Susceptibility to male infertility: replication study in Japanese men looking for an association with four GWAS-derived loci identified in European men

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Abstract

Purpose A previous genome-wide association study in European men identified four single nucleotide polymorphism (SNP) loci associated with male infertility. Our aim was to replicate, if possible, the association of these SNPs with Japanese male infertility.

Methods We genotyped four SNPs (rs5911500, rs10246939, rs2059807, and rs11204546) in 517 Japanese patients with male infertility and 369 fertile controls using SNP-specific real-time polymerase chain reaction TaqMan assays. Subsequently, we divided patients with male infertility into azoospermia ($n=417$) and oligospermia subgroups ($n=70$).

Results The four SNPs previously identified in European men showed no significant association with collective male infertility in our Japanese cohort. However, allele frequency analysis did indicate a significantly higher frequency of the rs11204546

C allele of the *OR2W3* gene in the oligospermia subset of infertility patients compared with controls ($p=0.0037$; odds ratio=1.74; 95 % confidence interval, 1.21–2.53).

Conclusions Although this study was somewhat limited by overall sample size, the *OR2W3* gene polymorphism rs11204546 was significantly associated with oligospermia in Japanese men, suggesting that *OR2W3* might be involved in genetic susceptibility to Japanese male infertility as well as in European males.

Keywords Male infertility · Azoospermia · Oligospermia · Japanese · European · SNP · GWAS

Introduction

Infertility affects approximately one out of every six couples and is a growing health problem for the Japanese public that urgently needs to be addressed. Male infertility accounts for approximately half of all couple-infertility cases. Major causes of male infertility are idiopathic azoospermia, oligospermia, and asthenospermia [1]. Patients with azoospermia or severe oligospermia can be treated by a combination of sperm retrieval by testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI), although this treatment is not always successful. Clarifying the molecular pathogenesis of male infertility should lead to the development of better treatments.

Although it is well known that men with a deletion of the azoospermia factor (AZF) region of the long arm of the Y chromosome are infertile [2], this condition accounts for only about 10 % of male infertility cases. Researchers have also reported associations between male infertility and various polymorphisms in genes such as methylenetetrahydrofolate reductase (*MTHFR*) [3–5] or ADP-ribosyltransferase 3 (*ART3*) [6, 7].

Capsule *OR2W3* might be involved in genetic susceptibility to Japanese male infertility.

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Table 1 Clinical characteristics of the study population

Characteristics	Azoospermia	Oligospermia	Control
Number of cases	447	70	369
Age (years)	34.8±6.5*	38.6±6.8*	43.6±12.9
BMI	23.7±3.0	23.4±1.7	NA
Sperm concentration (x10 ⁶ /ml)	0±0	3.2±5.7	NA

Continuous variables are expressed as Mean±SD

NA: not available

*p<0.001 (compared to controls)

However, genetic association studies often lead to different risks for male infertility among different ethnic populations. For instance, Wang et al. have conducted meta-analysis using seven previously published studies of the relationship between male infertility and polymorphisms in the Cytochrome P4501A1 (*CYP11A1*) gene and they showed an association of *CYP11A1**2A genotypes with susceptibility to idiopathic male infertility in Asians, but not in Caucasians [8].

Recently, genome-wide association studies (GWAS) using single-nucleotide polymorphism (SNP) arrays have begun to identify some of the underlying genetic susceptibilities for male infertility [9, 10]. However, one replication study for GWAS-derived male infertility susceptibility loci identified in Chinese men to susceptibility in Japanese men has shown that the four Chinese SNPs (rs12097821 near *PRMT6*; rs2477686 near *PEX10*; rs10842262 near *SOX5*; rs6080550 at *SIRPA*-

Table 2 Replication of previous GWAS SNPs reported to be associated with male infertility risk in European men (Aston et al., 2010)

SNP		Azo- and oligospermia		Control		OR* (95 % CI*)	p-value†
		No.	%	No.	%		
rs5911500							
Genotype	T/T	3	0.6	3	0.8		0.69
	T/C	58	11.1	35	9.5		
	C/C	456	88.4	331	89.7		
Allele frequencies	T	64	6.1	41	5.6	1.10	0.98
	C	984	93.9	697	94.4	(0.74 to 1.67)	
rs10246939							
Genotype	C/C	167	31.9	114	30.9		0.095
	C/T	269	51.3	172	46.6		
	T/T	88	16.8	83	22.5		
Allele frequencies	C	603	57.5	400	54.2	1.14	0.18
	T	445	42.5	338	45.8	(0.95 to 1.38)	
rs2059807							
Genotype	T/T	280	53.4	179	48.5		0.31
	T/C	209	39.9	161	43.6		
	C/C	35	6.7	29	7.9		
Allele frequencies	T	769	73.4	519	70.3	1.16	0.36
	C	279	26.6	219	29.7	(0.94 to 1.43)	
rs11204546							
Genotype	C/C	132	25.5	81	22.0		0.38
	T/C	248	48.0	179	48.5		
	T/T	137	26.5	109	29.5		
Allele frequencies	C	512	49.5	341	46.2	1.14	0.18
	T	522	50.5	397	53.8	(0.95 to 1.38)	

*OR and CI denote odds ratio and confidence interval, respectively

†p-values were calculated by the chi-square test

AIRPG) were not significantly associated with non-obstructive azoospermia (NOA) in Japanese men [10, 11].

In this replication study, we have looked for an association between four GWAS-derived loci recently identified in European men with susceptibility to male infertility in Japanese men, for the purpose of identifying common genetic risks for male infertility across different ethnicities.

Materials and methods

Subjects

Patients with male fertility were registered at the Niigata University, Tachikawa, and St. Mother's Hospitals. In total, 417 azoospermia patients, 70 oligospermia patients and 369 fertile controls were enrolled in this study (Table 1). Semen analysis was carried out according to the World Health

Organization's *Laboratory Manual for the Examination of Human Semen*. All patients with male infertility were diagnosed with primary infertility based on semen analysis (i.e., absence of competent sperm in ejaculate), hormone analysis, medical history and physical examination. Patients were diagnosed with azoospermia or oligospermia based on semen analysis, serum hormone levels and the results of physical examinations. We excluded patients with other identified causes of male infertility, such as varicocele, cryptorchidism, hypogonadotropic hypogonadism, and gross chromosomal abnormalities based on clinical records. Y microdeletion status was not included in exclusion criteria of this study. The control group consisted of 369 fertile Japanese men who had at least one child conceived without the use of assisted reproduction techniques. We excluded from the controls fertile men with any disease, based on clinical records at Niigata University. The ethics committees of the two participating institutions approved the study protocol, and each participant gave his written informed consent.

Table 3 Association of the four SNPs with azoospermia in Japanese men

SNP		Azoospermia		Control		OR* (95%CI*)	p-value†
		No.	%	No.	%		
rs5911500							
Genotype	T/T	2	0.4	3	0.8	1.09 (0.72 to 1.67)	1.00
	T/C	50	11.2	35	9.5		
	C/C	395	88.4	331	89.7		
Allele frequencies	T	54	6.0	41	5.6		
	C	840	94.0	697	94.4		
rs10246939							
Genotype	C/C	143	32.0	114	30.9	1.14 (0.94 to 1.40)	0.18
	C/T	229	51.2	172	46.6		
	T/T	75	16.8	83	22.5		
Allele frequencies	C	515	57.6	400	54.2		
	T	379	42.4	338	45.8		
rs2059807							
Genotype	T/T	247	55.3	179	48.5	1.21 (0.97 to 1.51)	0.10
	T/C	169	37.8	161	43.6		
	C/C	31	6.9	29	7.9		
Allele frequencies	T	663	74.2	519	70.3		
	C	231	25.8	219	29.7		
rs11204546							
Genotype	C/C	106	23.7	81	22.0	1.07 (0.88 to 1.30)	0.53
	T/C	216	48.3	179	48.5		
	T/T	125	28.0	109	29.5		
Allele frequencies	C	428	47.9	341	46.2		
	T	466	52.1	397	53.8		

*OR and CI denote odds ratio and confidence interval, respectively

†p-values were calculated by the chi-square test

Selection of the four SNPs

We selected the four SNPs (rs5911500, rs10246939, rs2059807 and rs11204546) from previous GWAS papers on European men [9, 12]. We confirmed that the minor allele frequencies (MAF) of these nine SNPs were greater than 0.05, based on Japanese polymorphism data in the National Center for Biotechnology Information SNP Database (<http://www.ncbi.nlm.nih.gov/snp>). The details of the SNPs for each gene are presented in Supplementary Table 1.

SNP genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA Blood Maxi Kit (QIAGEN, Tokyo, Japan), or from saliva using a phenol/chloroform extraction. To analyze these SNPs in the DNA of our study subjects, real-time polymerase chain reaction (PCR) TaqMan SNP genotyping assays were performed on an ABI PRISM 7900HT Sequence Detection System (Applied BioSystems,

Foster City, CA, USA) [13]. In brief, a PCR assay was conducted using TaqMan Universal Master Mix (Applied BioSystems) in a 5 µl final reaction volume using 20 ng of genomic DNA. Thermal cycling conditions were 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min. The genotype was automatically attributed in each sample by measuring the allele-specific fluorescence with the ABI Prism 7900 HT Sequence Detection System and using SDS 2.1 software (Applied BioSystems) for allele discrimination. The TaqMan probes used for identification of each allele are listed in Supplementary Table 1.

Statistical analysis

As quality controls for this association study, the Hardy–Weinberg equilibriums were calculated. The Hardy–Weinberg equilibrium was tested using the chi-square test by comparing the expected and actual allelic frequencies. The relative proportions of alleles between cases and controls were compared using the chi-square test. A difference in age

Table 4 Association of the four SNPs with oligospermia in Japanese men

SNP		Oligospermia		Control		OR* (95%CI*)	p-value†
		No.	%	No.	%		
rs5911500							
Genotype	T/T	1	1.4	3	0.8		0.77
	T/C	8	11.4	35	9.5		
	C/C	61	87.1	331	89.7		
Allele frequencies	T	10	7.1	41	5.6	1.32	0.73
	C	130	92.9	697	94.4	(0.61 to 2.61)	
rs10246939							
Genotype	C/C	21	30.0	114	30.9		0.81
	C/T	36	51.4	172	46.6		
	T/T	13	18.6	83	22.5		
Allele frequencies	C	78	55.7	400	54.2	1.06	0.81
	T	62	44.3	338	45.8	(0.74 to 1.53)	
rs2059807							
Genotype	T/T	30	42.9	179	48.5		0.46
	T/C	36	51.4	161	43.6		
	C/C	4	5.7	29	7.9		
Allele frequencies	T	96	68.6	519	70.3	0.92	0.75
	C	44	31.4	219	29.7	(0.63 to 1.37)	
rs11204546							
Genotype	C/C	26	37.1	81	22.0		0.011
	T/C	32	45.7	179	48.5		
	T/T	12	17.1	109	29.5		
Allele frequencies	C	84	60.0	397	53.8	1.74	0.0037
	T	56	40.0	341	46.2	(1.21 to 2.53)	

*OR and CI denote odds ratio and confidence interval, respectively

†p-values were calculated by the chi-square test

between cases and controls was examined by Welch's *t*-test. All analyses were performed using an R statistical environment (version 2.13.0); *p*-values of <0.05 were considered to be statistically significant.

Results

The clinical characteristics of the infertility cases are listed in Table 1. The controls were statistically older than the cases because we enrolled fertile men who had at least one child conceived without the use of assisted reproduction techniques as control. The observed frequencies of each SNP passed Hardy–Weinberg equilibrium analysis in both the case and control subjects. The allele frequencies of the controls in our study are in line with those from the polymorphism database for Japanese. Analysis showed that there were no significant differences between the collective male infertility cases and controls in the allele frequency of the four European-linked SNPs (Table 2). In addition, three of the four SNPs (rs5911500, rs10246939, and rs2059807) displayed allelic risk for male infertility in our Japanese set that was different (lower) from that seen in the previous European GWAS.

Subsequently, we divided our male infertility cases into two subgroups, “azoospermia” and “oligospermia”, and investigated for associations between the four SNPs and each disease subgroup (Tables 3 and 4). Our analysis showed that there was a significant difference between the oligospermia cases and the controls in the frequency of allele rs11204546 of the *OR2W3* gene ($p=0.0037$). On the other hand, there were no significant differences between the azoospermia or oligospermia cases and the controls in the allelic frequencies of the remaining three SNPs.

Discussion

A recent GWAS found that four SNP loci were associated with male infertility in Eastern European men. In the current study, we conducted a replication study of that body of work for the association of four European GWAS-derived SNPs with susceptibility to male infertility in Japanese men. We have identified the rs11204546 SNP in the *OR2W3* gene as being significantly associated with oligospermia in Japanese men. Thus, three studies have now demonstrated that the rs11204546 SNP in *OR2W3* is significantly associated with male infertility, in Chinese [14], in European men, and now in Japanese men, suggesting that the *OR2W3* gene might be a common risk factor in infertile men across widely different ethnicities.

OR2W3 gene codes for a member of a large family of G-protein-coupled receptors that belong to the seven-transmembrane-domain receptor family related to olfactory

receptors (OR) arising from unique single-coding-exon genes. The super family of OR genes are broadly expressed in a variety of tissues, including thyroid or testis [15]. Although *OR2W3* protein expression in testis has been validated (<http://www.proteinatlas.org/ENSG00000238243-OR2W3/tissue>), its function there remains a mystery. Zhang et al. have found that the rs11204546 genotype of *OR2W3* was correlated with the FSH level in infertile Chinese men. Further study is required to understand the full significance of *OR2W3* in infertile men. Especially, sequencing the *OR2W3* gene might lead to discover mutations associated with infertility.

In our study we could not validate associations with infertility between the other three SNPs we examined. It is noted that the four European GWAS-derived loci [9, 12] were not detected as being genetic factors for male infertility in a previous Chinese study [10] unrelated to the one that detected *OR2W3* [14]. This discrepancy might be related simply to differences in the SNP array platforms or analysis methods used, or, more likely, to the ethnicity of the three study populations. Further investigations are necessary to clarify the roles of *OR2W3* and this specific SNP in spermatogenesis.

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