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Follicle-stimulating hormone receptor polymorphisms in women with normogonadotropic anovulatory infertility

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Objective: To assess the incidence of different FSH receptor genotypes in normogonadotropic anovulatory infertile women (World Health Organization class II) and normo-ovulatory controls and to correlate these genotypes with baseline characteristics and ovarian responsiveness during ovulation induction.

Design: Cross-sectional study.

Setting: University hospital.

Patient(s): Thirty normo-ovulatory controls and 148 normogonadotropic anovulatory infertile women.

Intervention(s): All participants underwent a standardized evaluation that included cycle history, body mass index measurement, and transvaginal ultrasonography of ovaries. Fasting blood samples were obtained for endocrine evaluation. Ovarian responsiveness to FSH in normogonadotropic anovulatory infertile women was assessed during ovulation induction, and DNA was analyzed to determine the FSH receptor genotype.

Main Outcome Measure(s): Prevalence of FSH receptor polymorphisms, baseline serum FSH levels, amount of FSH administered, duration of stimulation, and ovarian response dose.

Result(s): The Thr/Thr 307 genotype was significantly less prevalent (52% vs. 23%) and the Ser/Ser 680 polymorphism was significantly more prevalent (40% vs. 16%) in patients compared with controls. Normogonadotropic anovulatory infertile women with the Ser/Ser 680 polymorphism presented with higher median FSH serum levels (5.2 IU/L [range, 2.4–9.7 IU/L]) than did those with the Asn/Asn 680 (4.6 IU/L [range, 1.4–5.8 IU/L) and Asn/Ser 680 (4.5 IU/L [range, 1.8–9.7 IU/L) variants. However, ovarian responsiveness to FSH was similar among anovulatory women with the various polymorphisms.

Conclusion(s): Normogonadotropic anovulatory infertile patients have a different FSH receptor genotype than do normo-ovulatory controls. Although this characteristic is associated with increased baseline FSH serum levels, altered ovarian sensitivity to exogenous FSH during ovulation induction could not be established. (Fertil Steril® 2003;80:986–92. ©2003 by American Society for Reproductive Medicine.)

Key Words: FSH receptor, polymorphisms, anovulation, ovulation induction, ultrasonography, endocrinology, WHO-type II, PCOS

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Follicle-stimulating hormone plays a crucial role during folliculogenesis by stimulating granulose-cell estrogen production through induction of aromatase activity (1). The action of FSH is mediated by the FSH receptor, which belongs to the large family of G-protein–coupled receptors. These receptors are characterized by a transmembrane domain consisting of seven membrane transversing α-helices connected by three extracellular and three intracellular loops (2, 3). The FSH receptor gene is located at chromosome 2p21 to 16 (2–4).

Several naturally occurring mutations in the FSH receptor gene have been found. In a sample of Finnish women with hypergonadotropic ovarian dysgenesis, a loss-of-function mutation was found that resulted from a ($Ala^{189}Val$) missense mutation that segregated perfectly with the phenotype (5). Patients with the lowest remaining FSH receptor activity have hypergonadotropic primary amenorrhea with atrophic ovaries (5), whereas carriers of mutations that less severely affect receptor function present with secondary amenorrhea, normal-sized ova-

ries, and follicular development up to the antral stage (3, 6–8). However, inactivating mutations of the FSH receptor are rarely found in premature ovarian failure (POF) (9–11).

The only activating FSH receptor mutation was identified in a hypophysectomized man who remained fertile despite undetectable gonadotropin levels (12). Symptoms of activating FSH receptor mutations might resemble the phenotype of patients with McCune–Albright disease (13). In these patients, the constitutive activation of $G_s\alpha$ leads to symptoms of combined LH and FSH hyperactivity. In addition, enlarged ovaries with multiple cysts have been described in women with FSH-producing pituitary tumors (14).

Recently, two polymorphisms of the FSH receptor gene have been identified. One is located in the extracellular domain at position 307, occupied either by alanine or threonine. The second one is located in the intracellular domain at position 680, occupied either by asparagine or serine. Both polymorphic sites are within exon 10 and give rise most frequently to two discrete allelic variants of the FSH receptor: Thr307/Asn680 and Ala307/Ser680 (4, 15). No distinct differences could be found in the distribution of these two allelic variants in infertile men or women compared with normal persons (16, 17).

From the combination of the two polymorphisms in both positions, two more allelic variants are possible: Thr307/Ser680 and Ala307/Asn680. Their frequency distribution in persons of different ethnic background has not been systematically analyzed. Whether FSH receptor polymorphisms have pathophysiologic significance with regard to ovarian dysfunction or ovarian response to stimulation is uncertain. Some investigators did not find differences in the distribution of these polymorphisms in polycystic ovary syndrome (PCOS) and POF (16, 18), whereas others did (19).

In normogonadotropic normoestrogenic anovulatory infertility (World Health Organization class II), the response of the ovary to exogenous FSH administration varies considerably among patients (20, 21). In a recent study, the individual FSH response dose for gonadotropin induction of ovulation in anovulatory infertile women could be predicted on the basis of initial screening characteristics, such as the initial FSH serum level (22). Recent observations in patients undergoing IVF suggest that the FSH receptor genotype is associated with different requirements for exogenous FSH (15).

We analyzed the frequency distribution of the two FSH receptor polymorphisms and their combination into four discrete allelic variants and compared their occurrence in normogonadotropic anovulatory infertile women with that in normo-ovulatory controls of different ethnic origin. In addition, we studied the correlation between the observed FSH receptor genotype and the response to exogenous FSH for ovulation induction in normogonadotropic anovulatory infertile women.

MATERIALS AND METHODS

Patients

This study was conducted as part of a research line that was approved by the Institutional Review Board of the Erasmus Medical Center. Informed consent was obtained from all participants.

We included 148 white Dutch patients who attended our infertility outpatient clinic between 1994 and 1999. No immigrants (persons of Mediterranean, Latin American, or southeast Asian origin) were included.

Inclusion criteria for patients were infertility, oligomenorrhea (interval between periods >35 days) or amenorrhea (absence of vaginal bleeding for at least 6 months), serum FSH concentrations within normal limits (1 to 10 IU/L) (23, 24), positive withdrawal bleeding after progestagen administration in patients with amenorrhea, and age 20 to 40 years. Standardized initial screening (clinical examination, transvaginal ultrasonography, and fasting blood sampling) was performed on a random day between 9 AM and 11 AM, as described elsewhere (23).

The control group consisted of 30 healthy volunteers selected by advertisement and paid for participation, as described elsewhere (25). Like the patients, controls were Dutch and not immigrants. Inclusion criteria were a regular menstrual cycle (26 to 30 days), age 20 to 35 years, normal body mass index (18 to 25 kg/m^2), no history of endocrine disease, and no use of medication or oral contraceptives for at least 3 months before study entry. Transvaginal ultrasonography and blood sampling were performed during the early follicular phase (cycle day 3, 4, or 5). The controls are described in detail elsewhere (25).

Ovulation Induction

In a subgroup of 89 women who failed to ovulate or conceive after clomiphene citrate administration, gonadotrophin treatment was commenced within 3 to 5 days after initiation of spontaneous or progestagen-induced withdrawal bleeding. Patients received daily SC injections of recombinant FSH (Gonal-F; Ares-Serono, Geneva, Switzerland). During all first cycles, a low-dose step-up protocol was used with a starting dose of FSH of one ampoule (75 IU) per day. The daily dose was increased by 0.5 ampoule if ovarian response (≥1 follicle ≥10 mm) was lacking after 14 days. Thereafter, the dose was increased by 0.5 ampoule every 7 days if required. The FSH response dose was defined as the dose at which an ovarian response was observed. If sufficient ovarian response was observed, the dose was kept constant until administration of hCG (Profasi; Ares-Serono).

Hormone Assays

Blood samples were obtained by venipuncture and processed within 2 hours after withdrawal. Serum was stored at −20°C and assayed for LH, FSH, androstenedione, T, inhibin B, E_2, and P. Serum LH and FSH levels were measured

by immunofluorometric assay (Amerlite; Ortho-Clinical Diagnostics, Amersham, United Kingdom), whereas serum E_2, P, T, androstenedione, and sex hormone–binding globulin levels were measured by RIA provided by Diagnostic Products Corp. (Los Angeles, CA), as described elsewhere (26). Intraassay and interassay coefficients of variation were less than 5% and 15% for LH, less than 3% and 8% for FSH, less than 8% and 11% for androstenedione, less than 3% and 5% for T, less than 5% and 7% for E_2, less than 16% and 17% for P, and less than 4% and 5% for sex hormone–binding globulin.

Dimeric inhibin B levels were assessed by using an immuno-enzymometric assay obtained from Serotec (Oxford, United Kingdom), as described elsewhere (24). The detection limit of the assay, defined as the amount of inhibin equivalent with the signal of the blank plus 3 SDs of this signal, was 3.4 ng/L. Intraassay and interassay coefficients of variation for inhibin B were less than 9% and 15%, respectively.

DNA Isolation and Analysis

Genomic DNA was obtained from peripheral blood leukocytes, as described elsewhere (12). Polymerase chain reaction amplification of fragments of exon 10 encompassing amino acid positions 307 and 680 were analyzed by single-stranded conformation polymorphism gel electrophoresis, as described elsewhere (4, 17, 27). The results of single-stranded conformation polymorphism analysis were confirmed by direct sequencing of about 10% of randomly chosen DNA samples.

Statistical Analysis

Statistical analysis was performed by using a commercially available software package (SPSS; SPSS Inc, Chicago, IL). Data were analyzed for normal distribution. Data are presented as the mean (±SD) if distributed normally or as the median and range if distributed non-normally. To detect differences between groups, Mann–Whitney or Kruskal–Wallis tests were used if data were not normally distributed. Normally distributed data were subjected to one-way analysis of variance. $P \leq .05$ was considered statistically significant.

RESULTS

The Ala/Ala 307 variant was found in approximately 16% of controls, whereas the Ala/Thr and Thr/Thr variants were found in 32% and 52% of controls. As for the polymorphism at position 680, 23% of controls had Asn/Asn, 61% had Asn/Ser, and 16% had Ser/Ser.

In normogonadotropic anovulatory infertile women, the overall frequency distribution for polymorphism at position 307 was 20% for Ala/Ala, 57% for Ala/Thr, and 23% for Thr/Thr; for polymorphism at position 680, 16% for Asn/Asn, 44% for Asn/Ser, and 40% for Ser/Ser. The distribution of both polymorphisms differed significantly between anovulatory patients and normo-ovulatory controls ($P<.01$ for position 307 and $P<.01$ for position 680) (Fig. 1). However, the prevalence of the combined genotype (polymorphisms at position 307 and 680) did not significantly differ ($P<.9$) between controls and anovulatory infertile women (Fig. 2).

FIGURE 1

Distribution of the three possible FSH receptor genotypes at positions 307 (**A**) and 680 (**B**) in exon 10 of the FSH receptor gene among normogonadotropic anovulatory women and normo-ovulatory controls. The difference in distribution between anovulatory patients and normo-ovulatory controls was significant for both polymorphisms ($P<.05$ for position 307 and $P<.05$ for position 680).

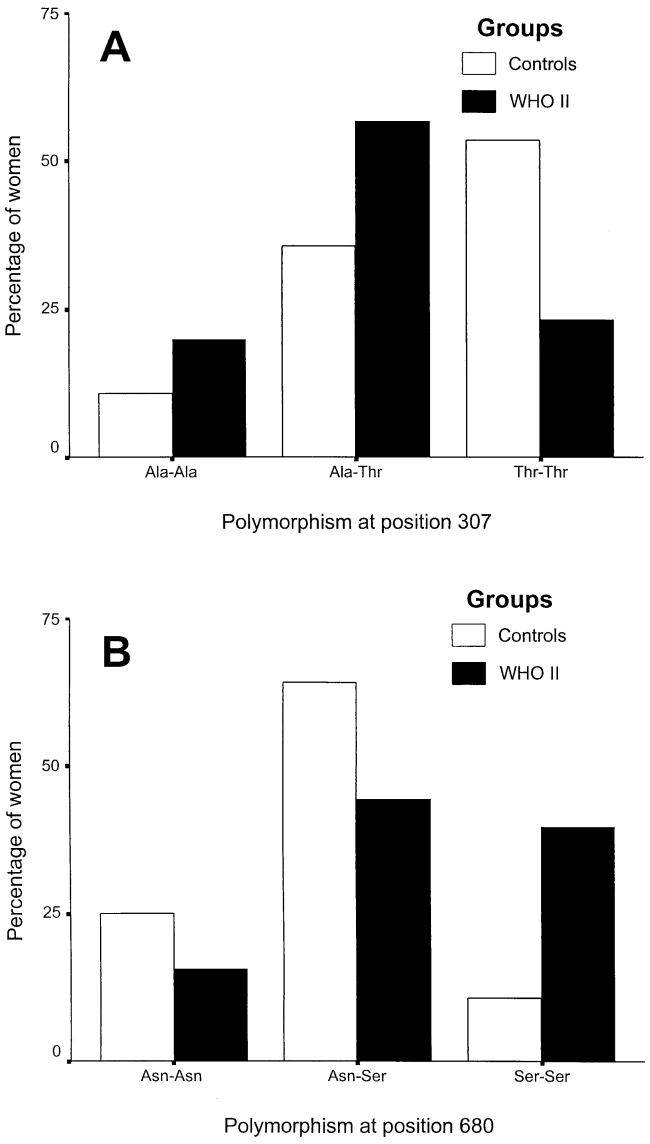

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FIGURE 2

The similarity in distribution of several FSH receptor genotypes among normogonadotropic anovulatory women and normo-ovulatory controls. Since the Ala307/Ser680–Thr307/Asn680 and Ala307/Asn680–Thr307/Ser680 genotype cannot be distinguished by the methods used, they are considered together and designated as Ala307/Ser680–Thr307/Asn680.

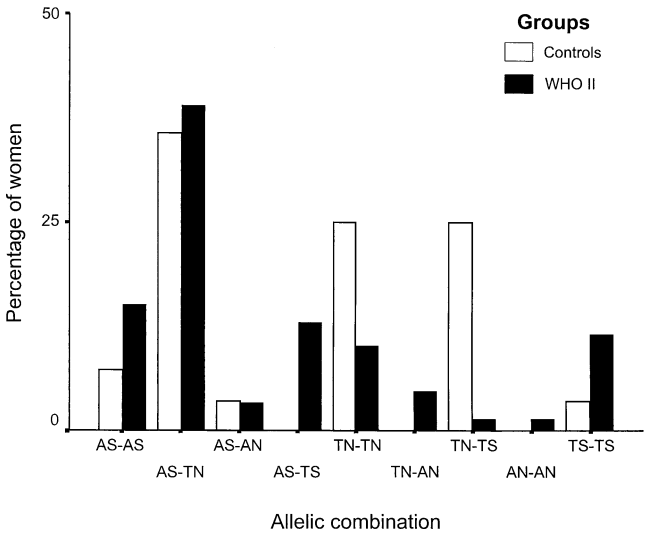

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Table 1 shows the prevalence of the different alleles of the FSH receptor in controls and anovulatory patients. The distribution of alleles did not significantly differ between (P=.8) or within groups (P= 0.7 in controls and P= 0.9 in anovulatory patients).

Table 2 shows clinical, endocrine, and ultrasonographic variables of anovulatory patients with the various FSH receptor polymorphisms. Except for the initial serum FSH concentration, no statistically significant differences were found among the three polymorphisms for position 307 or 680. The FSH serum concentrations were 5.2 IU/L in patients with the Ser/Ser 680 variant; this value was significantly higher than FSH serum levels in patients with the Asn/Ser 680 variant (4.5 IU/L) and those with the Asn/Asn 680 variant (4.6 IU/L).

Data on ovulation induction were available for 89 (60%) anovulatory women. The frequency of polymorphisms at position 307 in clomiphene citrate–resistant patients was 16% for those with the Ala/Ala variant, 54% for those with the Ala/Thr 370 variant, and 30% for those with the Thr/Thr variant. The frequency of polymorphisms at position 680 in these patients was 9% (Asn/Asn), 40% (Asn/Ser), and 51% (Ser/Ser 680). The frequencies of polymorphisms at position 307 or 680 did not differ between clomiphene citrate–resistant patients and those who failed to conceive during previous successful ovulation induction with clomiphene citrate. In addition, the distribution of different alleles and allelic combinations were similar between clomiphene citrate–resistant patients and those who ovulated after clomiphene citrate administration (data not shown).

The FSH response dose could be determined in 77 (87%) of the 89 clomiphene citrate–resistant patients. There were no statistically significant differences among patients with different subtypes of FSH receptor variants for the polymorphism at position 307 or 680 in terms of the FSH dose at the beginning of the stimulation cycle, the number of ampoules of FSH used, the duration of stimulation, the median daily dose of FSH, or the response dose (Table 2). Moreover, the number of cancelled cycles (due to poor response or hyperstimulation) was the same in all three groups for both receptor polymorphisms.

Patients with polycystic ovaries (defined as an ovarian volume ≥10.8 mL, mean number of follicles >10, or mean ovarian stroma score >3) (23) and hyperandrogenemia, defined as a free androgen index (T level/100 × sex hormone–binding globulin level) exceeding 4.5, were classified as having PCOS. In 61 women with PCOS, a similar distribution as in anovulatory patients was found; this distribution differed significantly from that in controls. In patients with PCOS and polymorphism at position 307, the overall frequency distribution was 21% for position Ala/Ala, 58% for Ala/Thr, and 21% for Thr/Thr; corresponding values for anovulatory women without PCOS were 22%, 51%, and 27%, respectively. The frequencies among patients with polymorphism at position 680 and PCOS were 15% for Asn/Asn, 50% for Asn/Ser, and 35% for Ser/Ser; values in patients with this polymorphism but no PCOS were 19%, 38%, and 44%, respectively. For both polymorphisms, the distribution differed significantly among anovulatory women with PCOS, anovulatory women without PCOS on one hand and normo-ovulatory controls on the other hand (P<.01 for position 307 and P<.01 for position 680).

TABLE 1

Distribution of allelic FSH receptor variants in normogonadotropic anovulatory patients and normo-ovulatory controls.

	Allele (%)			
Group	AS	TN	AN	TS
Anovulatory patients (n = 148)	126 (43%)	97 (33%)	17 (5%)	56 (19%)[a]
Controls (n = 30)	17 (28%)	32 (53%)	2 (3%)	9 (15%)

Note: Data are expressed as number of participants (percentage).

[a] Differences in distribution of the four distinct alleles between controls and anovulatory patients were not statistically significant (P<.8).

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TABLE 2

Clinical, endocrine, ultrasonographic, and stimulation characteristics of anovulatory infertile patients among polymorphic variants of the FSH receptor.

	Polymorphism 307			Polymorphism 680		
Variable	Ala/Ala (n = 30)	Ala/Thr (n = 84)	Thr/Thr (n = 34)	Asn/Asn (n = 24)	Asn/Ser (n = 66)	Ser/Ser (n = 58)
Clinical						
Age	28.8 (22.3–35.8)	28.1 (19.6–35.8)	27.7 (21.8–35.3)	27.6 (21.8–35.3)	28.3 (19.4–35.3)	28.8 (22.3–35.8)
Body mass index	26.5 (17.9–42.6)	24.7 (17.7–52.3)	25.5 (17.3–39.8)	26.5 (17.9–39.8)	26.5 (17.7–52.9)	24.6 (17.3–42.6)
Cycle duration	90 (35–199)	60 (35–199)	51 (35–199)	75 (39–199)	60 (35–199)	57 (35–199)
Endocrine						
FSH level	4.8 (3.2–9.0)	4.7 (1.8–9.7)	4.9 (1.4–7.1)	4.6 (1.4–5.8)	4.5 (1.8–9.7)	5.2 (2.4–9.7)[a]
LH level	8.3 (1.1–23.5)	8.1 (1.4–23.5)	6.9 (2.4–20.6)	7.6 (2.9–23.6)	7.3 (2.1–22.5)	7.6 (2.9–20.6)
T level	2.3 (0.6–5.0)	2.5 (0.7–6.8)	2.3 (0.6–4.3)	2.5 (0.6–4.0)	2.4 (0.7–6.8)	2.3 (0.6–5.0)
Free androgen index	5.8 (0.6–26.9)	4.7 (1.4–34.0)	4.4 (0.8–18.1)	5.0 (0.8–34.0)	4.8 (1.4–26.9)	4.3 (0.6–29.3)
E_2 level	188 (81–1868)	223 (39–1062)	222 (47–864)	218 (47–864)	227 (81–1062)	204 (39–1868)
FSH: E_2 ratio	0.03 (0.01–0.07)	0.02 (0.01–0.18)	0.02 (0.01–0.11)	0.03 (0.01–0.11)	0.02 (0.01–0.07)	0.03 (0.01–0.18)[b]
Inhibin B level	122 (25–326)	127 (9–541)	138 (37–506)	138 (59–316)	126 (33–541)	134 (9–506)
Ultrasonographic						
Ovarian volume	8.1 (3.5–17.9)	9.5 (2.6–23.0)	8.3 (5.4–21.5)	8.0 (5.4–19.9)	9.5 (2.6–23.0)	9.2 (2.6–22.9)
Mean follicle number	13.5 (4.5–25.0)	13.5 (2.0–34.5)	12.5 (5.5–23.5)	11.0 (5.5–23.5)	13.0 (4.5–34.5)	15.0 (2.0–30.0)
PCOS (%)	30 (20%)	81 (55%)	38 (25%)	23 (15%)	68 (46%)	58 (39%)
FSH treatment						
Duration of stimulation	11 (8–22)	12 (6–28)	12 (8–29)	11 (8–24)	12 (6–27)	14 (6–29)
Total number of ampoules	12.3 (7.0–33.5)	14.5 (5.0–59.0)	15.0 (6.0–49.0)	15.0 (6.0–38.0)	13.5 (5.0–44.0)	13.5 (5.0–59.0)

Note: Data are medians (ranges). Comparisons are made between three different genotypes within either polymorphism 307 or 680. PCOS = polycystic ovary syndrome.

[a] $P<.01$.

[b] $P<.05$.

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Figure 3 summarizes the data for patients with the polymorphism at position 680. No statistically significant differences in clinical, endocrine, or ultrasonographic variables were found among anovulatory women with PCOS, anovulatory women without PCOS, and controls. Stimulation characteristics in patients with PCOS and those without PCOS patients were similar (data not shown).

DISCUSSION

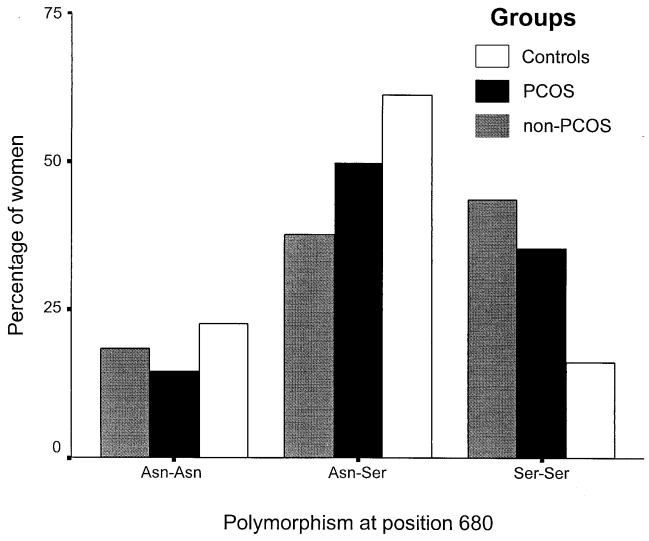
We found differences in distribution of the different FSH receptor genotypes between normogonadotropic anovulatory patients (those with WHO class II disease and PCOS) and normo-ovulatory controls. The FSH receptor variants Ala/Thr 307 and Ser/Ser 680 were significantly more prevalent among anovulatory women. The Thr/Thr 307 polymorphism was significantly more prevalent in controls. Earlier reports failed to establish differences in prevalence of FSH receptor genotypes in fertile or infertile men (17) and in infertile women (16).

Few studies have addressed the distribution of the allelic variants in patients with PCOS, and results are conflicting. Two studies revealed no differences in prevalence between a limited number of patients with PCOS and normo-ovulatory women (16, 18). Recently, Sudo et al. (19) reported a significant increase in the Ala/Thr 307 and Asn/Ser 680 genotype in a large cohort of women with PCOS. Because the distribution of the polymorphism at position 680 among our normo-ovulatory controls is similar to the previously reported incidence in normal women (16, 19, 28), the observed difference in this large cohort of anovulatory women and patients with PCOS seems to be real.

The FSH receptor polymorphism combination Ser/Ser 680 was associated with higher basal FSH levels compared with the Asn/Asn 680 and Asn/Ser 680 variants. This might indicate that the Ser/Ser 680 FSH receptor polymorphism is associated with decreased FSH sensitivity. In a recently published prediction model, the individual FSH response dose during ovulation induction therapy was determined in part by the initial basal FSH serum concentration (29). Moreover, in normo-ovulatory IVF patients, ovarian response to exogenous FSH stimulation was determined by the FSH receptor genotype. Normo-ovulatory patients exhibiting the Ser/Ser 680 allelic variant had higher levels of basal FSH and required a significantly higher daily FSH dose for suc-

FIGURE 3

Distribution of the three possible FSH receptor genotypes at both positions 307 and 680 in exon 10 of the FSH receptor gene among women without the polycystic ovary syndrome (*non-PCOS*), women with PCOS (*PCOS*), and normo-ovulatory controls. The distribution of both polymorphisms significantly differed between women with PCOS and those without PCOS on one hand and controls on the other hand.



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cessful hyperstimulation in a routine IVF/intracytoplasmic sperm injection (ICSI) program (15).

Similarly, in our study, the highest basal FSH serum levels in anovulatory infertile patients were associated with the Ser/Ser 680 allelic variant. However, neither the response dose nor the total number of ampoules FSH used or duration of stimulation differed among patients with the different FSH receptor genotypes. This lack of association between FSH receptor genotype and ovarian sensitivity might be due to differences in stimulation protocols. Because ovulation induction protocols in anovulatory patients aim for monofollicular development, the amount of exogenous FSH used may be within the physiologic range. In contrast, exogenous FSH administered during IVF stimulation protocols extends the physiologic range since multifollicular development is the goal.

Because previous in vitro experiments failed to establish significant differences in binding characteristics and receptor activation between several FSH receptor polymorphisms (17, 19), differences in FSH receptor activity might become apparent only after supraphysiologic stimulation (i.e, IVF stimulation protocols). However, the association between the FSH receptor phenotype and the basal FSH serum levels might also be coincidental. Moreover, the FSH receptor polymorphism might merely constitute a genetic marker for a nearby gene (not the FSH receptor) that increases the risk for anovulation.

More clinical and experimental data are necessary to establish the exact relationship between the dose of exogenous FSH and FSH receptor genotypes. Although about 50% of anovulatory infertile patients in our study were clomiphene resistant and not normo-ovulatory, which might make them not readily comparable to normo-ovulatory women, a dose dependent relationship between the FSH receptor polymorphism and the magnitude of the post-receptor signal cannot be ruled out.

In conclusion, normogonadotropic anovulatory patients and women with PCOS have a different FSH receptor genotype compared with normo-ovulatory controls. Despite these differences, FSH receptor genotypes are not associated with altered ovarian sensitivity to exogenous FSH during ovulation induction in anovulatory patients.

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