Association of molecular variants of luteinizing hormone with male infertility

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Luteinizing hormone (LH) stimulates the interstitial Leydig cells to produce testosterone, which is essential for spermatogenesis. Abnormalities in the function of LH may affect the process of spermatogenesis and thus result in infertility. The aim of this study was to determine the association of three known variants of LH (Gln54Arg [Trp8Arg; Ile15Thr] and Gly102Ser) with male infertility. A total of 145 infertile men and 200 healthy fertile men were recruited and screened for the presence of these three LH variants. The Gln54Arg variant could not be detected in either of the groups studied. Twelve infertile (8.2%) and 15 fertile (7.5%) men were found to carry the [Trp8Ile; I15Thr] variant, but its occurrence did not show any significant difference between the patient and control groups. The Gly102Ser variant was detected in five patients with infertility (3.4%), but not in the control subjects (P = 0.013). This study showed that the Gln54Arg and [Trp8Ile; I15Thr] variants in the LHβ gene were not associated with male infertility, whereas the Gly102Ser variant might be implicated in infertility in some Singapore Chinese men.

Key words: DNA sequencing/LH β-subunit/male infertility/PCR–RFLP variant

Introduction

According to the World Health Organization (WHO, 1993), ~8–10% of couples experience some forms of infertility problem, in which male factors may account for up to 50%. Conditions known to cause impaired fertility in the male include endocrine disorders, genetic factors, environmental factors, and varicocele (Feichtinger, 1991; Thielemans et al., 1998). However, in a great number of cases of male infertility caused by inadequate spermatogenesis or sperm defects, the origin of the problem still remains unexplained.

Luteinizing hormone (LH) is one of the three important gonadotrophin hormones essential to human reproduction. Its primary role in the male is to stimulate the production of testosterone by the Leydig cells which then, together with follicle stimulating hormone (FSH), regulate spermatogonial cell formation and spermatogenesis in the Sertoli cells of the testes (Griffin and Wilson, 1985). Gonadal failure, a cause of infertility, is indicated by elevated concentrations of LH and FSH, and by low concentrations of gonadal steroids (Beastdall, 1987). In the male, elevated concentrations of LH can result from hypergonadotrophic hypogonadism which could be due to various causes such as primary testicular failure, seminiferous tubule dysgenesis (Klinefelter syndrome), Sertoli cell failure, and anorchia (Franchimont, 1973; Marshall, 1975). In sexually matured adults, low concentrations of LH, FSH and steroids are observed in gonadotrophin deficiency. LH regulates male sexual differentiation, pubertal androgenization, male sexual function, and fertility through the function of Leydig cells. Thus, abnormalities or defects in the LH would disrupt the regulatory function of Leydig cells and result in male infertility.

LH, a member of the glycoprotein hormone family, is a heterodimer composed of a common α-subunit and a specific β-subunit that confers biological specificity for the hormone receptor in the target organ (Pierce and Parsons, 1981; Gharib et al., 1990). The LHβ gene is a member of CGβ/LHβ gene cluster that resides on chromosome 19q (Talmadge et al., 1984; Hollenberg et al., 1994).

There has been a report of a homozygous LHβ gene mutation in an adolescent with pubertal delay, increased serum concentration of LH, normal FSH and low testosterone. This mutation caused an amino acid change Gln54Arg in exon 3 (Weiss et al., 1992). A second variant of LH in exon 2 which caused two single amino acid substitutions [Trp8Ile; I15Thr] has been identified in both healthy and female infertile patients (Furui et al., 1994; Okuda et al., 1994). A third 1502G|CG mutation in exon 3 of LHβ gene causing a replacement of glycine by serine at amino acid 102 (Roy et al., 1996) has been found to be associated with female infertility (Liao et al., 1998; Ramanujam et al., 1999).

In this study, we examined the relationship of these LHβ variants with male infertility in Singapore Chinese men.

Materials and methods

One hundred and forty-five Chinese patients with male infertility participated in this study. Their ages ranged from 24 to 65 years (35.6 ± 6.0 years; mean ± SD). Their semen parameters ranged from azoospermia to oligozoospermia (Table I). Two patients with azoospermia due to varicocele (obstruction of sperm transport occurring in conjunction with varicocele) presented a normal testicular size and consistency and possible swelling of the epididymal head as well as normal FSH concentration in blood, whereas spermatogenic cells were absent from the ejaculate after varicocelectomy.

Two hundred fertile men aged between 23 and 48 years (34.3 ± 5.6 years; mean ± SD), were used as control subjects. Their semen analysis was normal and fertility was positively established 2 years before the trial; they fathered full term pregnancies with normal infants. Approval by ethical committee was obtained.
Aetiologies of male infertility in sample of 145 Singapore Chinese men

<table>
<thead>
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<th>Semen abnormalities</th>
<th>Causes</th>
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| Azoospermia (13)    | Genetic: Klinefelter’s syndrome (2) | Testicular: cryptorchidism (5), primary hypogonadism due to orchi
testic factors: vasectomy (1) |
|                     | Vascular: varicocele (2) | Testicular: mumps orchitis (1) |
|                     | Vascular: varicocele (7) | Idiopathic (26) |
| Asthenozoozoospermia (34) | Testicular: orchitis (2) | Idiopathic (12) |
| Teratozoospermia (12) | Central: hypogonadotropic hypogonadism (1) | Testicular: orchitis (1) |
|                     | Vascular: varicocele (1) | Idiopathic (10) |
| Oligozoospermia (16) | Testicular: orchitis (2), impending testicular failure (1) | Vascular: varicocele (1) |
|                     | Vascular: varicocele (1) | Idiopathic (12) |
| Asthenoteratozoospermia (14) | Vascular: varicocele (3) | Idiopathic (11) |
| Oligoasthenozoospermia (23) | Genetic: chromosomal abnormality (3) | Testicular: testicular failure (2) |
|                     | Vascular: varicocele (6) | Idiopathic (12) |
| Oligoteratozoospermia (9) | Vascular: varicocele (2) | Idiopathic (7) |
| Oligoasthenoteratozoospermia (24) | Testicular: orchitis (3), testicular failure (5) | Vascular: varicocele (16) |

Numbers in parentheses indicate number of patients.

Routine investigations included a complete haemogram, urine analysis and chest roentgenogram, and determination of fasting blood sugar, blood urea nitrogen, serum creatinine, serum bilirubin, and alkaline phosphatase.

Specific investigations performed were semen analysis, urological evaluation, testicular biopsy, hormone estimations, and scrotal exploration with vasography (where indicated). Semen samples were analysed according to the method described in the World Health Organization manual (WHO, 1993). Azoospermia was defined as total absence of sperm in the semen, oligozoospermia as a sperm concentration of $<20\times10^6$/ml. Asthenozoospermia was defined as $<50\%$ spermatozoa with forward progression or $<25\%$ spermatozoa with rapid progression, teratozoospermia as reduced percentage ($<30\%$) of morphologically normal spermatozoa; oligoasthenoteratozoospermia signifies disturbance of all three variables.

Semen cultures were done in relevant cases. Genital infections with Chlamydia or Mycoplasm species were particularly sought. Hormonal estimations consisted of LH, FSH, prolactin and TSH. Testosterone was measured in relevant cases. Testicular biopsy was done for patients with an abnormal semenogram who had normal gonadotrophin values and no obvious cause for semen abnormality.

Plasma concentrations of FSH, LH, prolactin and testosterone were analysed by radioimmunoassay using reagents provided by the WHO under the Matched Reagent Programme (Goh et al., 1979). Three blood samples were taken from each patient in the morning at an interval of 20 min, and the sera pooled and stored at $-20\,^{\circ}\text{C}$ until assayed.

Nuclear DNA was extracted from peripheral leukocytes by a standard procedure and used as template DNA for polymerase chain reaction (PCR). PCR for DNA amplification was carried out using specific primers as described previously (Roy et al., 1996). Restriction fragment length polymorphism (RFLP) analysis was employed to detect the presence of individual variants. Bsu36I was used to detect the existence of the first variant (Gln54Arg). RFLP analyses using NcoI, FokI and Eco0109I were carried out as described previously (Ramanujam et al., 1998). The mutations were confirmed by PCR-mediated direct DNA sequencing using a DNA Sequencer (ABI Prism TM 377, Perkin Elmer, Norwalk, CT, USA) with Big Dye Cycle Sequencing Ready Reaction Kit (Perkin Elmer).

Statistical analysis

Statistical tests of significance and by $\chi^2$-analyses and Fisher’s exact test (two-sided) were carried out using SPSS for windows, version 8.0. $P < 0.05$ was considered statistically significant.

Results

$LHB$ subunit gene was analysed in the patients ($n = 145$) and control subjects ($n = 200$) using PCR-based RFLP and confirmed by DNA sequencing. The first variant (Gly54Arg) could not be detected in any of the infertile and fertile men studied. However, 12 infertile and 14 fertile subjects were found to be heterozygous for the second [Trp8Arg; Ile15Thr] variant. In addition, one fertile subject was found to be homozygous for this variant. Thus, the prevalence of this variant was seen to be 8.2% (12/145) and 7.5% (15/200) in infertile and fertile men respectively, which did not differ significantly ($P = 0.634$).

In contrast, a heterozygous Gly102Ser variant was detected in five patients with male infertility (3.4%, 5/145), but none in the control subjects. Thus, the difference in occurrences of this variant between infertile and fertile men was significant ($P = 0.013$). Out of these five cases detected, two had oligoasthenozoospermia due to varicocele, two were with asthenozoospermia due to varicocele and orchitis and one was with oligoasthenoteratozoospermia due to varicocele. All of these patients had a history of primary infertility. They had normal LH concentrations, except one patient (case 4) who presented low LH value. Testosterone concentrations were low in two patients, while the other three had normal concentrations. Their semen parameters, clinical and hormone profiles are outlined in Table II.

Discussion

Many studies have indicated the importance of LH in male reproductive function. It was observed that plasma LH, FSH and testosterone concentrations were increased in patients with sperm counts of $<10\times10^6$/ml (Urry et al., 1976). These results suggest that there is not only impaired spermatogenesis but also impaired Leydig cell function in severely oligozoospermic and azoospermic patients. It was demonstrated that LH concentrations were several-fold higher in seminal plasma than in serum, which were significantly higher in oligozoospermia and normozoospermia than azoospermia samples (Sheth et al., 1976; Biswas et al., 1978). The enhancement by LH of sperm fructosylation, and adenyl cyclase activity, which are important means by which spermatozoa derive energy for their motility, indicates a possible role of seminal plasma LH in sperm motility and metabolism. Bennet et al. (1991) observed that male patients with idiopathic oligozoospermia were found to
have a higher mean LH pulse frequency and a lower mean bioactive/immunoactive LH ratio than the controls. These results suggested that abnormal LH secretions might be related to male infertility.

Molecular variants of LH have recently been identified and found to be associated with male infertility. Weiss et al. (1992) detected a homozygous Gln54Arg variant in exon 3 of LHβ gene in a male with hypergonadotrophic hypogonadism, elevated immunoreactive LH but low bioactive LH, low testosterone concentration and low sperm count. Although the proband received chorionic gonadotrophin and testosterone therapy on several occasions, he had decreased spermatogenesis and was infertile. This mutation eliminates the ability of lutinizing hormone to bind to its receptors, resulting in the failure of puberty to develop spontaneously and subsequent infertility. In heterozygous men, this mutation caused impaired steroidogenesis and a high incidence of infertility (3/4) despite normal development of secondary sexual characteristic (Weiss et al., 1992).

An immunologically anomalous form of LH ([Trp8Arg; Ile15Thr]) was described in a Finnish woman (Pettersson et al., 1992) and another case subsequently (Furui et al., 1994). Many studies have been carried out to determine the importance of this variant in female reproduction. In two studies, it was found to be related to menstrual disorders, premature ovarian failure and female infertility (Suganuma et al., 1995; Takahashi et al., 1998, 1999) but not in other studies (Haavisto et al., 1995; Rajkhowa et al., 1995; Ramanujam et al., 1999). Thus, the role of this variant in female infertility remains controversial. Raivio et al. (1996) examined the possible correlation of this LH variant with the onset and progression of puberty in healthy boys and found to be associated with delayed pubertal maturation. However, the clinical significance of this LH variant in patients with male infertility is still not known.

In a recent study Liao et al. (1998) have found the 1502G → A mutation in exon 3 of LHβ-subunit gene described by Roy et al. (1996) in two unrelated infertile Chinese women with endometriosis but not in the controls. This finding suggested implication of this mutation in female infertility in some women.

In the present study, we investigated whether there was any correlation of these variants with male infertility. The Gln54Arg variant could not be detected in control subjects and infertile patients studied. However, we found 12 heterozygous cases of immunologically anomalous exon 2 mutations in infertile male patients (8.2%), and 14 heterozygous and one homozygous cases in the control subjects (7.5%). Our data showed an allele frequency of 0.0413 in infertile males and 0.04 in the control group but the difference was not statistically significant. These findings were found to be in agreement with those of Haavisto et al. (1995). Therefore, the Gln54Arg and [Trp8lle; I15Thr] variants are unlikely to play a significant role in male infertility in the Singapore Chinese population.

For the Gly102Ser variant (Roy et al., 1996; Liao et al., 1998), out of 170 patients with male infertility, five were found to be carriers (3.4%). On the contrary, none of the control subjects studied were carriers of this variant. The variant showed an allele frequency of 0.01 which was significantly higher in infertile males than fertile subjects (P = 0.013). These results suggest that this LH variant may be associated with male infertility in some men. Interestingly, all five men were found to have varicocele.

Varicocele is the dilatation of pampiniform plexus. Some reports have suggested that varicocele was the reason for the abnormal semen analysis and reduced fertility potential in men.
(Stewart, 1974; Dubin and Amelar, 1975). Abnormal LH values have been found in the semen samples of the patients with varicocele (Abdalla et al., 1981; Micic et al., 1986). Freire and Nahoum (1981) observed that serum LH concentrations were higher in infertile patients with varicocele than fertile males. However, the role of these abnormal LH values in infertile men with varicocele remains unclear.

In our study, since all the five patients carrying this exon 3 variant had varicoceles, it is possible that a man carrying the LH variant with varicocele may eventually become infertile. This can be due to combined effects of both LH variant and varicocele. However, further study is warranted to investigate the exact role of this LH variant in varicocele-associated infertility.

In conclusion, in this study, the Gln54Arg variant and immunologically anomalous [Trp88le; I15Thr] variant of LH were not seen to be associated with male infertility, whereas the Gly102Ser variant of LH, previously implicated in female infertility (Liao et al., 1998), appeared to be involved in male infertility. Thus, this variant may play a role in both male and female infertility.

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References

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