Association of *TUSC1* and *DPF3* gene polymorphisms with male infertility

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Abstract

Purpose Recently, a genome-wide association studies of a Hutterite population in the USA revealed that five single nucleotide polymorphisms (SNPs) with a significant association with sperm quality and/or function in ethnically diverse men from Chicago were significantly correlated with family size. Of these, three SNPs (rs7867029, rs7174015, and rs12870438) were found to be significantly associated with the risk of azoospermia and/or oligozoospermia in a Japanese population. In this study, we investigated whether the rs10966811 (located in an intergenic region between the *TUSC1* and *IZUMO3* genes) and rs10129954 (located in the *DPF3* gene) SNPs, previously related to family size, are associated with male infertility. In addition, we performed association analysis between rs12348 in *TUSC1* and rs2772579 in *IZUMO3* and male infertility.

Methods We genotyped 145 patients with infertility (including 83 patients with azoospermia, and 62 with oligozoospermia) and 713 fertile controls by PCR-RFLP technique for polymorphism. Because rs10966811 has no restriction sites, the SNP rs12376894 with strong linkage disequilibrium was selected as an alternative to rs10966811.

Results There was a statistically significant association between rs12376894 proxy SNP of rs10966811, and oligozoospermia. A statistically significant association between rs10129954 and azoospermia, and oligozoospermia were observed. When we assessed the relationship between rs12348 in *TUSC1* and rs2772579 in *IZUMO3* and male infertility traits, we found that rs12348 in *TUSC1* was significantly associated with azoospermia and oligozoospermia, but rs2772579 in *IZUMO3* was not associated with male infertility.

Conclusion We found that the polymorphisms in *TUSC1* and *DPF3* displayed strong associations with male infertility.

Keywords: single nucleotide polymorphism · male infertility · azoospermia · oligozoospermia · tumor suppressor candidate 1 · double plant homeodomain fingers, family 3

Introduction

The main cause of male infertility is spermatogenic failure, of which there are three general classifications: azoospermia, oligozoospermia, and asthenozoospermia. It is thought that lifestyle factors such as cigarette smoking and alcohol abuse, as well as environmental factors such as exposure to certain chemicals are potential causes of spermatogenic failure [1]. In addition, genetic factors such as gene mutations and chromosomal aberrations can also be related to male infertility. Previously, three genome-wide association studies (GWASs) revealed that several single nucleotide polymorphisms (SNPs) were associated with male infertility including azoospermia and/or oligozoospermia [2–4]. Another GWAS, including 269 members of a Hutterite population in the USA, showed that of 41 SNPs associated with family size or birth rate, nine were also associated with reduced sperm quality and/or function in ethnically diverse men from Chicago [5]. Recently, we conducted a case-control association study to assess whether four SNPs, associated with semen parameters in men from Chicago with minor allele frequencies > 0.05 in the HapMap-JPT population, are associated with infertility (including azoospermia and oligozoospermia) in Japanese males. The SNPs assessed and their locations were as follows: rs7867029 (downstream of phosphoserine aminotransferase 1 [*PSAT1*], rs7174015 (in the ubiquitin specific peptidase 8 [*USP8*] gene, rs12870438 (in the epithelial stromal interaction protein 1 [*EPSTI1*] gene, and rs724078 (upstream of MAS1 oncogene-like protein [*MAS1L*] and downstream of ubiquitin D [*UBD*]). We found that rs7867029, rs7174015, and rs12870438 were significantly associated with the risk of azoospermia and/or oligozoospermia [6]. These SNPs were previously related to family size in the Hutterite GWAS. Conversely, rs724078, which was not associated with male infertility, was associated with birth rate in the

previous GWAS. From this data, we predicted that other SNPs, in which significant association with family size was indicated in the previous Hutterite GWAS, may also be related to male infertility in the Japanese population. Of nine SNPs associated with reduced sperm quality and/or function in the men from Chicago, five SNPs were associated with family size in the previous GWAS [5]. These included rs10966811 (downstream of the tumor suppressor candidate 1 [*TUSC1*] gene) and rs10129954 (located in the D4, zinc and double plant homeodomain fingers, family 3 [*DPF3*] gene), as well as three SNPs (rs7867029, rs7174015 and rs12870438), which were analyzed previously [6]. In this study, we assessed whether rs10966811 and rs10129954 are associated with male infertility including azoospermia, and oligozoospermia.

Materials and Methods

Subjects and clinical trait measurements

In total, 145 consecutive patients who presented with infertility at the Department of Urology, St. Mariana University Hospital, Kanagawa Prefecture, Japan, were recruited from 2000 to 2011. Of these patients, 83 (aged 33.0 ± 5.6 years; mean ± SD) were diagnosed as having azoospermia, and 62 (aged 34.8 ± 6.2 years; mean ± SD) were diagnosed as having oligozoospermia. Some of the individuals in this study and diagnostic criteria have been described in previous reports [6–8]. Briefly, semen analysis was performed in accordance with the fourth edition of World Health Organization (WHO) Laboratory Manual for the Examination of Human Semen [9]. According to the criteria described in the fourth edition WHO guidelines (1999), patients with azoospermia were diagnosed on the basis of semen analysis (absence of sperm in ejaculate), serum hormone levels, and the results of physical examinations. Oligozoospermia was defined as a sperm concentration of less than 20×10^6/mL. We excluded patients with any other known cause of infertility (i.e., obstructive azoospermia, varicocele, cryptorchidism, hypogonadotropic hypogonadism, karyotype abnormalities, or complete deletion of *AZF* a, b, or c). Deletions in *AZF* a, b, and c were analyzed according to European Academy of Andrology and the European Molecular Genetics Quality Network best practice guidelines [10].

The 713 fertile Japanese men (aged 31.1 ± 4.8 years; mean ± SD) used as the control group were the partners of pregnant women who attended obstetric clinics in four cities in Japan (Sapporo, Kanazawa, Osaka, and Fukuoka). Some of the fertile individuals in this study and the eligibility criteria for participants have been described in previous reports [11–15]. Briefly, the eligibility criteria for the male participants were as follows:

age 20–45 years at the time of invitation by the hospital, and that both the participant and his mother were born in Japan and currently living there as well. In addition, the pregnancy of their partner had to have been the result of conception by sexual intercourse and not through fertility treatment.

SNP selection and genotyping

For the association analysis, the SNP rs12376894 was selected as an alternative to rs10966811, because rs10966811 has no restriction sites, and rs10966811 and rs12376894 are in strong linkage disequilibrium (LD) (r^2=0.91, |*D'*|=1), according to data from JPT in 1000 genomes phase 1 release version 3 dataset [16]. rs12348 and rs2772579, located in *TUSC1* and IZUMO family member 3 (*IZUMO3*), respectively, were selected as tag SNPs using SNPinfo Web Server (http://snpinfo.niehs.nih.gov/) [17], owing to their strong LD ($r^2 > 0.8$), minor allele frequency > 0.05, and a minimum of two SNPs tagged each.

Genomic DNA was extracted from the peripheral blood samples of subjects using a QIAamp DNA blood kit (Qiagen; Tokyo, Japan) as previously described [6–8, 12–15]. The rs12376894, rs10129954, rs12348, and rs2772579 SNPs were detected by restriction fragment length polymorphism PCR using the following primer sets: rs12376894, 5'-CGGTGTAGAAAATGCGACCAA-3' (forward) and 5'-ATCACCCCATCGTCAGTCAT-3' (reverse); rs10129954, 5'-TTGGCTCTGAACCCAAGCAA -3' (forward) and 5'-TGATTGGTCTCCCAGCCTCT-3' (reverse); rs12348, 5'-TCGCCGCAACCTTTCAGATA-3' (forward) and 5'-CCGCGGATACCTTGGACTAC-3' (reverse); and rs2772579, 5'-GCGGGAAAAGTGGAGTCAGA-3' (forward) and 5'-

AGATTTCCCAGCAAGGAGCC-3' (reverse). DNA from each subject was amplified using EmeraldAmp MAX PCR Master Mix (TaKaRa Bio Inc., Otsu, Japan) under the following PCR cycling parameters: initial denaturation at 94°C for 3 min; 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min; and final extension for 3 min at 72°C. The resulting PCR products were then digested at 37°C for 3 hours using the following restriction enzymes: EarI (rs12376894), HpyCH4IV (rs10129954), PvuII (rs12348), and Hpy166II (rs2772579); all of which were sourced from New England Biolabs Japan Inc., Tokyo, Japan. The digested products were separated by electrophoresis on 2.5% agarose gel. The following fragment sizes were used for allele identification on gels: for rs12376894, 594 bp (A-allele) and 287 + 307 bp (G-allele); for rs10129954, 738 bp (T-allele) and 365 + 373 bp (C-allele); for rs12348, 633 bp (T-allele) and 269 + 364 bp (C-allele); and for rs2772579, 250 bp (G-allele) and 74 + 176 bp (C-allele).

Statistical analysis

The fertile cohort was used as the control, and odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using logistic regression analysis. All statistical analyses were performed using R version 3.1.2 (The R Project for Statistical Computing [http://www.r-project.org]), and statistical significance was declared at *P*-value < 0.05. Hardy–Weinberg equilibrium (HWE) was assessed in both patients with infertility and fertile control samples with a threshold of 0.05 by using Pearson chi-square test for genotypes. LD was calculated using PLINK version 1.07 [18] with JPT in 1000 genomes phase 1 release version 3 dataset [16] or genotyped fertile control data in this study.

Results

The genotype and allele frequencies of rs12376894 and rs10129954 SNPs among 713 fertile controls and 83 patients with azoospermia, and 62 with oligozoospermia are summarized in Table 1. Two SNPs were in HWE in the both patients with infertility and fertile controls.

To verify whether rs12376894 and rs10129954 SNPs are associated with male infertility traits, we carried out a case-control study using a logistic regression analysis under three comparative genetic models (additive, recessive, or dominant). The results of the logistic regression analysis are summarized in Table 2. There was a statistically significant association between rs12376894 and oligozoospermia in the recessive model (OR = 2.03, 95% CI = 1.17–3.52, P = 0.012). rs10129954 displayed significant association with azoospermia in all three models: log-additive (OR = 2.05, 95% CI = 1.21–3.46, $P = 7.4 \times 10^{-3}$), recessive (OR = 17.60, 95% CI = 1.58–196.00, P = 0.020), and dominant (OR = 1.92, 95% CI = 1.09–3.38, P = 0.025); and with oligozoospermia in two models: log-additive (OR = 2.83, 95% CI = 1.61–4.97, $P = 2.9 \times 10^{-4}$), and dominant (OR = 2.83, 95% CI = 1.57–5.11, $P = 5.6 \times 10^{-4}$).

rs10129954 is located in an intronic region of *DPF3*. Therefore, DPF3 protein may affect spermatogenesis, although there is no evidence. rs12376894 is located in an intergenic region between *TUSC1* and *IZUMO3*. Then, we speculate that TUSC1 or IZUMO3 may be related to male infertility. We performed a further association analysis between *TUSC1* and *IZUMO3* polymorphisms and male infertility; rs12348 and rs2772579 were selected as tag SNPs respectively, according to the pairwise LD measurement. rs12348 is located in a 3' untranslated region (UTR) of *TUSC1*, and rs2772579 is located in a 5' UTR of *IZUMO3*. The genotype and allele frequencies of

rs12348 and rs2772579 among 713 fertile controls, as well as 83 patients with azoospermia, and 62 with oligozoospermia are summarized in Table 3. These SNPs were in HWE in the both patients with infertility and fertile control group. Using a logistic regression analysis, a statistically significant association between rs12348 and azoospermia was observed in the recessive model (OR = 1.82, 95% CI = 1.07–3.10, P = 0.028), and oligozoospermia in two models: log-additive (OR = 1.87, 95% CI = 1.29–2.71, $P = 8.8 \times 10^{-4}$), and recessive (OR = 3.16, 95% CI = 1.82–5.50, $P = 4.5 \times 10^{-5}$) (Table 4). However, no association was found between rs2772579 and male infertility traits in three genetic models.

To understand the relationship between rs12376894 and rs12348, we performed a conditional logistic regression analysis for azoospermia, and oligozoospermia adjusted for each SNP. The strength of association between two SNPs and azoospermia or oligozoospermia was not reduced (Table 5). Additionally, we calculated the pairwise LD between rs12376894 and rs12348 using genotyped fertile control data in this study Pairwise r^2 values were low ($r^2 = 0.005$). Therefore, it is suggested that rs12376894 and rs12348 are independently related to azoospermia and/or oligozoospermia.

Discussion

In this study, we identified that the SNPs rs12376894 and rs10129954, which have been associated with family size in a genome-wide association study (GWAS) of a Hutterite population in the USA [5], were significantly correlated with azoospermia, and/or oligozoospermia in a Japanese population. rs12376894 is located 445.4 kb downstream of *TUSC1* and 687.8 kb upstream of *IZUMO3*. Therefore, we speculate that TUSC1 or IZUMO3 may be contribute to spermatogenesis, and assessed whether *TUSC1* and *IZUMO3* polymorphisms are related to male infertility. We found that rs12348 in *TUSC1* was associated with azoospermia and oligozoospermia; an especially strong association was identified in the recessive model (TT versus TT+CC). However, rs2772579 in *IZUMO3* was not associated with male infertility. *TUSC1* has been suggested as a candidate tumor suppressor gene in several cancers [19–23], and it is reported that variation in *TUSC1* and the surrounding region is associated with risk of cancers [24, 25]. It is also reported that *TUSC1* variants are related to tanning ability [24], supernumerary teeth occurrence [26], amongst other traits. However, no evidence that *TUSC1* contributes to spermatogenesis has yet been published. *TUSC1* is an intronless gene, and major transcripts TUSC1-L (2.0-kb long) and TUSC1-S (1.5-kb long) have been detected in a wide range of human adult tissues [19]. Interestingly, two transcripts were most strongly expressed in human testes and another 1.1-kb transcript has been detected only in this organ [19]. Similarly, in mouse tissues, TUSC1-S was most strongly expressed in the testis [19]. Therefore, TUSC1 may have an important function in the testis. On the other hand, IZUMO3 was also strongly expressed in the testis. However, IZUMO3 is essential for the function of the gamete fusion rather than spermatogenesis [27].

rs12376894, which displayed significant association with oligozoospermia, is located 445.4 kb downstream of *TUSC1*. According to LD and conditional logistic regression analysis adjusted for each SNP, rs12376894 and rs12348 are individually related to male infertility. It is known that SNPs within non-coding regions (intronic, intergenic, etc.) may influence expression regulation of genes both local to and distant from the locus [28]. Though significant expression quantitative trait loci (eQTL) of rs12376894 were not found in the GTEx Portal database [29], this SNP may contribute to the regulation of *TUSC1* expression.

Liu *et al*. reported that rs10129954 is strongly associated with idiopathic male infertility (asthenozoospermia, oligozoospermia, and oligoasthenozoospermia) in Chinese Han people [30]. In the current study, we found a similar relationship in a Japanese population. rs10129954 is located in an intronic region of *DPF3*. DPF3 protein is a member of the BRG1-associated factor (BAF) chromatin remodeling complex (SWI/SNF-like complex), with binding acetylated and methylated H3 and H4 lysine residues, and regulates the transcription during heart and muscle development [31]. It is known that the SWI/SNF chromatin remodeling complex has an important role throughout spermatogenesis [32, 33], especially during male meiosis [34]. *DPF3* is strongly expressed in the human testis as well as in the ovaries, heart, and brain [35]. It is suggested that DPF3 may contribute to spermatogenesis via chromatin remodeling. However, it is unclear whether the intronic variant rs10129954 influences DPF3.

In conclusion, we found that polymorphisms in *TUSC1* and *DPF3* displayed strong associations with male infertility. The effect of these polymorphisms on spermatogenesis remains unknown. To study this, functional analyses using genetically modified animals are required. In addition, in this study, the sample size of case subject

was not very large. Therefore, further studies with larger sample size are also required to achieve the sufficient statistical power.

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Compliance with ethical standards

This study was approved by the ethics committees of the University of Tokushima and St. Marianna Medical University. All participants provided written informed consent.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J. The role of oxidative stress and antioxidants in male fertility. Cent European J Urol. 2013;66:60–7.

2. Aston KI, Carrell DT. Genome-wide study of single-nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. J Androl. 2009;30:711–25.

3. Hu Z, Xia Y, Guo X, Dai J, Li H, Hu H, et al. A genome-wide association study in Chinese men identifies three risk loci for non-obstructive azoospermia. Nat Genet. 2011;44:183–6.

4. Zhao H, Xu J, Zhang H, Sun J, Sun Y, Wang Z, et al. A genome-wide association study reveals that variants within the HLA region are associated with risk for nonobstructive azoospermia. Am J Hum Genet. 2012;90:900–6.

5. Kosova G, Scott NM, Niederberger C, Prins GS, Ober C. Genome-wide association study identifies candidate genes for male fertility traits in humans. Am J Hum Genet. 2012;90:950–61.

6. Sato Y, Tajima A, Tsunematsu K, Nozawa S, Yoshiike M, Koh E, et al. An association study of four candidate loci for human male fertility traits with male infertility. Hum Reprod. 2015;30:1510–4.

7. Sato Y, Shinka T, Iwamoto T, Yamauchi A, Nakahori Y. Y chromosome haplogroup

D2* lineage is associated with azoospermia in Japanese males. Biol Reprod. 2013;88:107.

8. Sato Y, Jinam T, Iwamoto T, Yamauchi A, Imoto I, Inoue I, et al. Replication study and meta-analysis of human non-obstructive azoospermia in Japanese populations. Biol Reprod. 2013;88:87.

9. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction, 4th edn. Cambridge, Cambridge University Press. 1999.

10. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. Int J Androl. 2004;27:240–9.

11. Iwamoto T, Nozawa S, Yoshiike M, Namiki M, Koh E, Kanaya J, et al. Semen quality of fertile Japanese men: a cross-sectional population-based study of 792 men. BMJ Open. 2013;3:e002223.

12. Sato Y, Iwamoto T, Shinka T, Nozawa S, Yoshiike M, Koh E, et al. Y chromosome gr/gr subdeletion is associated with lower semen quality in young men from the general Japanese population but not in fertile Japanese men. Biol Reprod. 2014;90:116.

13. Sato Y, Shinka T, Nozawa S, Yoshiike M, Koh E, Kanaya J, et al. Y chromosome haplogroup D2a1 is significantly associated with high levels of luteinizing hormone in

Japanese men. Andrology. 2015;3:520–5.

14. Sato Y, Tajima A, Tsunematsu K, Nozawa S, Yoshiike M, Koh E, et al. Lack of replication of four candidate SNPs implicated in human male fertility traits: a large-scale population-based study. Hum Reprod. 2015;30:1505–9.

15. Sato Y, Tajima A, Katsurayama M, Nozawa S, Yoshiike M, Koh E, et al. A replication study of a candidate locus for follicle-stimulating hormone levels and association analysis for semen quality traits in Japanese men. J Hum Genet. 2016;61:911–5.

16. 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012;491:56–65.

17. Xu Z, Taylor JA. SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res. 2009;37 (Web Server issue):W600–5.

18. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al
PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81:559–75.

19. Shan Z, Parker T, Wiest JS. Identifying novel homozygous deletions by microsatellite analysis and characterization of tumor suppressor candidate 1 gene, TUSC1, on chromosome 9p in human lung cancer. Oncogene. 2004;23:6612–20.

20. Shan Z, Shakoori A, Bodaghi S, Goldsmith P, Jin J, Wiest JS. TUSC1, a putative tumor suppressor gene, reduces tumor cell growth in vitro and tumor growth in vivo. PLoS One. 2013;8:e66114.

21. Shimizu D, Kanda M, Nomoto S, Oya H, Takami H, Hibino S, et al. Identification of intragenic methylation in the TUSC1 gene as a novel prognostic marker of hepatocellular carcinoma. Oncol Rep. 2014;31:1305–13.

22. Kanda M, Shimizu D, Nomoto S, Hibino S, Oya H, Takami H, et al. Clinical significance of expression and epigenetic profiling of TUSC1 in gastric cancer. J Surg Oncol. 2014;110:136–44.

23. Zhang R, Yu W, Liang G, Jia Z, Chen Z, Zhao L, et al. Tumor Suppressor Candidate 1 Suppresses Cell Growth and Predicts Better Survival in Glioblastoma. Cell Mol Neurobiol. 2017;37:37–42.

24. Yang XR, Liang X, Pfeiffer RM, Wheeler W, Maeder D, Burdette L, et al. Associations of 9p21 variants with cutaneous malignant melanoma, nevi, and pigmentation phenotypes in melanoma-prone families with and without CDKN2A mutations. Fam Cancer. 2010;9:625–33.

25. Li WQ, Pfeiffer RM, Hyland PL, Shi J, Gu F, Wang Z, et al. Genetic polymorphisms in the 9p21 region associated with risk of multiple cancers. Carcinogenesis. 2014;35:2698–705.

26. Takahashi M, Hosomichi K, Yamaguchi T, Yano K, Funatsu T, Adel M, et al. Whole-exome sequencing analysis of supernumerary teeth occurrence in Japanese individuals. Hum Genome Var. 2017;4:16046.

27. Ellerman DA, Pei J, Gupta S, Snell WJ, Myles D, Primakoff P. Izumo is part of a multiprotein family whose members form large complexes on mammalian sperm. Mol Reprod Dev. 2009;76:1188–99.

28. Farrall M. Quantitative genetic variation: a post-modern view. Hum Mol Genet. 2014;13:R1–7.

29. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. Nat Genet. 2013;45:580–5.

30. Liu SY, Zhang CJ, Peng HY, Sun H, Lin KQ, Huang XQ, et al. Strong association of SLC1A1 and DPF3 gene variants with idiopathic male infertility in Han Chinese. Asian J Androl. 2017;19:486–92.

31. Lange M, Kaynak B, Forster UB, Tönjes M, Fischer JJ, Grimm C, et al. Regulation

of muscle development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex. Genes Dev. 2008;22:2370–84.

32. Wang J, Gu H, Lin H, Chi T. Essential roles of the chromatin remodeling factor BRG1 in spermatogenesis in mice. Biol Reprod. 2012;86:186.

33. Serber DW, Runge JS, Menon DU, Magnuson T. The mouse INO80 chromatin-remodeling complex is an essential meiotic factor for spermatogenesis. Biol Reprod. 2016;94:8.

34. Kim Y, Fedoriw AM, Magnuson T. An essential role for a mammalian SWI/SNF chromatin-remodeling complex during male meiosis. Development. 2012;139:1133–40.

35. Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics. 2014;13:397–406.

Table 1. Genotype and allele frequencies of rs12376894 and rs10129954 SNPs in the subjects.

							Control		Case			
		Position	Closest				(n = 713)		Azoospermia (n = 83)		Oligozoospermia (n = 62)	
SNP	Chr	(NCBI Build 37)	Genes	Location	Dist. (kb)	Allele[a]	Genotypes[b]	AF[c]	Genotypes[b]	AF[c]	Genotypes[b]	AF[c]
rs12376894	9	25,268,867	*TUSC1*	dwnst.	445.4	A/G	152/364/197	0.47	13/45/25	0.43	22/25/15	0.56
			IZUMO3	upst.	687.8							
rs10129954	14	73,150,701	*DPF3*	intron	−	C/T	1/89/623	0.06	2/16/65	0.12	1/17/44	0.15

[a]Allele indicates control subject minor/major allele.

[b]Genotypes indicate control subject minor homozygote/heterozygote/major homozygote.

[c]AF indicates the allele frequency of the control subject minor alleles.

Abbreviations: Chr, chromosome; dwnst., downstream; upst., upstream; Dist., distance.

Gene names: *TUSC1*, tumor suppressor candidate 1; *IZUMO3*, IZUMO family member 3; *DPF3*, D4, zinc and double plant homeodomain fingers, family 3.

Table 2. The associations derived from different comparative genetic models (additive, recessive, and dominant) between two SNPs (rs12376894 and rs10129954) and azoospermia, and oligozoospermia.

		Azoospermia		Oligozoospermia	
SNP	Model	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs12376894	Log-additive *(Risk allele, A)*[a]	0.84 (0.61–1.17)	0.31	1.43 (0.99–2.07)	0.60
	Recessive *(AA vs. AG+GG)*	0.69 (0.37–1.27)	0.23	**2.03 (1.17–3.52)**	**0.012**
	Dominant *(AA+AG vs. GG)*	0.89 (0.54–1.46)	0.63	1.20 (0.65–2.19)	0.56
rs10129954	Log-additive *(Risk allele, C)*[a]	**2.05 (1.21–3.46)**	**7.4×10^{-3}**	**2.83 (1.61–4.97)**	**2.9×10^{-4}**
	Recessive *(CC vs. CT+TT)*	**17.60 (1.58–196.00)**	**0.020**	11.70 (0.72–189.00)	0.084
	Dominant *(CC+CT vs. TT)*	**1.92 (1.09–3.38)**	**0.025**	**2.83 (1.57–5.11)**	**5.6×10^{-4}**

Data are shown as odds ratio (OR), 95 % confidence interval (CI), and *P*-value.

Bold numbers indicate *P*-value < 0.05.

[a]Log-additive, additive model in log-odds scale.

Table 3. Allele and genotype frequencies of rs12348 in *TUSC1* and rs2772579 in *IZUMO3* in the study group.

SNP	Position (NCBI Build 37)	Genes	Function	Allele[a]	Control (n = 713)		Case			
							Azoospermia (n = 83)		Oligozoospermia (n = 62)	
					Genotypes[b]	AF[c]	Genotypes[b]	AF[c]	Genotypes[b]	AF[c]
rs12348	25,677,217	*TUSC1*	3'-UTR	T/C	112/345/256	0.40	21/37/25	0.48	23/23/16	0.56
rs2772579	24,545,695	*IZUMO3*	5'-UTR	G/C	28/250/435	0.21	1/25/57	0.16	3/21/38	0.22

[a]Allele indicates control subject minor/major allele.

[b]Genotypes indicate control subject minor homozygote/heterozygote/major homozygote.

[c]AF indicates the frequency of the control subject minor alleles.

Gene names: *TUSC1*, tumor suppressor candidate 1; *IZUMO3*, IZUMO family member 3.

Table 4. The associations derived from different comparative genetic models (additive, recessive, and dominant) between the SNPs, rs12348 (in *TUSC1*) and rs2772579 (in *IZUMO3*), and azoospermia and oligozoospermia.

		Azoospermia		Oligozoospermia	
SNP	Model	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs12348	Log-additive *(Risk allele, T)*[a]	1.37 (0.99–1.89)	0.058	**1.87 (1.29–2.71)**	**8.8×10^{-4}**
	Recessive *(TT vs. TC+CC)*	**1.82 (1.07–3.10)**	**0.028**	**3.16 (1.82–5.50)**	**4.5×10^{-5}**
	Dominant *(TT+TC vs. CC)*	1.30 (0.79–2.13)	0.30	1.61 (0.89–2.90)	0.11
rs2772579	Log-additive *(Risk allele, G)*[a]	0.70 (0.45–1.09)	0.11	1.02 (0.65–1.60)	0.93
	Recessive *(GG vs. GC+CC)*	0.30 (0.04–2.22)	0.24	1.24 (0.37–4.21)	0.73
	Dominant *(GG+GC vs. CC)*	0.71 (0.44–1.16)	0.18	0.99 (0.58–1.68)	0.97

Data are shown as odds ratio (OR), 95 % confidence interval (CI), and *P*-value.

Bold numbers indicate *P*-value < 0.05.

[a]Log-additive, additive model in log-odds scale.

Table 5. Conditional logistic regression analysis for azoospermia, and oligozoospermia under three genetic models (additive, recessive, and dominant).

SNP	Model	Azoospermia		Oligozoospermia	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs12376894	Log-additive *(Risk allele, T)*[a]	0.86 (0.62-1.19)	0.36	**1.47 (1.01–2.14)**	**0.042**
	Recessive *(TT vs. TC+CC)*	0.69 (0.37-1.28)	0.23	**2.10 (1.20–3.68)**	**9.2×10^{-3}**
	Dominant *(TT+TC vs. CC)*	0.90 (0.55-1.47)	0.67	1.23 (0.67–2.25)	0.50
rs12348	Log-additive *(Risk allele, T)*[a]	1.36 (0.98-1.87)	0.065	**1.91 (1.32–2.77)**	**6.4×10^{-4}**
	Recessive *(TT vs. TC+CC)*	**1.82 (1.06-3.10)**	**0.029**	**3.24 (1.85–5.66)**	**3.6×10^{-5}**
	Dominant *(TT+TC vs. CC)*	1.29 (0.79-2.12)	0.31	1.63 (0.90–2.94)	0.11

Data are shown as odds ratio (OR), 95 % confidence interval (CI), and *P*-value with adjusted with each SNPs.

Bold numbers indicate *P*-value < 0.05.

[a]Log-additive, additive model in log-odds scale.