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DETECTION of FSHβ gene -211 G>T POLYMORPHISM and FSHR GENE 2039 A>G

AMPLI set FSHβ - FSHR Cat. N. 1.504

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 βof the resulting protein is 123 amino acids. The inactivating mutations of FSHβ are 5 and are present in exon 3 of the gene. They cause the formation of a truncated protein, or the loss of a cysteine residue. Polymorphism -211 G> T (rs 10,835,638) influences the FSH levels in serum. This polymorphism, which causes the substitution of a G with a T, is placed in the promoter of the gene -211bp upstream of the transcript start site of mRNA. A statistically significant association between serum levels of FSH and genotype dell'FSHβ was found: heterozygotes (GT) or homozygous (TT) have serum FSH significantly lower than subjects WT (GG). Many studies, including Tüttelmann in 2012, found a significant correlation between the subjects carriers of T with serum levels of FSH (lower 24% in TT than GG), the relationship with FSH / LH, with the testicular volume and concentration and sperm counts (lower by 36% and 34% in TT than GG). 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 βof 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 2039 A> G (rs 6166) characterizes the haplotype of exon 10. In the protein, the replacement 2039 A> G causes the substitution asparagine with serine. 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. In the study of Tüttelmann of 2012 was evaluated the combined effect of polymorphisms -211G> T FSHβ of 2039 to> G of FSHR on male reproductive parameters. The increase of FSH is significant when the three genotypes of the SNP in 2039 A> G of FSHR are associated with the genotype of -211TT FSHβ. The increase of LH is statistically significant when the three genotypes of the SNP in 2039 A> G FSHR gene are associated with the genotype FSHβ -211GT. It has been also shown a significant decrease of testicular volume related to the various genotypes of SNP 2039 A> G and -211GT - 211TT of FSHβ. From these results, Tüttelmann concluded that homozygous carriers for the minor alleles of both SNPs have the worst phenotype characterized by lower testicular volume. Conversely, a high sensitivity receptor given by AA genotype in FSHR can partially offset the reduced serum levels of FSH due to reduced transcriptional activity of subunit FSHβdata genotype TT for the SNP -211G> T. The GG genotype for SNP -211G> T, which is expressed in a high transcriptional activity of the gene FSHβ can compensate the reduced sensitivity of the receptor for the GG genotype of FSHR SNP 20139 A> G . The FSHR SNP 2029 A > G, while having no effect if analyzed singularly, can alter male reproductive function when analyzed simultaneously with the FSHβ SNP -211G> T. 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 C) enzymatic digestion D) detection on agarose gel.
Applicability: On extracted and purified genomic DNA from whole blood samples.
Tests: 25 x 2

REAGENTS AND STORAGE

AMPLIFICATION and DIGESTION	
PCR mix FSH β	-20°C
PCR mix FSHR	-20°C
sterile H_2O	-20°C
Taq Polymerase (5U/µl)	-20°C
BSRI Enzyme(U/µl)	-20°C
Digestion BUFFER BSRI 10 X	-20°C
RSAI Enzyme (U/µl)	-20°C
Digestion BUFFER RSAI 10 X	-20°C
WT FSHβ control	-20°C
FSHR mutated homozigous control	-20°C

Stability: over 12 months if correctly stored (Agarose gels, if protected by light, can be stored 1 year at room temperature).

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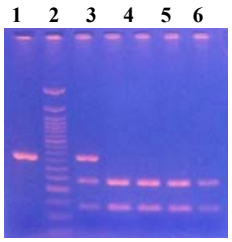
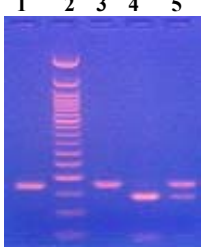
ANALYSIS OF RESULTS

The PCR product of **FSHβ** is a fragment of 364 bp. The next restriction section made by the RSAI enzyme can provide the following results:
Restriction digestion with **RSAI** of WT sample: 234bp and 130bp
Restriction digestion with **RSAI** of heterozigous sample: 364bp, 234bp e 130bp. No restriction digestion with **RSAI** of homozigous sample: 364bp.



1: PCR PRODUCT
2: MW LADDER 5O BP
3:HETEROZIGOUS SAMPLE
4-7: WT SAMPLES

The PCR product of **FSHR** is a fragment of 172 bp. The next restriction section made by the BSRI enzyme can provide the following results:
No restriction digestion with **BSRI** of WT sample: 172bp
Restriction digestion with **BSRI** of heterozigous sample: 172bp, 133bp and 39bp
Restriction digestion with **BSRI** of homozigous sample: 133 and 39bp.



1: PCR PRODUCT
2: MW LADDER 5O BP
3: WT SAMPLE
4: HOMOZIGOUS SAMPLE
5: HETEROZIGOUS SAMPLE

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