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IDENTIFICATION OF POLYMORPHISMS 919 A>G (T307A) and 2039 A>G (N680S) of FSHR gene

AMPLI-FSHR T307A-N680S REAL-TIME Cat. n.1.507 RT

The FSH or Follicle Stimulating Hormone is a glycoprotein hormone produced and secreted by adenohypophysis and contributes, in both sexes, the regulation of the development, and pubertal maturation of reproductive process. FSH is a heterodimer composed of two subunits, the α (shared with other glycoprotein hormones such as LH, hCG and TSH) and the β subunit that confers specificity of action. The β subunit is encoded by the gene FSH β (4.2 kb) placed in position 11p13 containing 3 coding exons. The subunit β of the resulting protein is 123 amino acids. FSH is able to carry out its stimulatory effects on gametogenesis only if it binds to a specific receptor (FSHR). This receptor is located on the surface of the Sertoli cells in the testis and on the surface of the ovary granulosa. The FSHR is encoded by a gene consists of 10 exons and 9 introns (54kb) on chromosome 2 in position p21-p16. The FSHR gene encodes a protein of 678 amino acids that form an extracellular domain, seven transmembrane domains and an intracellular domain. The extracellular domain of FSHR has the shape of a cylinder slightly curved, and it is the inner concave surface that interacts with the α subunit and β of FSH. As a result of the dimerization, the receptor is able to transduce the signal from the ligand within the cell. In literature mutations are described that cause constitutive activation of the receptor or loss of binding specificity. Among the various SNPs identified in the FSHR gene, some are common and able to influence the activity of the receptor itself. Researchers found 731 SNPs in FSHR gene (NCBI source). One SNP is present in the promoter of the gene, five are in the coding regions and the others are in non-coding regions. Exon 10 encodes the C-terminal part of the extracellular domain, the entire transmembrane domain and the intracellular domain of FSHR. Exon 10 is critical for signal transduction. Two common SNPs (c.919 A> G and c.2039 A> G) are located in exon 10 of FSHR. The variant 919 A>G (rs 6165) causes the substitution T307A, while the variant 2039 A> G (rs 6166) causes the substitution asparagine with serine (N680S). Polymorphisms mentioned above have been extensively studied and it has been shown that in women FSHR genotype related to these SNPs is the factor that most influences the ovarian responsiveness to FSH-treatment necessary for ovulation induction in the assisted fertilization techniques. The variant 2039 A> G reflects a reduced ovarian sensitivity in women requesting a dose of exogenous FSH higher in assisted fertilization techniques. FSHR genotype can be considered a predictor of ovarian responsiveness allowing to adjust the dose of FSH to be administered and the time of ovarian hyperstimulation. The studies by Selice (2011) and Ferlin (2011) have shown that the analysis of FSHR and FSHβ genes are valid pharmacogenetic approaches in male as the treatment with FSH is able to induce an improvement in semen parameters only in a subgroup of oligospermic patients with a specific genotype related to these two genes. Oligospermic men with normal levels of FSH will have a better response to treatment with FSH if carriers of a serine in position 680 in FSHR and a thymidine at position -211 in FSHβ.

Principle of assay: A) extraction of genomic DNA; B) amplification and detection using real-time PCR equipment; **Applicability:** of genomic DNA extracted and purified from whole blood samples and tissue samples from fresh and paraffin.
Numero di Test: 25x2.

REAGENTS and STORAGE

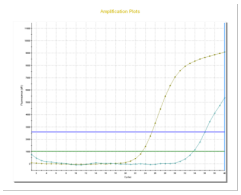
AMPLIFICATION	
PCR mix	+4°C
H_2O RNase/DNase free	-20°C
Primer-probe mix 20 X T307A FSHR	-20°C
Primer-probe mix 20 X N680S FSHR	-20°C
Control A/A	-20°C
Control G/G	-20°C
Control A/G	-20°C

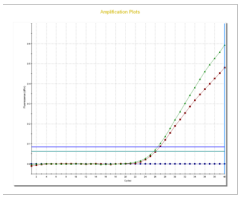
Stability: over 18 months if correctly stored.

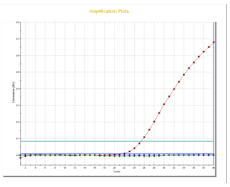
References:
Ann of Hum Gen (2007)71, 18-28
Endocrinology (2013) 154,3016-3021
Mol Hum Reprod (2002) 8, 893-899
JCEM (2012) 97, 3639-3647
JCEM (2010) 95, 100-108

ANALYSIS OF RESULTS

After an Allelic Discrimination post-read run, the software analyzes raw data using the AD specific program. Anywhere it is useful analyzing the amplification plots, in order to check the amplification reaction.


Sample AA


Sample AG


Sample GG