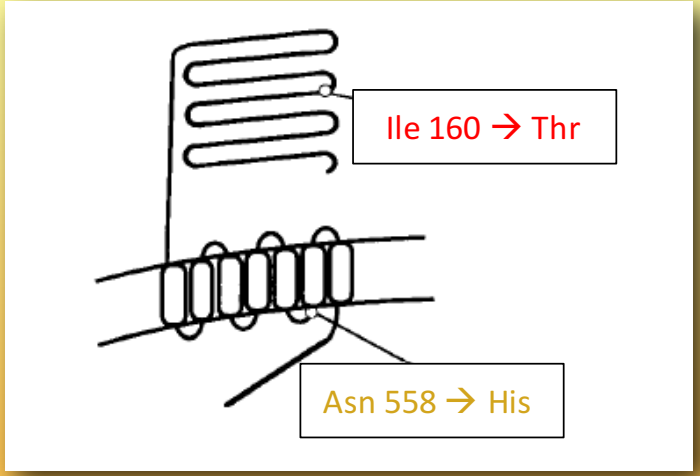


Figure 3: FSH receptor, showing the localization of the two mutations of interest I160T and N558H. Figure adapted from Beau et al. J Clin Invest. 1998 Oct 1;102(7):1352-9.

Conclusion

In conclusion, thanks to the efforts of Dr. Pierre Miron's laboratory, it has been shown that IVM should always be offered as first-line treatment in infertile women with GROS, particularly in the presence of mutations in the FSH receptor. The N558H mutation observed in the 3rd intracellular loop of the receptor is a new discovery but its functional significance, if any, has not been resolved and requires further investigation. The RQR is proud to have been able to connect Dr. Pierre Miron and Dr. Daniel Bernard and that a new collaboration has been established through this network.

References

Aittomaki, K., et al., Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. J Clin Endocrinol Metab, 1996. 81(10): p.3722-6.

Huhtaniemi, I.T. and K. Aittomaki, Mutations of follicle- stimulating hormone and its receptor: effects on gonadal function. Eur J Endocrinol, 1998. 138(5): p. 473-81.

Bramble, M.S., et al., A novel follicle-stimulating hormone receptor mutation causing primary ovarian failure: a fertility application of whole exome sequencing. Hum Reprod, 2016. 31(4): p. 905-14.

Binder, H., et al., Assessment of FSHR variants and antimullerian hormone in infertility patients with a reduced ovarian response to gonadotropin stimulation. Fertil Steril, 2012. 97(5): p.1169-75.

Binder, H., et al., Association of FSH receptor and CYP19A1 gene variations with sterility and ovarian hyperstimulation syndrome. Reproduction, 2008. 135(1): p. 107-16.

Beau et al. A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. J Clin Invest. 1998 Oct 1;102(7):1352-9.

RQR awards for knowledge translation

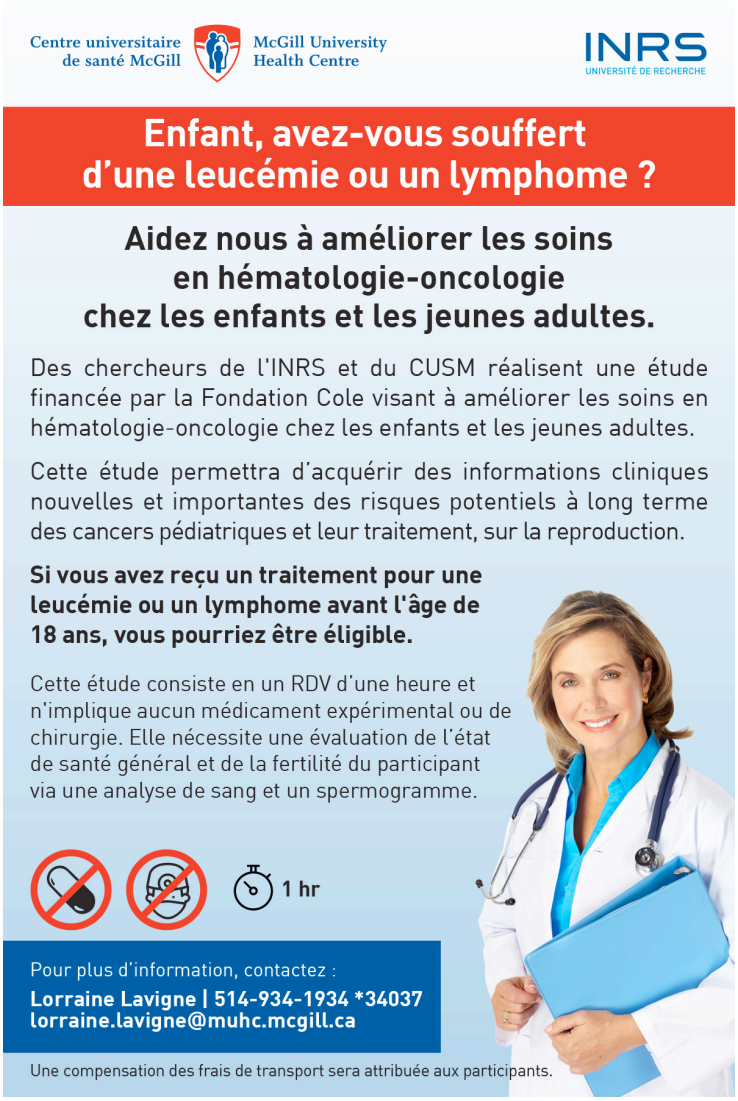
The knowledge translation efforts of two RQR members towards research end-users or the lay public were rewarded during the last RQR Symposium. The recipients were Dr. Cathy Vaillancourt from the INRS - Institut Armand Frappier and Bélinda Crobeddu, PhD student at the INRS- Institut Armand Frappier.

At the 11th annual Symposium of the RQR, Dr. Claude Robert, chair of the KT Committee, awarded Bélinda Crobeddu for her knowledge translation efforts towards reproductive biology research end-users.

Knowledge translation activities may be targeted at multiple end-users and not just the scientific community. These may include:

- the lay public,
- pharmaceutical and biotechnology companies,
- veterinarians,
- clinicians,
- government,
- and others relevant targets.

On behalf of all RQR members, congratulations to both awardees !



Coming events

- May 10 - 11, 2019 : 24 hours of science. This year's theme is « **Tomorrow, the Earth** » . As each year, the RQR wishes to participate in this event. If you are interested in organizing such an activity, please contact Charlène Rico, RQR manager, at charlene.rico@umontreal.ca