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Abstract. Aims. Dysfunction of the FSH receptor (FSHR) may be involved in some form of male infertility with azoospermia or oligozoospermia. We assessed the discrete codon combination with homo/heterozygous variation of the exon 10 in the FSHR gene. Methods. The genotype of codon 307 and codon 680 were analysed in 352 patients with idiopathic male infertility and 145 men with proven fertility. Results and Conclusion. There was no significant difference in the distributions of each homozygous codon 307 or 680 between these two groups as reported in the literature. However, the population with heterozygous combinations Thr/Ala (codon 307) and Ser/Asn (codon 680) comprised 26% (38/146) and 44.9% (157/343) in subjects with proven fertility and idiopathic infertile men, respectively. Moreover, the heterozygous genotype Thr/Ala-Ser/Asn was significantly increased in fertile patients compared with the controls. This finding showed that the combination of heterozygous FSHR can be responsible for male infertility.

Key words: FSH receptor, Male infertility, Polymorphism, Japanese, Codone

THE INTERACTION between follicular stimulating hormone (FSH) and the FSH receptor (FSHR) is essential for human reproduction [1, 2]. This pituitary gonadotropin stimulates spermatogenesis by binding to a specific receptor located on the surface of Sertoli cells in the testis. The FSHR is a G-protein-coupled receptor whose main signal-transduction mechanism involves activation of adenyly cyclase [3]. Recent studies have demonstrated that naturally occurring mutations of the FSHR are very rare [4, 5]. However, several single nucleotide polymorphisms (SNP) in the FSHR that affect the FSH level have been associated with risk of gametogenesis [6]. In women, SNPs of the FSHR determine the ovarian response [7-12]. In men, the impact of the FSHR SNPs is unclear [13, 14]. Recent genetic studies have shown that two SNPs of exon 10 of the FSHR gene are associated with the discrete FSH receptor allelic variants, codon 307 and codon 680 [6, 15, 16]. We investigated the frequency and distribution of FSHR variants at positions 919 and 2038 corresponding to codon 307 and codon 680, and examined the contribution of these SNPs to the susceptibility of Japanese to male infertility.

Materials and Methods

Subjects

Polymorphic analysis of the FSH receptor gene
was performed in Japanese men consulting Kanazawa University Hospital and Kiba Park Clinic (Tokyo). The present study being performed by our institution and collaborative clinic was approved by the Kanazawa University Institutional Review Board. Informed consent was obtained from each participant. All patients presented with non-obstructive azoospermia, without Y chromosome microdeletion and normal karyotypes. Using a computerized database of patients registered after this date January 1, 2007, we identified a cohort of 343 patients with elevated basal FSH concentrations (>10.0 mIU/mL).

Blood samples were collected from AM 9:00 to AM 12:00. All patients underwent routine examinations, including a detailed history, physical examination, and semen analyses, endocrinology profile testing. In this study, we used the FSH value and semen concentration at the first visit of our outpatient clinic. The DNA samples from 145 men with proven fertility were used as a control. The average age [mean ± standard deviation (SD)] was 34.2 ± 4.3 years. Fertility status was proven by the fact that each of the control subjects had fathered one or more children. Semen analysis was performed according to the standard methods outlined by the World Health Organization.

DNA isolation

Genomic DNA was prepared from peripheral blood lymphocytes with a commercial extraction kit (Quiagen, Hilden, Germany) according to the manufacturer’s protocol.

Analysis of the codon 307 and 680 variants

PCR amplification of the two DNA portions containing SNP at nucleotide positions 919 (codon 307) and 2039 (codon 680) from the translational start codon within exon 10 of the FSHR gene was performed.

The SNP at position 919 or 2039 creates an AGT (Thr) to AGC (Ala) substitution or AAG (Asn) to AGT (Ser) substitution, respectively. The presence of the position nucleotide 919T/C and 2039A/G variant and introduces a restriction site. The region of nucleotide number 857 to 1041 in the FSHR gene for Thr307Ala was amplified by PCR using genomic DNA as templates and a set of primers (5'-ACAGTCCGAGCCCAACAT-3' and 5'-CATGATACATTAGGAAAGC-3') that amplified a DNA fragment of 185 bp in size. The region of nucleotide number 1662 to 2262 in the FSHR gene for Asn680Ser was amplified by PCR using genomic DNA as templates and a set of primers (5'-ACAGTCCGAGCCCAACAT-3' and 5'-CATGATACATTAGGAAAGC-3') that amplified a DNA fragment of 600 bp in size. The PCR amplicon for the nucleotide position following Hpy188I digestion demonstrated three different patterns, two and three digested fragments show the homozygous and heterozygous DNA, respectively (Fig. 1). Based on this analysis, patients were classified into three groups, namely, TT (Thr307Thr), TA (Thr307Ala) and AA (Ala307Ala) (Fig. 1). According to codon 680, the PCR amplicon is digested by BsrI. No digested pattern (one band) showed a homologous amplicon, namely, NN (Asn680Asn) and digested site (two bands) representing the SS (Ser680Ser) homozygous pattern. Heterozygous DNA representing SN (Ser680Asn) showed three bands (Fig. 2). Selected samples were sequenced and their sequence identities were confirmed.

The FSHR gene allele combinations of 307 and 680 codons

To evaluate the effect of allelic variants, we used the homozygous and heterozygous status in both codon 307 and codon 680. The combinations of homozygous and heterozygous codons yielded nine possible allelic variation as follows, Thr307Thr-Ser680Ser (TT-SS), Thr307Thr-Asn680Asn (TT-NN), Thr307Thr-Ser680Asn (TT-SN), Ala307Ala-Ser680Ser (AA-SS), Ala307Ala-Asn680Asn (AA-NN), Ala307Ala-Ser680Asn (AA-SN), Thr307Ala-Ser680Asn (TA-SS), Thr307Ala-Asn680Asn (TA-NN), Thr307Ala-Ser680Asn (TA-AN). We classified nine groups.

Statistical analysis

Results of age and hormonal levels are shown as mean±SD. Data were analyzed using Statistical Package for the Social Sciences statistical software version 11.0 (SPSS, Chicago, IL, USA). Mann-Whitney’s U test was used to determine whether there was any significant difference in genotype frequencies between the normal control and infertile male groups. A value of $P < 0.05$ was considered significant. Comparisons of hormonal profiles between two groups
Fig. 1. Identification of homo/heterozygosity at position of codon 307
TT: Thr307Thr, TA: Thr307Ala, AA: Ala307Ala

Fig. 2. Identification of homo/heterozygosity at position of codon 680
NN: Asn680Asn, SS: Ser680Ser, SN: Ser680Asn
were made with unpaired \(t\)-test. A \(p\) value < 0.05 was considered significant.

**Results**

**The characteristics and hormonal profiles of infertile men**

FSHR seems to be important in determining the response to FSH stimulation. Of the 343 patients, 298 showed azoospermia. The remaining 45 patients had severe oligospermia. The number of patients showing FSH levels of 10 to 20 mIU/mL, 20 to 30 mIU/mL, 30 to 40 mIU/mL and more than 40 mIU/mL was 142, 95, 65 and 41, respectively. The average patient age was 35.1 ± 5.4 years (mean ± SD) in the overall patient group. The levels of FSH, LH (luteinizing hormone) and testosterone were 25.2 ± 11.6 mIU/mL, 6.9 ± 4.3 mIU/mL and 5.2 ± 3.4 ng/mL, respectively.

**Frequency of the variants codon 307 of the FSHR gene**

Concerning SNP of codon 307, there were three genotypes as follows, homozygous genotypes \(TT\), \(AA\) and heterozygous genotype \(TA\). All patients and fertile controls were classified into these three genotype groups. In fertile controls, the frequencies of \(TT\), \(AA\) and \(TA\) were 47.0%, 12.0% and 42.0%, respectively. In the infertile patients, the frequencies were 34.7%, 14.6% and 50.7%, respectively (Table 1). There was no significant difference among these subjects. The FSH levels of these three variants were 24.4 ± 11.4, 27.5 ± 10.4 and 24.9 ± 12.3 mIU/mL, respectively. There was no significant difference among these subjects. Interestingly, there was a high proportion of \(TA\) heterozygous genotype in codon 307 in both the fertile controls (42.0%) and infertile patients (50.7%).

**Frequency of the variants codon 680 of the FSHR gene**

There were two homozygous genotypes \(SS\), \(NN\) and heterozygous genotype \(SN\) in codon 680. The frequency of \(SS\), \(NN\) and \(SN\) was 8.0%, 49.0% and 42.0% in fertile controls, respectively. The frequency was 13.1%, 38.2% and 47.8% in infertile patients, respectively (Table 2). Moreover, the FSH levels of these three variants demonstrated 26.3 ± 10.2, 25.3 ± 10.6 and 24.8 ± 12.2 mIU/mL, respectively. There was no significant difference among these subjects. A high proportion of \(SN\) heterozygous pattern was also present in both the fertile controls (42.0%) and infertile patients (47.8%).

**Frequency of the allelic variants linkage between 307 and 680 codons**

To analyze the allelic variants combinations between 307 and 680 codons, we could evaluate nine possible allelic combinations, such as \(TT-SS\), \(TT-NN\), \(TT-SN\), \(AA-SS\), \(AA-NN\), \(AA-SN\), \(TA-SS\), \(TA-NN\), \(TA-SN\) (Table 3). In both the control and infertile groups, there were too few subjects with the combinations of \(TT-SS\), \(AA-NN\), \(AA-SN\), and \(TA-SS\) to be evaluated. However, the combination of \(TT-NN\), \(TT-SN\), \(AA-SS\), \(TA-NN\) and \(TA-SN\) showed relatively high frequency. The population with \(TT-NN\) combinations comprised 36% (52/146) of the subjects with proven fertility and 31.8% (109/343) of the idiopathic infertile men, respectively and there was no significant difference in the distributions of these combinations between these two groups (\(p=0.55\)). However, the population with \(TA-SN\) combinations comprised 26% (38/146) and 44.9% (154/343) in subjects with proven fertility and idiopathic infertile men, respectively. Moreover, the genotype distributions of \(AA-SS\) and \(TA-NN\) were also similar in the two groups (\(p=0.076\) and \(p=0.158\), re-
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The proportions of genotype NN, NS and SS were 41.0, 46.9 and 12.1%, respectively [20]. In our study, the proportions of NN, NS and SS were 49, 42 and 8% respectively in subjects with proven fertility and 38.2, 47.3 and 13.1% respectively in infertile patients, and there were no significant differences between the two. This was consistent with these reports. The screening for SNP at position codon 307 has not previously been reported, but our study showed no difference in genotype in this position.

As for two discrete codons 307 and 680, Simoni et al. also report the combinations TT-NN and AA-SS comprise 60% and 40%, respectively and the combination of TT-SS and AA-NN is very rare in Caucasians [21]. In Japanese patients, Sudo et al. report the combinations of TT-NN and AA-SS comprise 64% and 36%, respectively, while the combinations of TT-SS and AA-NN are very rare [20]. This study did not demonstrate any significant differences within the group or in subjects with different allelic variants, even when only homozygous genotypes.

Our study showed that the positions of codon 307 and 680 indicated heterozygosity beyond the expected in both fertile controls and infertile patients. Thus, the patients were classified as having hetero/homozygous FSHR. This study confirmed that in idiopathic infertility patients, the combinations with TA-SN heterozygosity led to the possibility of male infertility.

Discussion

Currently, measuring basal FSH is a routine procedure during the diagnostic work-up of subfertile couples for prognostic evaluation and to predict spermatogenesis. Serum FSH levels in the male act as an indicator of male infertility, since testicular failure leads to relatively high FSH levels [15]. Considering the role of FSHR, we postulated that dysfunction of the FSHR may be involved in some form of male infertility with azoospermia or oligozoospermia. However, critical SNP site has not yet been identified, despite screening of a large population [4, 17, 18].

The SNP at position codon 680 was the first to be confirmed and has been well studied [19]. Simoni et al. evaluated NN, NS and SS (37.2, 45.4 and 17.4% respectively) in populations with proven fertility and (32, 48.0 and 20.0% respectively) infertility and no significant differences were observed [15]. In Japan, Sudo et al. reported 522 ovulating woman who visited University hospital. The proportions of genotype NN, NS and SS were 41.0, 46.9 and 12.1%, respectively [20]. In our study, the proportions of NN, NS and SS were 49, 42 and 8% respectively in subjects with proven fertility and 38.2, 47.3 and 13.1% respectively in infertile patients, and there were no significant differences between the two. This was consistent with these reports. The screening for SNP at position codon 307 has not previously been reported, but our study showed no difference in genotype in this position.

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Exon 10 of FSHR encodes the C-terminal end of the extracellular domain, the entire transmembrane domain
and the intracellular domain of FSHR. Residue 307 is in the hinge region of the receptor and residue 680 is in the intracellular region, which is associated with phosphorylation. However, there were no roles identified for the heterozygous amino acid substitution in residues 307 and 680. The functional characteristics of the two FSH receptors were assessed in vitro, and the data suggested similar functional characteristics for both receptor isoforms [21]. In the functional point of view, our data has been speculated the combination of heterozygous FSHR may couple to physiological response through different signal transduction.

In conclusion, there was no association between two SNPs of FSHR at the positions of codon 307 and 607 and the occurrence of male infertility in Japanese subjects. However, the combination of TA-SVN heterozygous genotype linkage codon 307 and 607 of FSHR may influence FSH response to the receptor in some idiopathic male fertility.

References

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