An association study of four candidate loci for human male fertility traits with male infertility

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Running title: Candidate polymorphisms associate with male infertility

Abstract

STUDY QUESTION: Are the four candidate loci (rs7867029, rs7174015, rs12870438, and rs724078) for human male fertility traits, identified in a genome-wide association study (GWAS) of a Hutterite population in the USA, associated with male infertility in a Japanese population?

SUMMARY ANSWER: rs7867029, rs7174015, and rs12870438 are significantly associated with the risk of male infertility in a Japanese population.

WHAT IS KNOWN ALREADY: Recently, a GWAS of a Hutterite population in the USA revealed that 41 single nucleotide polymorphisms (SNPs) were significantly correlated with family size or birth rate. Of these, four SNPs (rs7867029, rs7174015, rs12870438, and rs724078) were found to be associated with semen parameters in ethnically diverse men from Chicago.

STUDY DESIGN, SIZE, DURATION: This is a case-control association study in a total of 917 Japanese subjects, including 791 fertile men, 76 patients with azoospermia, and 50 patients with oligozoospermia.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Azoospermia was diagnosed on the basis of semen analysis (absence of sperm in ejaculate), serum hormone levels, and physical examinations. Oligozoospermia was defined as a sperm concentration of less than 20×10^6/mL. We excluded patients with any known cause of infertility (i.e., obstructive azoospermia, varicocele, cryptorchidism, hypogonadotropic hypogonadism, karyotype abnormalities, or complete deletion of *AZF* a, b, or c). The SNPs rs7867029, rs7174015, rs12870438, and rs724078 were genotyped using DNA from peripheral blood samples and either restriction fragment length polymorphism PCR or TaqMan probes. Genetic associations between the four SNPs and male

infertility were assessed using a logistic regression analysis under three different comparative models (additive, recessive, or dominant)

MAIN RESULTS AND THE ROLE OF CHANCE: The genotypes of all four SNPs were in HWE in the fertile controls. The SNPs rs7867029 and rs7174015 are associated with oligozoospermia (rs7867029: odds ratio [OR] = 1.70, 95% confidence interval [CI] = 1.07–2.68, *P* = 0.024 [log-additive]; rs7174015: OR = 6.52, 95% CI = 1.57–27.10, *P* = 0.0099 [dominant]), and rs12870438 is associated with azoospermia (OR = 10.90, 95% CI = 2.67-44.60, *P* = 0.00087 [recessive]) and oligozoospermia (OR = 8.54, 95% CI = 1.52-47.90, *P* = 0.015 [recessive]). The association between rs7174015 and oligozoospermia under a dominant model and between rs12870438 and azoospermia under additive and recessive models remained after correction for multiple testing. There were no associations between rs724078 and azoospermia or oligozoospermia.

LIMITATIONS, REASONS FOR CAUTION: Even though the sample size of case subjects was not very large, we found that three SNPs were associated with the risk of male infertility in a Japanese population.

WIDER IMPLICATIONS OF THE FINDINGS: The three infertility-associated SNPs may be contributing to a quantitative reduction in spermatogenesis.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported in part by the Ministry of Health and Welfare of Japan (1013201) (to T. I.), Grant-in-Aids for Scientific Research (C) (23510242) (to A.T.) from the Japan Society for the Promotion of Science, the European Union (BMH4-CT96-0314) (to T. I.), and the Takeda Science Foundation (to A.T.). None of the authors has any competing interests to declare.

Keywords: case-control association study/ male infertility/ azoospermia/ oligozoospermia/ Japanese population

Introduction

Infertility is a major problem worldwide that affects approximately 10% of couples, and 40–50% of these problems are due to male-factor etiology (Skakkebaek *et al*., 1994; McLachlan and Kretser, 2001; Maduro and Lamb, 2002). The main cause of male infertility is spermatogenic failure such as azoospermia and oligozoospermia. In terms of the genetic background underlying male infertility, deletion of the three azoospermia factor (*AZF*) regions (termed *AZF*a, b, and c) of the long arm of the Y chromosome (Yq) has been detected in 10–15% of men with nonobstructive azoospermia or severe oligozoospermia (Vogt *et al*., 1996; Vogt, 1998; Krausz and McElreavey, 1999; Maurer and Simoni, 2000; McElreavey *et al*., 2000). Aside from the genes in the Y chromosome, polymorphisms in certain genes, such as those encoding glutathione S-transferases (Pajarinen *et al*., 1996; Chen *et al*., 2002; Finotti *et al*., 2009; Polonikov *et al*., 2010), 5-methylenetetrahydrofolate reductase (Bezold *et al*., 2001; Park *et al*., 2005; Singh *et al*., 2005), and ADP-ribosyltransferase 3 (Okada *et al*., 2008; Norambuena *et al*., 2012), have been reported to be associated with male infertility.

To date, there have been four genome-wide association studies (GWASs) regarding male fertility and infertility (Aston *et al*., 2009; Hu *et al*., 2012; Zhao *et al*., 2012; Kosova *et al*., 2012). Of these GWASs, a GWAS in a Hutterite population in the USA revealed that 41 single nucleotide polymorphisms (SNPs) are significantly correlated with family size or birth rate ($P < 1 \times 10^{-4}$). Hutterites comprise a founder population of European descent that traditionally proscribes contraception and uniformly desires large families. Of 41 SNPs, the following were found to be associated with sperm concentration or total sperm count in ethnically diverse men from Chicago, USA: rs7867029, which is downstream of *PSAT1*, the gene that encodes phosphoserine

aminotransferase 1; rs7174015, which is in *USP8*, the gene that encodes ubiquitin specific peptidase 8; rs12870438, which is in *EPSTI1*, the gene that encodes the epithelial stromal interaction protein 1; and rs724078, which is upstream of *MAS1L*, the gene that encodes the MAS1 oncogene-like protein, and downstream of *UBD*, the gene that encodes ubiquitin D (Kosova *et al*., 2012).

Associated conditions, azoospermia and oligozoospermia, were defined as the absence of sperm in ejaculate and a sperm concentration of less than 20×10^6/mL, respectively. We hypothesized that these four aforementioned SNPs might also be associated with the risk of male infertility in a Japanese population. Hence, in this study, we conducted a case-control association study to assess whether the SNPs rs7867029, rs7174015, rs12870438, and rs724078 were associated with infertility in Japanese males.

Materials and Methods

Subjects

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

The 791 fertile Japanese men (31.2 ± 4.8 years; mean ± SD) were used as the control sample. The fertile subjects in this study have been described in previous reports (Iwamoto *et al*., 2013). Briefly, fertile men were recruited from the partners of pregnant women who attended obstetric clinics in four cities in Japan (Sapporo, Kanazawa, Osaka, and Fukuoka). The eligibility criteria for the male participants were as follows: the participants had to have been aged 20–45 years at the time of invitation by the hospital at which they were recruited, and both the man and his mother had to have been born in and living in Japan. In addition, the pregnancy of the female partner had to have been the result of conception by sexual intercourse and not by fertility treatment.

Some of the subjects in this study have been described in previous reports (Sato *et al*., 2013). Briefly, 126 patients who consecutively presented as infertile at the Department of Urology, St. Mariana University Hospital, Kanagawa Prefecture, Japan, were enrolled from 2000 to 2011; of these patients, 76 (aged 33.2 ± 5.6 years; mean ± SD) were diagnosed as having azoospermia and 50 (aged 35.1 ± 6.1 years; mean ± SD) were diagnosed as having oligozoospermia. Semen analysis was performed in accordance with the 4th edition WHO Laboratory Manual for the Examination of Human Semen (World Health Organization, 1999). According to the 4th edition WHO guidelines (1999) criteria, azoospermia patients 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 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 (Simoni *et al*., 2004).

Genotyping

Genomic DNA was extracted from the peripheral blood samples of subjects using a QIAamp DNA blood kit (Qiagen; Tokyo, Japan). From SNPs previously reported to show associations with sperm concentration, semen volume, total sperm count, total motile sperm count, or sperm motility (Kosova *et al*., 2012), 4 SNPs (rs7867029, rs12870438, rs7174015, and rs724078) with minor allele frequencies > 0.05 in the HapMap-JPT population were selected for genotyping. The rs12870438 SNP was detected by restriction fragment length polymorphism -PCR using the following primer sets: 5′ - GCAAACAGGAGAAGGGTGTT -3′ (forward) and 5′ - GCTTTGGAGCATGTTTTCCC -3′ (reverse). DNA from each subject was amplified using Taq DNA polymerase (Promega; Tokyo, Japan) under the appropriate amplification conditions. The resulting PCR products were then digested using the *Hha*I restriction enzyme (New England Biolabs Japan Inc.; Tokyo, Japan). The digested products were separated by electrophoresis on a 2.5% agarose gel. The following fragment sizes were used for allele identification on gels: 488 bp (A-allele) and 278 + 210 bp (G-allele). The rs7867029, rs7174015, and rs724078 SNPs were genotyped using TaqMan probes rs7867029 (C_31364474_20), rs7174015 (C_32072246_10), and

rs724078 (C_2500858_10; Applied Biosystems; Tokyo, Japan) with the ABI 7900HT real-time PCR system (Applied Biosystems).

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was assessed in control samples by using an internet-based HWE calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using logistic regression analysis. All statistical analyses were performed using R version 3.0.2 (The R Project for Statistical Computing [http://www.r-project.org]), and statistical significance was considered at P-value < 0.05. Correction for multiple testing was performed with a factor of eight (four SNPs and two phenotypes).

Results

The genotype and allele frequencies of the four SNPs among 791 fertile controls, and 76 azoospermia and 50 oligozoospermia patients are shown in Table I. The genotyping of the SNPs was complete except for rs12870438 (the missing genotyping rate was 0.3%), and the genotypes of all four SNPs were in HWE in the fertile controls. Next, we assessed genetic associations between the four SNPs and male infertility in a case-control study design using a logistic regression analysis under three different comparative models (additive, recessive, or dominant) to verify whether the genetic model effects were consistent with the male fertility trait associations reported previously. The results of the logistic regression analysis from different comparative genetic models are summarized in Table II. There was a statistically significant association between rs7867029 and oligozoospermia in two models: log-additive (OR = 1.70, 95% CI = 1.07–2.68, P = 0.024) and recessive (OR = 3.14, 95% CI = 1.16–8.55, P = 0.025). However, there was no association between rs7867029 and azoospermia. Similarly, rs7174015 showed a significant association with oligozoospermia in two models: log-additive (OR = 1.56, 95% CI = 1.02–2.39, P = 0.042) and dominant (OR = 6.52, 95% CI = 1.57–27.10, P = 0.0099), but not with azoospermia. SNP rs12870438 showed significant associations with azoospermia in three models: log-additive (OR = 1.92, 95% CI = 1.21–3.05, P = 0.0059), recessive (OR = 10.90, 95% CI = 2.67–44.60, P = 0.00087), and dominant (OR = 1.71, 95% CI = 1.01–2.89, P = 0.046). In addition, rs12870438 showed a significant association with oligozoospermia in the recessive model (OR = 8.54, 95% CI = 1.52–47.90, P = 0.015). Among these, the association between rs7174015 and oligozoospermia under a dominant model and between rs12870438 and azoospermia under additive and recessive models remained after

correction for multiple testing (*P*-value < 0.0063). There were no associations between rs724078 and azoospermia or oligozoospermia.

Discussion

A recent GWAS found that 41 SNPs were significantly correlated with family size or birth rate ($P < 1 \times 10^{-4}$) in 269 Hutterite men in the USA. Of these SNPs, rs7867029, rs7174015, rs12870438, and rs724078 were found to be associated with semen parameters (including sperm concentration, semen volume, total sperm count, total motile sperm count, or sperm motility) in 123 ethnically diverse men from Chicago, USA (Kosova *et al*., 2012). Recently, we performed replication analyses of these four SNPs to assess their association with five semen parameters; however, none of the four SNPs displayed a significant association with any semen parameters in a total of 2015 Japanese men (Sato *et al*., submitted)). In contrast, we found that the polymorphisms rs7867029, rs7174015, and rs12870438 were significantly associated with more severe disease phenotype(s) in male infertility in this case-control study. SNPs rs7867029, rs7174015, and rs12870438 were associated with the risk for developing oligozoospermia, and rs12870438 was also associated with azoospermia. Meanwhile, there were no associations between rs724078 and either azoospermia or oligozoospermia. In the previous GWAS in 269 Hutterite men (Kosova *et al*., 2012), rs7867029, rs7174015, and rs12870438 were significantly associated with family size, and rs724078 was significantly associated with birth rate. There have been no previous studies that examined family size and oligozoospermia. This study therefore provides the first evidence that the family size-associated SNPs (rs7867029, rs7174015, and rs12870438), but not the birth rate-associated SNP (rs724078), are associated with the risk of oligozoospermia in a Japanese population.

Two (rs7174015 and rs12870438) of the three associated SNPs are located in the introns of *USP8* and *EPSTI1*, respectively. *Usp8* is highly expressed in male germ cells

and contributes to the formation of the mouse acrosome, which is indispensable for fertilization (Berruti *et al*., 2010), while *EPSTI1* is highly expressed in the testes (Nielsen *et al*., 2002). Although the relationship between these SNPs and the function of these genes is unknown, they may be biologically compelling candidates for further exploration into the genetics of human male infertility.

The present findings imply that three infertility-associated SNPs may be contributing to a quantitative reduction in spermatogenesis rather than to spermatogenesis failure. Although there has been no report available to indicate relationships between these three SNPs and sperm parameters in Hutterite men, men with these risk alleles might have associated reproductive outcomes, leading to a decrease in family size in the Japanese population.

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Authors' roles

Y.S. and A.T.: study design and data analysis; Y.S. and K.T.: genotyping; S.N., M.Y., E.K., J.K., M.N., K.M., A.T., K.K., N.I., J.E., and T.I.: cohort collection and characterization; Y.S., A.T., K.T., S.N., M.Y., E.K., J.K., M.N., K.M., A.T., K.K., N.I., J.E., I.I., A.Y., and T.I.: preparation and approval of the final version of the manuscript.

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Conflicts of interest

None declared.

References

Aston KI, Carrell DT. Genome-wide study of single-nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. *J Androl* 2009;**30**:711–725.

Bezold G, Lange M, Peter RU. Homozygous methylenetetrahydrofolate reductase C677T mutation and male infertility. *N Engl J Med* 2001;**344**:1172–1173.

Berruti G, Ripolone M, Ceriani M. USP8, a regulator of endosomal sorting, is involved in mouse acrosome biogenesis through interaction with the spermatid ESCRT-0 complex and microtubules. *Biol Reprod* 2010;**82**:930–939.

Chen SS, Chang LS, Chen HW, Wei YH. Polymorphisms of glutathione S-transferase M1 and male infertility in Taiwanese patients with varicocele. *Hum Reprod* 2002; **17**:718–725.

Finotti AC, Costa E Silva RC, Bordin BM, Silva CT, Moura KK. Glutathione S-transferase M1 and T1 polymorphism in men with idiopathic infertility. *Genet Mol Res* 2009;**8**:1093–1098.

Hu Z, Xia Y, Guo X, Dai J, Li H, Hu H, Jiang Y, Lu F, Wu Y, Yang X *et al*. A genome-wide association study in Chinese men identifies three risk loci for non-obstructive azoospermia. *Nat Genet* 2012;**44**:183–186.

Iwamoto T, Nozawa S, Yoshiike M, Namiki M, Koh E, Kanaya J, Okuyama A,

Matsumiya K, Tsujimura A, Komatsu K *et al*. Semen quality of fertile Japanese men: a cross-sectional population-based study of 792 men. *BMJ Open* 2013a;**3**:e002223.

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**:950–961.

Krausz C, McElreavey K. Y chromosome and male infertility. *Front Biosci* 1999;**4**:E1–E8.

Maduro MR, Lamb DJ. Understanding new genetics of male infertility. *J Urol* 2002;**168**:2197–2205.

Maurer B, Simoni M. Y chromosome microdeletion screening in infertile men. *J Endocrinol Invest* 2000;**23**:664–670.

McElreavey K, Krausz C, Bishop CE. The human Y chromosome and male infertility. *Results Probl Cell Differ* 2000;**28**:211–232.

McLachlan RI, de Kretser DM. Male infertility: the case for continued research. *Med J Aust* 2001;**174**:116–117.

Nielsen HL, Rønnov-Jessen L, Villadsen R, Petersen OW. Identification of EPSTI1, a novel gene induced by epithelial-stromal interaction in human breast cancer. *Genomics*

2002;**79**:703–710.

Norambuena PA, Diblik J, Krenkova P, Paulasova P, Macek M, Macek M, Sr. An ADP-ribosyltransferase 3 (ART3) variant is associated with reduced sperm counts in Czech males: case/control association study replicating results from the Japanese population. *Neuro Endocrinol Lett* 2012;**33**:48–52.

Okada H, Tajima A, Shichiri K, Tanaka A, Tanaka K, Inoue I. Genome-wide expression of azoospermia testes demonstrates a specific profile and implicates ART3 in genetic susceptibility. *PLoS Genet* 2008;**4**:e26.

Pajarinen J, Savolainen V, Perola M, Penttilä A, Karhunen PJ. Glutathione S-transferase-M1 'null' genotype and alcohol-induced disorders of human spermatogenesis. *Int J Androl* 1996;**19**:155–163.

Park JH, Lee HC, Jeong YM, Chung TG, Kim HJ, Kim NK, Lee SH, Lee S. MTHFR C677T polymorphism associates with unexplained infertile male factors. *J Assist Reprod Genet* 2005;**22**:361–368.

Polonikov AV, Yarosh SL, Kokhtenko EV, Starodubova NI, Pakhomov SP, Orlova VS. The functional genotype of glutathione S-transferase T1 gene is strongly associated with increased risk of idiopathic infertility in Russian men. *Fertil Steril* 2010;**94**:1144–1147.

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.

Sato Y, Tajima A, Tsunematsu K, Nozawa S, Yoshiike M, Koh E, Kanaya J, Namiki M, Matsumiya K, Tsujimura A *et al*. Lack of replication of four candidate SNPs implicated in human male fertility traits: a large-scale population-based study. *Hum Reprod* Submitted.

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**:240–249.

Singh K, Singh SK, Sah R, Singh I, Raman R. Mutation C677T in the methylenetetrahydrofolate reductase gene is associated with male infertility in an Indian population. *Int J Androl* 2005;**28**:115–119.

Skakkebaek NE, Giwercman A, de Kretser D. Pathogenesis and management of male infertility. *Lancet* 1994;**343**:1473–1479.

Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, Köhn FM, Schill WB, Farah S, Ramos C *et al*. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Human Mol Genet* 1996;**5**:933–943.

Vogt PH. Human chromosome deletions in Yq11, AZF candidate genes and male infertility: history and update. *Mol Hum Reprod* 1998;**4**:739–744.

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

Zhao H, Xu J, Zhang H, Sun J, Sun Y, Wang Z, Liu J, Ding Q, Lu S, Shi R *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**:900–906.

Table I. Allele and genotype frequencies of the subjects in a study of candidate loci for human male fertility traits

						Control		Case			
								Azoospermia		Oligozoospermia	
SNP	Chr	Position (NCBI Build 36.3)	Closest Genes[a]	Location	Allele[b]	Genotypes[b]	AF[b]	Genotypes[b]	AF[b]	Genotypes[b]	AF[b]
rs7867029	9	80,210,238	*PSAT1*	dwnst.	G	27/256/508	0.20	4/27/45	0.23	5/19/26	0.29
rs7174015	15	48,504,360	*USP8*	intron	T	226/396/169	0.54	22/38/16	0.54	16/32/2	0.64
rs12870438	13	42,378,205	*EPSTI1*	intron	A	4/148/638	0.098	4/18/54	0.17	2/6/40	0.10
rs724078	6	29,597,027	*MAS1L*, *UBD*	upst., dwnst.	T	61/334/396	0.29	7/27/42	0.27	2/26/22	0.30

SNP: single nucleotide polymorphism, Chr, chromosome; dwnst., downstream; upst., upstream.
[a]Gene names: *PSAT1*, phosphoserine aminotransferase 1; *EPSTI1*, epithelial stromal interaction 1; *USP8*, ubiquitin specific peptidase 8; *MAS1L*, MAS1 oncogene-like; *UBD*, ubiquitin D.
[b]"Allele" indicates the Hutterite minor allele reported in previous genome wide association studies (Kosova et al., 2012). "Genotypes" and "AF" indicate genotype counts (2/1/0) and the frequencies of the Hutterite minor alleles, respectively.

Table II. The associations from different comparative genetic models between four SNPs and azoospermia or oligozoospermia

Model	Case	OR (95% CI)	*P*
rs7867029			
Log-additive[a]	Azoospermia	1.23 (0.83–1.85)	0.31
(Risk allele, G)	Oligozoospermia	1.70 (1.07–2.68)	0.024
Recessive	Azoospermia	1.57 (0.54–4.62)	0.41
(GG vs. GC+CC)	Oligozoospermia	3.14 (1.16–8.55)	0.025
Dominant	Azoospermia	1.24 (0.77–2.00)	0.39
(GG+GC vs. CC)	Oligozoospermia	1.66 (0.93–2.94)	0.084
rs7174015			
Log-additive	Azoospermia	1.01 (0.73–1.42)	0.94
(Risk allele, T)	Oligozoospermia	1.56 (1.02–2.39)	0.042
Recessive	Azoospermia	1.02 (0.61–1.71)	0.95
(TT vs. TC+CC)	Oligozoospermia	1.18 (0.64–2.17)	0.60
Dominant	Azoospermia	1.02 (0.57–1.81)	0.95
(TT+TC vs. CC)	Oligozoospermia	**6.52 (1.57–27.10)**	**0.0099**
rs12870438			
Log-additive	Azoospermia	**1.92 (1.21–3.05)**	**0.0059**
(Risk allele, A)	Oligozoospermia	1.06 (0.54–2.11)	0.86
Recessive	Azoospermia	**10.90 (2.67–44.60)**	**0.00087**
(AA vs. AG+GG)	Oligozoospermia	8.54 (1.52–47.90)	0.015
Dominant	Azoospermia	1.71 (1.01–2.89)	0.046
(AA+AG vs. GG)	Oligozoospermia	0.84 (0.39–1.83)	0.66
rs724078			
Log-additive	Azoospermia	0.91 (0.62–1.33)	0.63
(Risk allele, T)	Oligozoospermia	1.06 (0.68–1.67)	0.80
Recessive	Azoospermia	1.21 (0.54–2.76)	0.64
(TT vs. TC+CC)	Oligozoospermia	0.50 (0.12–2.10)	0.34
Dominant	Azoospermia	0.81 (0.51–1.30)	0.39
(TT+TC vs. CC)	Oligozoospermia	1.28 (0.72–2.27)	0.41

Underlines indicate *P*-value < 0.05 and bold numbers indicate *P*-value < 0.0063 (0.05/8 test: four SNPs and two phenotypes) to account for multiple testing.
[a]Log-additive, additive model in log-odds scale.