Variants in the *SRD5A2* gene are associated with quality of semen

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Received February 13, 2012; Accepted June 19, 2012

DOI: 10.3892/mmr.2012.965

Abstract. Spermatogenesis and sperm maturation are androgen-dependent processes. Steroid 5α-reductase (SRD5A) is a key enzyme converting testosterone into the more active metabolite, dihydrotestosterone (DHT). We aimed to investigate the association between the genetic variants of *SRD5A2* (rs4952197, rs2268797, rs13395648, rs523349 and rs632148) and semen quality. Variant genotyping and semen analysis was performed in 708 males with definite idiopathic infertility by TaqMan SNP genotyping assays and computer-assisted semen analysis, respectively. It was found that the rs13395648 TC genotype was associated with a significantly lower semen volume compared with the TT genotype (P=0.016). The same trend was found between the combination of the TC and CC genotypes and the TT genotype (P=0.020). With regard to variant rs632148, subjects with the GC genotype had significantly lower sperm motility in comparison to those with the GG genotype (P=0.029). The sperm motility between the combination of the GC and CC genotypes and the GC genotype was also significantly different (P=0.033). These findings indicate that genetic variants in the *SRD5A2* gene may be associated with semen quality.

Introduction

Infertility has been defined as the failure of a couple to conceive and reproduce after 12 months of regular intercourse (1). Approximately 13-15% of couples of reproductive age worldwide are affected by this common clinical problem, and half of these cases are related to male dysfunction (2). Among all males with infertility, the cause of more than half is unknown (idiopathic). Irregularities in sperm function or spermatogenesis are believed to be associated with idiopathic male infertility (3).

Spermatogenesis and spermatozoa maturation is an androgen-dependent process (4). Dihydrotestosterone (DHT) is the main androgen responsible for spermatozoa maturation (5), as well as for the maintenance of normal spermatogenesis (6). The enzyme steroid 5α-reductase (SRD5A) is a key enzyme involved in converting testosterone into a more potent androgen, DHT, in male reproductive tissues. Human 5α-reductase mainly consists of type 1 (*SRD5A1*) and type 2 (*SRD5A2*) isozymes, but type 2 is more essential for development of the male reproductive organs (7) and thus may be associated with semen quality compared with type 1.

The *SRD5A2* gene is on chromosome 2p23 with 5 exons, encoding a 254 amino acid protein (7). Several missense polymorphisms affect enzyme activity (8): some normal variations in the *SRD5A2* gene may be associated with prostate cancer (9), while other serious mutations of *SRD5A2* reduce or eliminate enzymatic activity and cause deficient virilization of the male external genitalia or even pseudohermaphrodism during development (10-12). Of these, three single nucleotide polymorphisms (SNPs) alter other amino acids of SRD5A2 that are more noteworthy. The most common variation is a valine to leucine substitution at codon 89 (*V89L*, rs523349), which has been associated with prostate cancer. Due to this variation, there is an approximately 30% reduced activity of SRD5A2 in Asian men (13), which is not found in European populations (14-15). The other missense polymorphism is at codon 49, substituting an alanine with a threonine at codon 49 (*A49T*). The T-variant has mainly been associated with prostate cancer and was shown to increase the enzymatic activity to a great extent (16-17). The R227Q mutation substitutes arginine with glutamine, leading to SRD5A2 enzymatic activity being almost completely absent. With the exception of the above three mutations, other mutations are detected at a very low frequency (8). Nevertheless, all of the above shows that variations of *SRD5A2* are associated with an increased risk of developing diseases of the male reproductive organs and functions. Thus, we speculate whether the polymorphisms

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Key words: idiopathic male infertility, polymorphism, semen quality, *SRD5A2*

in the *SRD5A2* gene are associated with semen quality in the Han-Chinese population.

In the present study, we investigated the frequency distribution of five tagSNPs representing genetic variation across the entire *SRD5A2* gene in 708 patients with idiopathic infertility. This study may add to our knowledge regarding the impact of *SRD5A2* polymorphisms on male infertility. The SNP tagging method is used to select tagSNPs. Five tagSNPs were captured to present 648 SNPs of human *SRD5A2*. Therefore, the purpose of this study was to investigate the possible association between the genetic variants of *SRD5A2* and semen quality in 708 males with definite idiopathic infertility in a Han-Chinese population.

Materials and methods

Subjects. A total of 708 ethnic Han-Chinese males with definite idiopathic infertility (without diagnosed infertile wives) visiting the First Affiliated Hospitals of Nanjing Medical University were consecutively recruited between March 2010 and March 2011. All the subjects had been unable to conceive for at least 12 months. Those with a history of orchitis, obstruction, cryptorchidism, congenital bilateral absence of vas deferens, cytogenetic abnormalities and Y chromosome microdeletions were excluded from the study after a complete historical and physical examination (18-19). All participants provided informed consent and completed a questionnaire including information about age, smoking and drinking habits and other lifestyle factors. Each subject donated 5 ml of peripheral blood for genomic DNA extraction and an ejaculate for semen analysis. This study was approved by the ethics review board of Nanjing Medical University.

Semen analysis. The computer-assisted semen analysis system (WLJY-9000; Weili New Century Science and Tech Dev., Beijing, China) was used to perform the semen analysis according to the World Health Organization guidelines (World Health Organization, 1999). Four parameters of semen quality were selected for the statistical analysis: Semen volume, sperm concentration (10^6/ml), sperm number per ejaculate (10^6/ejaculate) and sperm motility. The sperm numbers and sperm motility characteristics provided a reliable estimation of the fertilizing ability of human spermatozoa (20). Strict quality control measures were enforced throughout the study. Each sample was assessed twice.

SNP selection. Genotype data obtained from unrelated Han Chinese individuals from Beijing in the HapMap (HapMap Data Rel 24/phase II nov08, on NCBI B36 assembly, dbSNP b126; http://hapmap.ncbi.nlm.nih.gov) were used to select the SNPs. SNPs with a minor allele frequency >0.05 in Han Chinese in Beijing were selected within the 56318 bp human *SRD5A2* gene, which was pinpointed to chromosome 2 from 31603218 to 31659535. Thirty SNPs were captured in this region. A linkage disequilibrium (LD) plot of this region was made using Haploview 4.0 software (21), based on the r^2 values, which indicate the ability of a certain SNP to predict another SNP (Fig. 1). Using Tagger and a tagging threshold of r^2>0.80, five tagSNPs were selected (rs4952197, rs2268797, rs13395648, rs523349 and rs632148) that could represent other SNPs of this region with a mean r^2 of 0.959 (Fig. 1).

Genotyping. Genomic DNA was extracted from peripheral blood leukocytes of 708 Han-Chinese males suffering from infertility according to standard protocols (Genomic DNA kit; Tiangen, Beijing, China). TaqMan SNP genotyping assays were performed using the *Taq* amplification method in a 7900 HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA).

PCR amplification of rs4952197, rs2268797 and rs13395648 was performed at 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec, 56˚C for 10 sec and 60˚C for 1 min, with one additional cycle at 60˚C for 10 min. The amplification conditions of rs523349 and rs632148 were similar to the above except that 55 cycles were used due to the higher relative GC content. Ten percent of study participants were randomly chosen and genotyped in duplicate to confirm the concordance of the genotyping results. In our study, the call rates for these SNP genotyping were >97% and the concordance of duplicates was 100%.

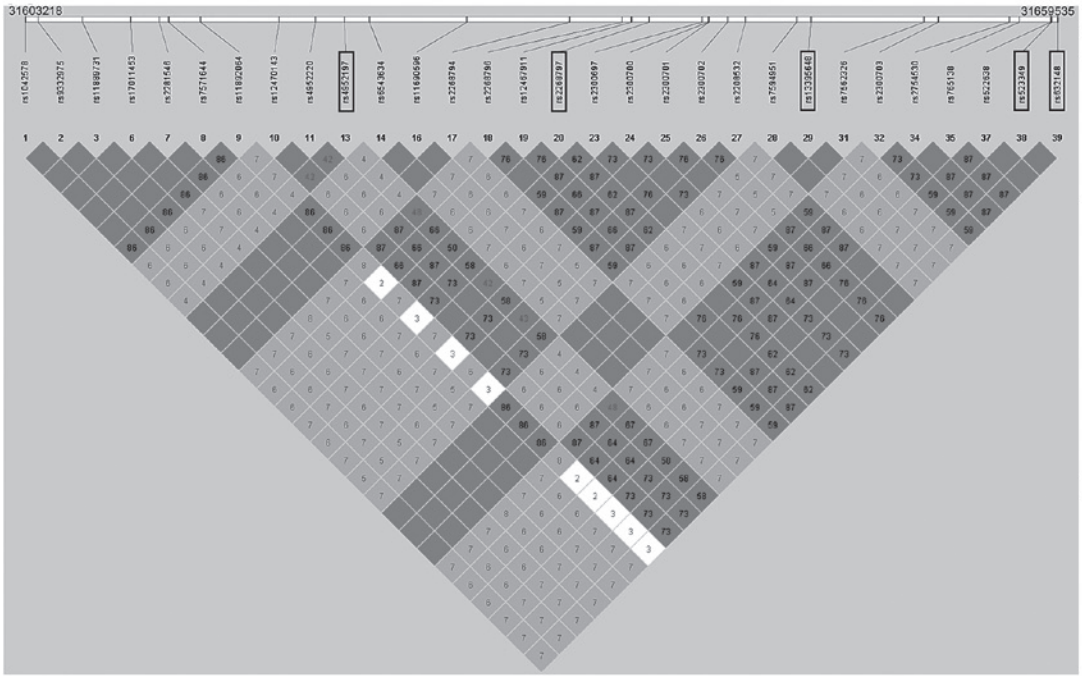
Statistical analysis. Logarithmic transformation of the sperm concentration and sperm number per ejaculate was undertaken to achieve homogeneity of variance and normal distribution of residuals. No transformation was performed for the remaining parameters. Differences in selected demographic variables, smoking and alcohol status were evaluated by the χ^2 test. The Student's t-test was used to evaluate continuous variables, including age, BMI and pack-years of cigarette smoking. For statistical analysis, a multiple linear regression analysis was applied for the comparison of semen parameters as considered for the genotypes of each SNP. Statistical analyses were carried out using Stata (Version 9.0, StataCorp, LP). P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of the study populations. The study population comprised 708 ethnic Han-Chinese males with definite idiopathic infertility at 30.57±5.14 years of age. The mean duration of sexual abstinence prior to semen collection was 5.23±3.52 days. The sperm parameters between the stratification of selected characteristics were analyzed (Table I). No significant differences were found in the stratification of age, smoking and BMI (body mass index) for all four parameters. However, the sperm number per ejaculate and the sperm motility were significantly higher in the never drinking group when compared with the ever drinking group (P=0.015 and 0.0425, respectively). No significant difference was observed in the remaining parameters (semen volume and concentration) between the ever drinking and never drinking groups. For the duration of sexual abstinence (Abs), the 4-7 days group and ≥7 days group were associated with a significantly higher semen volume (P<0.001 and P<0.001, respectively) and sperm number per ejaculate (P=0.025 and P<0.001, respectively) compared with the <4 days group. The sperm concentration between the ≥7 days group and the <4 days group was also significantly different (P=0.013). The sperm motility parameter among the three stratifications of the duration of sexual abstinence was similar.

Associations between SRD5A2 SNPs and semen quality. Table II shows the association of the genotype frequency and

A



B

TaqSNP	r^2 value	alleles	MAF
rs4952197		T / C	0.326
rs2268797		C / T	0.477
rs4952220	0.872		
rs12470143	0.872		
rs2300703	1.0		
rs2268796	1.0		
rs2300700	0.872		
rs2300702	0.872		
rs13395648		G / A	0.078
r6543634	1.0		
rs17011453	1.0		
rs11892064	0.864		
rs11889731	1.0		
rs11690596	1.0		
rs2281546	1.0		
rs 7594951	1.0		
rs1042578	1.0		
rs7571644	1.0		
rs7562326	1.0		
rs2268794	1.0		
rs9332975	1.0		
rs523349		C / G	0.478
rs632148		G / C	0.478
rs522638	1.0		
rs2754530	0.874		
rs2208532	1.0		
rs2300697	1.0		
rs765138	0.874		
rs12467911	0.872		
rs2300701	1.0		

Figure 1. Overview of selected tagSNPs and their characteristics. (A) Location of the 5 tagSNPs within the *SRD5A2* gene. The plot was generated by Haploview 4.0 software and a confidence intervals algorithm. (B) The 5 tagSNPs and SNPs that are indirectly measured by them are listed with corresponding r^2 values. TaqSNP, tagging single-nucleotide polymorphisms; MAF, minor allele frequency.

sperm parameters of each variant. The sperm concentration and sperm number per ejaculate were not significantly different between the genotypes of each genetic variant. The semen volume was also similar among the genotypes of three variants (rs4952197, rs2268797 and rs523349). For rs13395648, the subjects with the TC genotype presented a significantly lower semen volume compared with the TT genotype (P=0.016). The same trend was found between the combination of the TC and CC genotypes and the TT genotype (P=0.020) (Table II).

For rs632148, the GC genotype carriers had a significantly lower motility compared with the GG genotype (P=0.029). However, the difference between the CC and GG genotypes was not statistically significant (P=0.078), whereas the combination of GC and CC genotype carriers had a significantly lower motility compared with the GG genotype (P=0.033) (Table II). The sperm motility was not significantly different between the genotypes of rs4952197, rs2268797, rs13395648 and rs523349 (Table II).

Discussion

In the present study, five tagSNPs (rs4952197, rs2268797, rs13395648, rs523349 and rs632148) in the *SRD5A2* gene were selected using the tagSNP method. In total, 708 Han-Chinese males with idiopathic infertility were genotyped for these SNPs using TaqMan-based genotyping. Significant associations were detected between the genetic variant rs13395648 and semen volume, and between the genetic variant rs632148 and sperm motility. It was demonstrated that subjects with the rs13395648 TC genotype had significantly lower semen volume compared with those with the TT variant. The same trend was also found in subjects carrying a C allele at rs13395648 (TC and CC). Regarding rs632148, the sperm motility was significantly lower in subjects with the GC genotype compared with those with the GG genotype. The combination of GC and CC genotype carriers also had a significantly lower motility compared with the GG genotype. No association was found between any of the remaining three polymorphisms and semen quality.

The sperm number and motility characteristics are known to be the most informative parameters in semen quality analysis and may provide a reliable estimation of the fertilizing ability of human spermatozoa (20), which are dependent on normal spermatogenesis and spermatozoa maturation (2,22). Spermatogenesis and spermatozoa maturation are androgen-dependent processes (4-5,23). SRD5A is a key enzyme converting testosterone into a more potent androgen, DHT. Therefore, the SRD5A enzyme is crucial in these processes. Furthermore, it is more likely that milder polymorphic variations of *SRD5A2* are associated with semen quality. In this study, we examined the association of the *SRD5A2* gene with semen parameters in 708 Han-Chinese males with definite idiopathic infertility. Results showed that the heterozygote at rs13395648 and rs632148 in *SRD5A2* was significantly associated with semen volume and sperm motility, respectively.

As mentioned previously, *V89L*, *A49T* and *R227Q* are three special mutations that alter other amino acids of SRD5A2 among the 648 SNPs detected in the *SRD5A2* gene according to public genome databases. The *V89L* (rs523349) polymorphism has been extensively examined in relation to prostate cancer (24). Although certain studies have shown an association with prostate disease, the results are conflicting (25-27). As yet, there are no conclusions on whether *V89L* mutation is associated with the risk of prostate cancer (15,28), and there is no conclusive evidence of *V89L* polymorphism affecting serum

Table I. Association between the selected individual characteristics and sperm parameters in 708 males with definite idiopathic infertility.

	N (%)	Semen volume (ml)	Concentration[a]	Sperm number per ejaculate[a]	Motility (%)
Age (years)					
<28	224 (31.64)	3.76±1.53	3.50±1.23	4.62±1.31	52.32±24.50
28-32	216 (30.51)	3.41±1.42	3.65±1.16	4.79±1.28	53.52±22.94
≥32	268 (37.85)	3.45±1.33	3.72±1.13	4.89±1.24	49.23±25.21
Smoking					
Yes (ever)	364 (51.41)	3.35±1.36	3.57±1.22	4.71±1.33	51.99±24.00
No (never)	344 (48.59)	3.49±1.49	3.68±1.13	4.85±1.23	51.01±24.74
Drinking					
Yes (ever)	324 (45.76)	3.38±1.43	3.55±1.23	4.69±1.36[b]	49.49±24.88[b]
No (never)	384 (54.24)	3.45±1.42	3.69±1.13	4.85±1.20	53.23±23.78
BMI					
<20	82 (11.58)	3.23±1.40	3.46±1.25	4.57±1.32	51.78±24.56
20-25	396 (55.93)	3.39±1.36	3.60±1.20	4.74±1.31	50.72±24.86
≥25	230 (32.49)	3.52±1.53	3.73±1.10	4.91±1.19	52.79±23.41
Abs (days)					
<4	176 (24.86)	2.91±1.09	3.50±1.11	4.51±1.17	53.69±22.69
4-7	357 (50.42)	3.52±1.51[c]	3.60±1.15	4.76±1.28[c]	51.20±24.44
≥7	175 (24.72)	3.72±1.41[c]	3.82±1.29[c]	5.09±1.33[c]	49.97±25.71

Values are the means ± SD unless otherwise stated. BMI, body mass index; Abs, the duration of sexual abstinence. [a]Values were re-transformed following logarithmic transformation. [b]P<0.05 compared to the groups of drinking status. [c]P<0.05 compared the group of abstinence time <4 days.

hormone levels (25). Our study suggests that there is no association between rs523349 and semen quality. Concerning the *A49T* and *R227Q* mutations, they are not captured by tagSNP selection due to their very low frequency.

In their study, Elzanaty *et al* investigated the effect of *SRD5A2* polymorphisms on sperm parameters in Swedish military conscripts. Their results showed that variants of *A49T* and *V89L* are associated with sperm concentration and motility, respectively (29). Our results are not in agreement with those authors in that the mutation at rs632148 demonstrated an association with sperm motility, but not the rs523349 (*V89L*) mutation. This discrepancy may be due to ethnic differences. Another study investigating the effect of *SRD5A2* polymorphisms on male infertility in an Estonian population has shown that the *SRD5A2* polymorphism exhibited a significant association with sperm motility in normozoospermic men, while no correlation was found between any tagSNP in *SRD5A2* and the sperm number (30). Our results are generally consistent with that finding.

SRD5A2 is expressed in the adult epididymis (31), the prostate (32), the male external genitalia, seminal vesicles (33) and intact seminiferous tubules (23). Spermatogenesis and spermatozoa maturation are androgen-dependent processes (4-5,23). DHT is the main androgen responsible for spermatozoan maturation in the epididymis (34) and maintenance of normal spermatogenesis in the testis (23), as well as maintenance of normal function of the prostate and seminal vesicles (35). SRD5A is a key enzyme converting testosterone into DHT. Thus the activity of SRD5A2 may be associated with sperm motility, seminal volume and even sperm count.

In our study, the heterozygote at rs13395648 was significantly associated with semen volume. A possible explanation for this finding is that SRD5A2 activity affected by mutation leads to a reduction of DHT in the microenvironment of the sexual gland and accessory sexual gland, and causes seminal plasma secretion to be reduced.

Additionally, the heterozygote at rs632148 was significantly associated with sperm motility. Sperm progressive motility is necessary for the transit of spermatozoa through the female genital tract and fertilizing ova after ejaculation in the natural process of pregnancy. The epididymis performs is important in the maturation of spermatozoa, including their acquisition of progressive motility and fertilizing ability (36). DHT is the main androgen responsible for spermatozoan maturation in the epididymis (34). Seminal DHT is of primarily epididymal origin (37). Thus, the activity of SRD5A2 may be associated with sperm motility. A study in rats on epididymal sperm maturation has shown that the number of motile sperm is reduced following treatment with dual 5α-reductase inhibitor (38). This indicates the association between SRD5A2 activity and sperm motility.

The majority of the male pseudohermaphrodites with inherited SRD5A2 enzyme deficiency, and thereby decreased DHT production, have shown a low total sperm count,

Table II. Sperm parameters according to the genetic variants of the *SRD5A2* gene in 708 males with definite idiopathic infertility.

Variant	Genotype	N (%)	Semen volume (ml)	Concentration[a]	Sperm number per ejaculate[a]	Motility (%)
rs4952197						
	GG	228 (32.20)	3.40±1.45	3.63±1.11	4.78±1.23	52.75±25.27
	GA	340 (48.02)	3.40±1.41	3.66±1.20	4.80±1.29	51.57±24.38
	AA	140 (19.77)	3.47±1.42	3.54±1.23	4.70±1.33	49.38±22.71
	GA+AA	480 (67.80)	3.42±1.41	3.63±1.21	4.77±1.30	50.93±23.90
rs2268797						
	CC	299 (42.65)	3.37±1.37	3.55±1.21	4.69±1.30	50.86±23.58
	CT	301 (42.94)	3.41±1.38	3.69±1.14	4.85±1.24	51.15±24.85
	TT	101 (14.41)	3.51±1.66	3.65±1.18	4.79±1.33	54.15±25.25
	CT+TT	402 (57.35)	3.44±1.45	3.68±1.15	4.83±1.26	51.91±24.95
rs13395648						
	TT	583 (84.01)	3.46±1.44	3.62±1.19	4.78±1.30	51.88±24.40
	TC	97 (13.98)	3.07±1.32[b]	3.62±1.10	4.68±1.16	49.78±25.36
	CC	14 (2.20)	3.34±1.09	3.38±1.26	4.54±1.29	56.22±16.00
	TC+CC	111 (15.99)	3.10±1.29[c]	3.60±1.12	4.66±1.17	50.60±24.42
rs523349						
	CC	133 (19.44)	3.29±1.52	3.68±1.13	4.77±1.24	53.17±25.27
	CG	275 (40.20)	3.47±1.36	3.58±1.14	4.75±1.26	49.45±24.51
	GG	276 (40.35)	3.42±1.38	3.65±1.22	4.80±1.32	51.81±23.79
	CG+GG	551 (80.56)	3.44±1.37	3.61±1.19	4.78±1.29	50.63±24.16
rs632148						
	GG	132 (18.88)	3.27±1.34	3.70±1.10	4.80±1.20	55.60±24.29
	GC	307 (43.92)	3.50±1.49	3.61±1.16	4.79±1.26	49.47±24.82[d]
	CC	260 (37.20)	3.39±1.39	3.62±1.23	4.77±1.33	52.32±23.73[e]
	GC+CC	567 (81.12)	3.45±1.44	3.62±1.19	4.78±1.29	50.78±24.35[f]

P-values were adjusted for age, smoking, drinking, body mass index (BMI) and the duration of sexual abstinence (Abs). Values are the means ± SD unless otherwise stated. [a]Values are re-transformed following logarithmic transformation. [b]P<0.05 compared to rs13395648 TC and TT genotypes (P=0.016). [c]P<0.05 compared to rs13395648 TC + CC and TT genotypes (P=0.020). [d]P<0.05 compared to rs632148 GC and GG genotypes (P=0.029). [e]P=0.078 compared to rs632148 CC and GG genotypes. [f]P<0.05 compared to rs632148 GC + CC and GG genotypes (P=0.033).

although a few of them maintain normal sperm concentration and motility (39). The *A49T* mutation was associated with a significantly higher sperm concentration (29). Both of these findings indicate the role of SRD5A2 on spermatogenesis. However, we found no association between any studied polymorphism and sperm number or concentration. This observation may be explained by the fact that lower enzyme activities moderately altered by the *SRD5A2* polymorphisms studied may be sufficient for normal spermatogenesis (30), although they affected semen parameters dependent on prostate or epididymis functions, such as semen volume or sperm motility.

A previous study on finasteride and dutasteride revealed a small but significant reduction in semen volume and sperm motility in normal men during treatment and follow-up (40), indicating a role of SRD5A2 activity in producing seminal plasma and gaining sperm motility. Nevertheless, the *SRD5A2* polymorphisms have shown an association with seminal volume and sperm motility in Han-Chinese populations with definite idiopathic infertility in our study. However, our results should be interpreted with caution since there is no clear mechanism explaining the differentiation of *SRD5A2* expression in the testis, epididymis, prostate and other reproductive tissue, as well as how genetic variants associated with moderately altered enzyme activity or intronic polymorphisms with unknown function would affect semen quality.

We did not collect data to analyze reproductive hormones, but results of the studies by Elzanaty *et al* and Peters *et al* did not show any correlation between *SRD5A2* allelic variants and the serum levels of reproductive hormones (29,30). Moreover, the treatment of healthy men with 5α-reductase inhibitors did not cause significant changes in their serum gonadotropin levels (40). Thus, it is supposed that the studied polymorphisms or 5α-reductase inhibitors affect the concentrations of DHT in the microenvironment but not in the serum, and are unlikely to affect the serum levels of other reproductive hormones.

In conclusion, this study, to the best of our knowledge, is the first to examine the association of *SRD5A2* genetic

variants with semen quality in a Han-Chinese population with idiopathic male infertility. Our data suggest that the *SRD5A2* genetic variants rs13395648 and rs642138 are associated with seminal volume and sperm motility, respectively. The discrepancies in semen quality characteristics between the genotypes of some variants remain unclear. Thus, larger sample size studies and *in vivo* or *in vitro* functional studies are required to confirm the findings of this study.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (grant no. 30973199) and the National Eleventh-Five Science and Technology Support Program of China (grant no. 2006BAI03B12).

References

1. Krausz C and Forti G: Clinical aspects of male infertility. Results Probl Cell Differ 28: 1-21, 2000.
2. Huynh T, Mollard R and Trounson A: Selected genetic factors associated with male infertility. Hum Reprod Update 8: 183-198, 2002.
3. Jamsai D, Reilly A, Smith SJ, *et al*: Polymorphisms in the human cysteine-rich secretory protein 2 (CRISP2) gene in Australian men. Hum Reprod 23: 2151-2159, 2008.
4. Moore HD and Akhondi MA: In vitro maturation of mammalian spermatozoa. Rev Reprod 1: 54-60, 1996.
5. Viger RS and Robaire B: Steady state steroid 5 alpha-reductase messenger ribonucleic acid levels and immunocytochemical localization of the type 1 protein in the rat testis during postnatal development. Endocrinology 136: 5409-5415, 1995.
6. Steinberger E: Hormonal control of mammalian spermatogenesis. Physiol Rev 51: 1-22, 1971.
7. Sultan C, Lumbroso S, Paris F, *et al*: Disorders of androgen action. Semin Reprod Med 20: 217-228, 2002.
8. Makridakis NM, di Salle E and Reichardt JK: Biochemical and pharmacogenetic dissection of human steroid 5 alpha-reductase type II. Pharmacogenetics 10: 407-413, 2000.
9. Debes JD and Tindall DJ: Mechanisms of androgen-refractory prostate cancer. N Engl J Med 351: 1488-1490, 2004.
10. Can S, Zhu YS, Cai LQ, *et al*: The identification of 5 alpha-reductase-2 and 17 beta-hydroxysteroid dehydrogenase-3 gene defects in male pseudohermaphrodites from a Turkish kindred. J Clin Endocrinol Metab 83: 560-569, 1998.
11. Fernandez-Cancio M, Nistal M, Gracia R, *et al*: Compound heterozygous mutations in the SRD5A2 gene exon 4 in a male pseudohermaphrodite patient of Chinese origin. J Androl 25: 412-416, 2004.
12. Chavez B, Valdez E and Vilchis F: Uniparental disomy in steroid 5alpha-reductase 2 deficiency. J Clin Endocrinol Metab 85: 3147-3150, 2000.
13. Makridakis N, Ross RK, Pike MC, *et al*: A prevalent missense substitution that modulates activity of prostatic steroid 5alpha-reductase. Cancer Res 57: 1020-1022, 1997.
14. Allen NE, Forrest MS and Key TJ: The association between polymorphisms in the CYP17 and 5alpha-reductase (SRD5A2) genes and serum androgen concentrations in men. Cancer Epidemiol Biomarkers Prev 10: 185-189, 2001.
15. Hayes VM, Severi G, Padilla EJ, *et al*: 5alpha-reductase type 2 gene variant associations with prostate cancer risk, circulating hormone levels and androgenetic alopecia. Int J Cancer 120: 776-780, 2007.
16. Makridakis NM, Ross RK, Pike MC, *et al*: Association of mis-sense substitution in SRD5A2 gene with prostate cancer in African-American and Hispanic men in Los Angeles, USA. Lancet 354: 975-978, 1999.
17. Ross RK, Pike MC, Coetzee GA, *et al*: Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. Cancer Res 58: 4497-4504, 1998.
18. Ji G, Gu A, Xia Y, *et al*: ERCC1 and ERCC2 polymorphisms and risk of idiopathic azoospermia in a Chinese population. Reprod Biomed Online 17: 36-41, 2008.
19. Osborne EC, Lynch M, McLachlan R, Trounson AO and Cram DS: Microarray detection of Y chromosome deletions associated with male infertility. Reprod Biomed Online 15: 673-680, 2007.
20. Larsen L, Scheike T, Jensen TK, *et al*: Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. The Danish First Pregnancy Planner Study Team. Hum Reprod 15: 1562-1567, 2000.
21. Barrett JC, Fry B, Maller J and Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263-265, 2005.
22. Menkveld R, Wong WY, Lombard CJ, *et al*: Semen parameters, including WHO and strict criteria morphology, in a fertile and subfertile population: an effort towards standardization of in-vivo thresholds. Hum Reprod 16: 1165-1171, 2001.
23. Payne AH, Kawano A and Jaffe RB: Formation of dihydrotestosterone and other 5 alpha-reduced metabolites by isolated seminiferous tubules and suspension of interstitial cells in a human testis. J Clin Endocrinol Metab 37: 448-453, 1973.
24. Lindstrom S, Adami HO, Balter KA, *et al*: Inherited variation in hormone-regulating genes and prostate cancer survival. Clin Cancer Res 13: 5156-5161, 2007.
25. Ntais C, Polycarpou A and Ioannidis JP: SRD5A2 gene polymorphisms and the risk of prostate cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 12: 618-624, 2003.
26. Cicek MS, Conti DV, Curran A, *et al*: Association of prostate cancer risk and aggressiveness to androgen pathway genes: SRD5A2, CYP17, and the AR. Prostate 59: 69-76, 2004.
27. Salam MT, Ursin G, Skinner EC, Dessissa T and Reichardt JK: Associations between polymorphisms in the steroid 5-alpha reductase type II (SRD5A2) gene and benign prostatic hyperplasia and prostate cancer. Urol Oncol 23: 246-253, 2005.
28. Pearce CL, Makridakis NM, Ross RK, *et al*: Steroid 5-alpha reductase type II V89L substitution is not associated with risk of prostate cancer in a multiethnic population study. Cancer Epidemiol Biomarkers Prev 11: 417-418, 2002.
29. Elzanaty S, Giwercman YL and Giwercman A: Significant impact of 5alpha-reductase type 2 polymorphisms on sperm concentration and motility. Int J Androl 29: 414-420, 2006.
30. Peters M, Saare M, Kaart T, *et al*: Analysis of polymorphisms in the SRD5A2 gene and semen parameters in Estonian men. J Androl 31: 372-378, 2010.
31. Mahony MC, Swanlund DJ, Billeter M, Roberts KP and Pryor JL: Regional distribution of 5alpha-reductase type 1 and type 2 mRNA along the human epididymis. Fertil Steril 69: 1116-1121, 1998.
32. Berthaut I, Mestayer C, Portois MC, Cussenot O and Mowszowicz I: Pharmacological and molecular evidence for the expression of the two steroid 5 alpha-reductase isozymes in normal and hyperplastic human prostatic cells in culture. Prostate 32: 155-163, 1997.
33. Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD and Russell DW: Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression. J Clin Invest 92: 903-910, 1993.
34. Robaire B and Viger RS: Regulation of epididymal epithelial cell functions. Biol Reprod 52: 226-236, 1995.
35. Mahendroo MS, Cala KM, Hess DL and Russell DW: Unexpected virilization in male mice lacking steroid 5 alpha-reductase enzymes. Endocrinology 142: 4652-4662, 2001.
36. Cornwall GA: New insights into epididymal biology and function. Hum Reprod Update 15: 213-227, 2009.
37. Anderson RA, Kelly RW and Wu FC: Comparison between testosterone enanthate-induced azoospermia and oligozoospermia in a male contraceptive study. V. Localization of higher 5 alpha-reductase activity to the reproductive tract in oligozoospermic men administered supraphysiological doses of testosterone. J Androl 18: 366-371, 1997.
38. Henderson NA and Robaire B: Effects of PNU157706, a dual 5alpha-reductase inhibitor, on rat epididymal sperm maturation and fertility. Biol Reprod 72: 436-443, 2005.
39. Cai LQ, Fratianni CM, Gautier T and Imperato-McGinley J: Dihydrotestosterone regulation of semen in male pseudohermaphrodites with 5 alpha-reductase-2 deficiency. J Clin Endocrinol Metab 79: 409-414, 1994.
40. Amory JK, Wang C, Swerdloff RS, *et al*: The effect of 5alpha-reductase inhibition with dutasteride and finasteride on semen parameters and serum hormones in healthy men. J Clin Endocrinol Metab 92: 1659-1665, 2007.