2. Methods

2.1. Subjects and treatment

This study comprised 201 unrelated Japanese patients with major depression treated at the department of neuropsychiatry in Kansai Medical University. Some of the subjects included in this study were previously investigated using a candidate gene analysis (Wakeno et al., 2008; Kato et al., 2009). Patients with clinically significant unstable medical illness, pregnancy, a principal psychiatric disease other than major depression, a history of substance abuse or dependence within the last 6 months, and electroconvulsive therapy within the last 6 months were excluded. Major depression was diagnosed using a Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1995). The diagnoses were made by two independent senior psychiatrists and confirmed by a third psychiatrist, who was blind to the previous evaluations. A washout period of 10 days was required if patients took antidepressants, and the clinician decided to change the prescription to paroxetine, fluvoxamine, or milnacipran due to ineffectiveness. Eligible patients were either drug-free or taking ineffective antidepressants, and after 10 days of washout, paroxetine, fluvoxamine, or milnacipran was administered to reach therapeutic doses from days 8–11 until the end of trial. The maximum doses of paroxetine, fluvoxamine, and milnacipran were 40 mg/day, 200 mg/day, and 100 mg/day, respectively. Concomitant psychotropic drugs were not allowed, except for a low dose of sleep-inducing hypnotic agents at bedtime. Clinicians specifically assessed adverse drug reactions in patients for 6 weeks during each hospital visit. This study was approved by the appropriate Institutional Review Board or Research Administration of the institutions involved. Signed informed consent documents were obtained from all study participants before entry into the trial.

2.2. Genotyping and quality control (QC)

Genomic DNA was extracted from peripheral blood using a Qiagen blood Maxi kit (Qiagen, Tokyo, Japan), according to the manufacturer's protocol. Genotyping was performed with an Affymetrix GeneChip Human Mapping 250K Nsp Array (Affymetrix Inc., Santa Clara, CA) containing 262,264 SNPs, according to the standard protocol recommended by the manufacturer. Fluorescence intensities were quantified using the Affymetrix array scanner 3000 7G. Data management and analyses were performed using the Affymetrix GeneChip Operating Software 1.4 client. Genotyping calls were determined from fluorescent intensities using the GeneChip Genotyping Analysis Software 4.1 and the BRLMM algorithm (Rabbee and Speed, 2006). All subjects passed QC criteria for genotyping call rates greater than 0.95 and the heterozygosity rates of X genotypes for males with observable Y genotypes were less than 0.1 (except in the pseudoautosomal regions). From an initial full set of 262,264 SNPs, we discarded SNPs from the association analysis if they exhibited a call rate less than 0.95 for each SNP (17,294 SNPs), a minor allele frequency less than 0.01 (52,908 SNPs), or a deviation from Hardy–Weinberg equilibrium with an exact P value less than 0.001 (5,742 SNPs). These quality control steps removed a total of 75,944 SNPs. The final SNP set used in the association analysis contained 186,320 SNPs.

2.3. Association analysis

The statistical significance of the association between each SNP and sexual dysfunction induced by SSRI and SNRI was initially assessed using the Cochran–Armitage trend test. We used the false discovery rate (FDR)-based method of Benjamini and Hochberg to correct multiple comparisons (Benjamini and Hochberg, 1995). A threshold for genome-wide significance ($P < 0.05$) was estimated as $P = 4.29 \times 10^{-6}$ after FDR correction for 186,320 tests. Allelic risk ratios associated with each SNP and 95% confidence intervals (CI) were

calculated using the minor allele as a reference. The genomic control inflation factor (λ_{GC}) was estimated to evaluate population stratification (Devlin and Roeder, 1999). Multivariable logistic regression analysis was used to adjust for age and sex in the additive model, which was implemented using PLINK version 1.07 software. All other statistical analyses were performed using the R environment (version 2.7.2; The R Foundation for Statistical Computing). Manhattan plots and QQ plots were generated using Haploview 4.2 (Barrett et al., 2005) and the R environment, respectively.

3. Results

Descriptive characteristics of the subjects used in GWAS are shown in Table 1. A total of 201 Japanese major depression patients (106 male and 95 female) were included in this study. The administration of SSRI (paroxetine or fluvoxamine) or SNRI (milnacipran) resulted in 36 patients being affected with sexual dysfunction including decreased libido, delayed ejaculation, delayed orgasm, and erectile dysfunction. The four symptoms are nonidentical but known to be correlated. We performed GWAS for a set of the four symptoms to explore SNPs associated with sexual dysfunction. We genotyped the 201 DNA samples using an Affymetrix GeneChip Human 250K Nsp array. A total of 186,320 SNPs that satisfied the quality control criteria were used in the association study. Statistically significant SNPs were identified using the Cochran–Armitage trend test for additive allelic effects on the risk of SSRI/SNRI-induced sexual dysfunction. A quantile–quantile (QQ) plot for observed versus expected P values of the Cochran–Armitage trend test is shown in Figure 1. The observed genomic control inflation factor (λ_{GC}) was 1.042, suggesting that there was no substantial population stratification among the subjects. Figure 2 shows a Manhattan plot of the distribution of the log-transformed P values in the trend test across chromosomes. We observed relatively strong statistical signals ($P < 10^{-4}$) associated with sexual dysfunction in 54 SNPs (Table 2). Of these,

14 SNPs were tightly clustered into a distinct region on chromosome 14q21.3 (Fig. 2). After FDR correction for multiple testing, 11 contiguous SNPs met the requirement for association with genome-wide significance ($P < 4.29 \times 10^{-6}$). These SNPs were located in the intronic region of the *MDGA2* (MAM domain-containing glycosylphosphatidylinositol anchor 2) gene. The P value of the most significantly associated SNP was rs1160351 ($P = 3.04 \times 10^{-7}$) with a risk ratio of 2.92 (95% CI = 1.79–4.76). Furthermore, we found 3 contiguous SNPs with P values less than 10^{-4} in the intronic region of the *ADAMTS19* (a disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 19) gene on chromosome 5q23.3 (Fig. 2 and Table 2). Two of these SNPs (rs13436218 and rs6896924) had genome-wide significance with the most significant P value of 1.25×10^{-6} and a risk ratio of 5.92 (95% CI = 4.67–7.52). The remaining 3 SNPs, rs225848 (14q12), rs6603109 (19p13.2), and rs857228 (14q32.2), had genome-wide significance ($P = 1.17 \times 10^{-6}$, 1.92×10^{-6}, and 3.28×10^{-6}, respectively), but their closest genes were a processed pseudogene, a putative uncharacterized protein, and a predicted micro-RNA, respectively.

Table 1 shows that the differences in the distribution of sex and age between the two groups, i.e., those affected by sexual dysfunction and those unaffected, were statistically significant ($P = 0.028$ and $P = 0.007$ for sex and age, respectively). Therefore, the association was also assessed by logistic regression analysis with age and sex as covariates under the additive genetic model. Figures 3 and 4 show a QQ plot and Manhattan plot, respectively. The Manhattan plot shows that the strong association peak remained located in the intronic region of *MDGA2* on chromosome 14q21.3 after adjusting for sex and age, although the observed P values became increased slightly and they were nonsignificant after correction for multiple testing (Fig. 4 and Table 2). Of 16 SNPs with P values lower than 10^{-4}, 14 SNPs were mapped to the *MDGA2* gene (Table 2). The most significantly associated SNP was rs1160351 ($P = 3.55 \times 10^{-6}$), which also showed the strongest association before the adjustment for covariates

(Table 2). The two SNPs in the *ADAMTS19* gene, with *P* values that satisfied FDR-corrected significance showed no association with sexual dysfunction after adjustment for sex and age ($P = 0.998$) (Fig. 4 and Table 2).

4. Discussion

To our knowledge, this is the first GWAS to demonstrate significant associations between genetic variations and sexual dysfunction induced by SSRI/SNRI. We genotyped 201 Japanese major depression patients and found 16 SNPs that were significantly associated with SSRI/SNRI-induced sexual dysfunction at the FDR-corrected genome-wide significance level ($P < 4.29 \times 10^{-6}$) using the Cochran–Armitage trend test. Of these 16 SNPs, 11 SNPs (69%) with associations were located in the first intron of *MDGA2* and the most significant association was found for rs1160351 ($P = 3.04 \times 10^{-7}$). Two SNPs located in intron15 of the *ADAMTS19* gene also met the genome-wide significance requirement and rs13436218 had the most significant *P* value of 1.25×10^{-6}. Therefore, it is conceivable that two genes are involved in the sexual dysfunction pathology associated with SSRI/SNRI. However, adjustment of the association analysis for covariates (sex and age) demonstrated that a strong peak only remained in the association with *MDGA2* (Fig. 4), which suggested that the apparent statistical association with the *ADAMTS19* SNPs was caused by a patient bias related to sex and/or age. However, the association with the *MDGA2* SNPs was independent of sex and age. Unfortunately, the *P* values became nonsignificant after adjusting for sex and age, probably because of the small patient number. Furthermore, tight linkage of the assayed SNPs such as those found in *MDGA2* (Table 2) may increase the *P* value because multiple testing correction assumes the independence of the factors during comparisons. In the present study, unrelated subjects were used in GWAS. Therefore, it is not conceivable that the association between the *MDGA2* locus and SSRI/SNRI-induced sexual

dysfunction was affected by consanguinity. However, it is ~~not completely denied~~ possible that individuals who were genetically related to one another were accidentally included in the study subjects. According to the information in the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/), 7 missense SNPs and 1 frame-shift SNP have been reported in the *MDGA2* exons, although their minor allele frequencies were not available or not comparable to the detected *MDGA2* SNPs in this study. It is possible that these SNPs and/or the other unidentified functional SNPs may affect MDGA2 protein function or gene expression, resulting in sexual dysfunction by SSRI/SNRI. The functional mechanism whereby *MDGA2* might be involved in sexual dysfunction remains obscure and a replicate study will be necessary to validate our results, but *MDGA2* is a possible candidate gene for SSRI/SNRI- induced sexual dysfunction.

MDGA2, also known as *MAMDC1*, is a poorly characterized gene spanning 835 kb on chromosome 14q21.3 composed of 17 exons with two alternative first exons, which results in 2 distinct protein isoforms (956 or 727 amino acids). The 11 SNPs in *MDGA2* that were associated with sexual dysfunction were located in the first intron of the longer isoform, which is presumed to have an important function based on its high similarity (>97%) with orthologs in other mammalian species including the chimpanzee, mouse, rat, dog, and horse (Hellquist et al., 2009). *MDGA2* was first cloned from the rat neonatal basilar pons as a brain-derived immunoglobulin superfamily member with 6 Ig-like domains, a highly conserved MAM (meprin, A5 protein, receptor protein tyrosine phosphatase mu) domain, a C-terminal glycosylphosphatidylinositol (GPI) anchoring signal, and several potential N-glycosylation sites (Litwack et al., 2004). MDGA2 is predominantly expressed in the brain of the embryonic and neonatal rat, including the central and peripheral nervous system, and it was proposed that it regulates neural development and axonal guidance (Litwack et al., 2004). In humans, MDGA2 is known to be expressed in a variety of tissues including the brain

(Hellquist et al., 2009). Little is known about *MDGA2*, but it was recently reported to be a candidate susceptibility gene for autism (Bucan et al., 2009) and neuroticism (van den Oord et al., 2008; Heck et al., 2011).

Two pharmacogenetic studies have investigated SSRI-induced sexual dysfunction using a candidate gene approach. The first (Bishop et al., 2006) was a study of the serotonin receptor 2A (*HTR2A*) gene (−1438G/A), where subjects with a SNP of the GG genotype were significantly more associated with sexual dysfunction compared with persons possessing a GA or AA genotype (OR = 3.6; 95% CI = 1.03–12.6; $P = 0.046$). The second was a study of glutamatergic genes including *GRIA3, GRIA1, GRIN3A,* and *GRIK2*, where SNPs were associated with decreased libido (*GRIA3* and *GRIK2*), difficulty achieving orgasm (*GRIA1*), and difficulty achieving erection (*GRIN3A*) (Perlis et al., 2009). We examined the SNPs in and around these 4 genes in our GWAS, but found no evidence of association.

In summary, our GWAS suggests that *MDGA2* is a candidate gene for sexual dysfunction induced by SSRI/SNRI. Future work will be necessary to evaluate the role of this gene in SSRI/SNRI-induced sexual dysfunction, but this study provides important clues to understanding the mechanism of SSRI/SNRI-induced sexual dysfunction. SNPs found in the *MDGA2* gene are potential biomarkers indicating a risk of this side effect and may be utilized to manage the sexual dysfunction induced by SSRI/SNRI. The relatively small sample size with administration of a limited number of SSRI/SNRI and the lack of a replication study with other independent samples are potential limitations of this study. Therefore, additional studies with a larger sample size, wide varieties of SSRI/SNRI, and higher density SNP array will be required to validate these results. However, our exploratory study represents an important first step in applying GWAS to identify the genetic causes of SSRI/SNRI-induced sexual dysfunction.

Acknowledgments

We thank StaGen Co., Ltd. for assisting with statistical analysis. This work was supported by a grant from the Japan Health Sciences Foundation (Research on Publicly Essential Drugs and Medical Devices).

References

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263-265.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 57, 289-300.

Bishop, J.R., Moline, J., Ellingrod, V.L., Schultz, S.K., Clayton, A.H., 2006. Serotonin 2A -1438 G/A and G-protein Beta3 subunit C825T polymorphisms in patients with depression and SSRI-associated sexual side-effects. Neuropsychopharmacology 31, 2281-2288.

Bucan, M., Abrahams, B.S., Wang, K., Glessner, J.T., Herman, E.I., Sonnenblick, L.I., Alvarez Retuerto, A.I., Imielinski, M., Hadley, D., Bradfield, J.P., Kim, C., Gidaya, N.B., Lindquist, I., Hutman, T., Sigman, M., Kustanovich, V., Lajonchere, C.M., Singleton, A., Kim, J., Wassink, T.H., McMahon, W.M., Owley, T., Sweeney, J.A., Coon, H., Nurnberger, J.I., Li, M., Cantor, R.M., Minshew, N.J., Sutcliffe, J.S., Cook, E.H., Dawson, G., Buxbaum, J.D., Grant, S.F., Schellenberg, G.D., Geschwind, D.H., Hakonarson, H., 2009. Genome-wide analyses of exonic copy number variants in a family-based study point to novel autism susceptibility genes. PLoS Genetics 5, e1000536.

Daly, A.K., Donaldson, P.T., Bhatnagar, P., Shen, Y., Pe'er, I., Floratos, A., Daly, M.J., Goldstein, D.B., John, S., Nelson, M.R., Graham, J., Park, B.K., Dillon, J.F., Bernal, W., Cordell, H.J., Pirmohamed, M., Aithal, G.P., Day, C.P., 2009. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nature Genetics 41, 816-819.

Devlin, B., Roeder, K., 1999. Genomic control for association studies. Biometrics 55, 997-1004.

First, M.B., Spitzer, R.L., Gibbon, M., B, W.J. (1995). Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P, version 2.0) New York: Biometrics Research, New York State Psychiatric Institute.

Heck, A., Pfister, H., Czamara, D., Muller-Myhsok, B., Putz, B., Lucae, S., Hennings, J., Ising, M., 2011. Evidence for associations between MDGA2 polymorphisms and harm avoidance - replication and extension of a genome-wide association finding. Psychiatric Genetics 21, 257-260.

Hellquist, A., Zucchelli, M., Lindgren, C.M., Saarialho-Kere, U., Jarvinen, T.M., Koskenmies, S., Julkunen, H., Onkamo, P., Skoog, T., Panelius, J., Raisanen-Sokolowski, A., Hasan, T., Widen, E., Gunnarson, I., Svenungsson, E., Padyukov, L., Assadi, G., Berglind, L., Makela, V.V., Kivinen, K., Wong, A.,

Cunningham Graham, D.S., Vyse, T.J., D'Amato, M., Kere, J., 2009. Identification of MAMDC1 as a candidate susceptibility gene for systemic lupus erythematosus (SLE). PLoS ONE 4, e8037.

Ising, M., Lucae, S., Binder, E.B., Bettecken, T., Uhr, M., Ripke, S., Kohli, M.A., Hennings, J.M., Horstmann, S., Kloiber, S., Menke, A., Bondy, B., Rupprecht, R., Domschke, K., Baune, B.T., Arolt, V., Rush, A.J., Holsboer, F., Muller-Myhsok, B., 2009. A genomewide association study points to multiple loci that predict antidepressant drug treatment outcome in depression. Archives of General Psychiatry 66, 966-975.

Kato, M., Fukuda, T., Wakeno, M., Fukuda, K., Okugawa, G., Ikenaga, Y., Yamashita, M., Takekita, Y., Nobuhara, K., Azuma, J., Kinoshita, T., 2006. Effects of the serotonin type 2A, 3A and 3B receptor and the serotonin transporter genes on paroxetine and fluvoxamine efficacy and adverse drug reactions in depressed Japanese patients. Neuropsychobiology 53, 186-195.

Kato, M., Fukuda, T., Serretti, A., Wakeno, M., Okugawa, G., Ikenaga, Y., Hosoi, Y., Takekita, Y., Mandelli, L., Azuma, J., Kinoshita, T., 2008. ABCB1 (MDR1) gene polymorphisms are associated with the clinical response to paroxetine in patients with major depressive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry 32, 398-404.

Kato, M., Fukuda, T., Wakeno, M., Okugawa, G., Takekita, Y., Watanabe, S., Yamashita, M., Hosoi, Y., Azuma, J., Kinoshita, T., Serretti, A., 2009. Effect of 5-HT1A gene polymorphisms on antidepressant response in major depressive disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 150B, 115-123.

Kato, M., Serretti, A., 2010. Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. Molecular Psychiatry 15, 473-500.

Link, E., Parish, S., Armitage, J., Bowman, L., Heath, S., Matsuda, F., Gut, I., Lathrop, M., Collins, R., 2008. SLCO1B1 variants and statin-induced myopathy--a genomewide study. New England Journal of Medicine 359, 789-799.

Litwack, E.D., Babey, R., Buser, R., Gesemann, M., O'Leary, D.D., 2004. Identification and characterization of two novel brain-derived immunoglobulin superfamily members with a unique structural organization. Molecular and Cellular Neurosciences 25, 263-274.

Malhotra, A.K., Murphy, G.M., Jr., Kennedy, J.L., 2004. Pharmacogenetics of psychotropic drug response. American Journal of Psychiatry 161, 780-796.

Ozeki, T., Mushiroda, T., Yowang, A., Takahashi, A., Kubo, M., Shirakata, Y., Ikezawa, Z., Iijima, M., Shiohara,

T., Hashimoto, K., Kamatani, N., Nakamura, Y., 2011. Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. Human Molecular Genetics 20, 1034-1041.

Perlis, R.H., Laje, G., Smoller, J.W., Fava, M., Rush, A.J., McMahon, F.J., 2009. Genetic and clinical predictors of sexual dysfunction in citalopram-treated depressed patients. Neuropsychopharmacology 34, 1819-1828.

Rabbee, N., Speed, T.P., 2006. A genotype calling algorithm for affymetrix SNP arrays. Bioinformatics 22, 7-12.

Serretti, A., Artioli, P., 2004. The pharmacogenomics of selective serotonin reuptake inhibitors. Pharmacogenomics J 4, 233-244.

Serretti, A., Kato, M., De Ronchi, D., Kinoshita, T., 2007. Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. Molecular Psychiatry 12, 247-257.

Thomas, K.L., Ellingrod, V.L., 2009. Pharmacogenetics of selective serotonin reuptake inhibitors and associated adverse drug reactions. Pharmacotherapy 29, 822-831.

van den Oord, E.J., Kuo, P.H., Hartmann, A.M., Webb, B.T., Moller, H.J., Hettema, J.M., Giegling, I., Bukszar, J., Rujescu, D., 2008. Genomewide association analysis followed by a replication study implicates a novel candidate gene for neuroticism. Archives of General Psychiatry 65, 1062-1071.

Wakeno, M., Kato, M., Okugawa, G., Fukuda, T., Hosoi, Y., Takekita, Y., Yamashita, M., Nonen, S., Azuma, J., Kinoshita, T., 2008. The alpha 2A-adrenergic receptor gene polymorphism modifies antidepressant responses to milnacipran. Journal of Clinical Psychopharmacology 28, 518-524.

Figure legends

Fig. 1. Quantile–quantile (QQ) plot for the observed versus expected distribution of *P* values of Cochran–Armitage trend test. (λ_{GC} = 1.042). FDR- and Bonferroni (BF)-corrected significance levels are indicated by broken lines.

Fig. 2. The association of SNPs with SSRI/SNRI-induced sexual dysfunction across chromosomes. The $-\log_{10}P$ values of the Cochran–Armitage trend test were plotted across chromosomes. FDR- and Bonferroni (BF)-corrected significance levels are indicated by lines in blue and red, respectively.

Fig. 3. Quantile–quantile (QQ) plot for the observed versus expected distribution of *P* values from the logistic regression analysis using the additive models adjusted for age and sex. (λ_{GC} = 0.988).

Fig. 4. Association of SNPs with SSRI/SNRI-induced sexual dysfunction across chromosomes. The $-\log_{10}P$ values from the logistic regression analysis using the additive models adjusted for age and sex were plotted across chromosomes.

Table 1

Table 1. Descriptive characteristics of study subjects

Characteristics	All patients (n =201)	Patients with Sexual dysfunction (n = 36))	Patients without Sexual dysfunction (n =165)	P
Sex	female 95, male106	female 11, male25	female 84, male81	0.028[a]
Age ±SD, (years)	46.2 ± 15.1 (19-82)	40.0 ± 12.3 (19-73)	47.6 ± 15.3 (22-82)	0.007[b]
Dose ±SD, mg/day				
Paroxetine	22.6 ±5.1 (n = 101)	21.0 ±3.0 (n = 21)	23.1 ±5.4 (n = 80)	0.091[b]
Fluvoxamine	89.6 ±39.9 (n= 49)	60.0 ±22.4 (n = 10)	93.0 ±40.2 (n = 41)	0.069[b]
Milnacipran	54.8 ±13.5 (n = 51)	55.0 ±19.7 (n = 5)	54.8 ±11.8 (n = 44)	0.743[b]

[a] Fisher's exact test between patients with and without sexual dysfunction

[b] Mann–Whitney U test between patients with and without sexual dysfunction

Table 2

Table 2. SNPs associated with SSRI/SNRI-induced sexual dysfunction at $P < 1 \times 10^{-4}$ (trend)

Rank (trend)	SNP	Chr	Chr position	Closest gene	Distance (base pairs)	Allele	MAF	Total	Affected	Unaffected	*P* (trend)	Allele RR (95%CI)	*P* (logistic)	Rank (logistic)
1	rs1160351	14	48015982	MDGA2	0	G>T	0.381	73/99/26	3/19/13	70/80/13	3.04E-07	2.92(1.79-4.76)	3.55E-06	1
2	rs10467790	14	47910244	MDGA2	0	G>A	0.394	71/99/29	3/19/14	68/80/15	4.17E-07	2.88(1.78-4.69)	7.94E-06	4
3	rs8016913	14	47890381	MDGA2	0	A>T	0.396	72/99/30	3/19/14	69/80/16	5.64E-07	2.87(1.77-4.67)	8.38E-06	5
3	rs8007707	14	47899386	MDGA2	0	T>C	0.396	72/99/30	3/19/14	69/80/16	5.64E-07	2.87(1.77-4.67)	8.38E-06	5
5	rs9323139	14	47905531	MDGA2	0	T>A	0.398	71/99/30	3/19/14	68/80/16	6.64E-07	2.85(1.75-4.63)	1.02E-05	9
6	rs4900764	14	47922766	MDGA2	0	T>A	0.396	73/97/31	3/19/14	70/78/17	7.41E-07	2.87(1.77-4.67)	8.60E-06	7
7	rs2002293	14	47890816	MDGA2	0	A>C	0.400	70/100/30	3/19/14	67/81/16	7.85E-07	2.82(1.74-4.59)	9.30E-06	8
8	rs7159493	14	47889952	MDGA2	0	C>A	0.390	70/98/27	3/19/13	67/79/14	8.01E-07	2.82(1.73-4.59)	1.24E-05	10
9	rs8016611	14	48015425	MDGA2	0	T>C	0.388	74/98/29	3/20/13	71/78/16	1.03E-06	2.79(1.72-4.50)	5.77E-06	2
10	rs225848	14	30594657	RP11-269C4.1	69497	A>G	0.028	189/11/0	28/8/0	161/3/0	1.17E-06	4.42(2.12-9.26)	5.79E-05	14
11	rs6572432	14	47966854	MDGA2	0	T>C	0.391	73/99/29	3/20/13	70/79/16	1.22E-06	2.76(1.71-4.46)	6.55E-06	3
12	rs13436218	5	129000386	ADAMTS19	0	A>C	0.012	196/5/0	31/5/0	165/0/0	1.25E-06	5.92(4.67-7.52)	0.9983	184534
13	rs6896924	5	128995118	ADAMTS19	0	A>G	0.013	195/5/0	31/5/0	164/0/0	1.34E-06	5.88(4.63-7.46)	0.9983	184534
14	rs4898568	14	47922676	MDGA2	0	A>G	0.393	71/97/29	3/19/13	68/78/16	1.69E-06	2.78(1.70-4.52)	1.48E-05	11
15	rs6603109	19	7424528	AC119396.1	-3138	T>A	0.017	194/7/0	30/6/0	164/1/0	1.92E-06	5.13(2.22-11.76)	5.26E-04	66
16	rs857228	14	98670158	AL163760.3	127811	G>T	0.354	80/96/22	6/18/12	74/78/10	3.28E-06	2.56(1.60-4.08)	4.94E-05	12
17	rs8009848	14	47880960	MDGA2	0	A>G	0.391	74/92/31	5/17/14	69/75/17	8.15E-06	2.60(1.61-4.18)	5.07E-05	13
18	rs4121760	11	101090720	PGR	-90176	G>A	0.025	191/10/0	29/7/0	162/3/0	1.05E-05	4.22(1.93-9.17)	3.66E-04	43
19	rs606386	11	101052811	PGR	-52267	C>T	0.025	190/10/0	29/7/0	161/3/0	1.13E-05	4.20(1.93-9.17)	3.71E-04	44
20	rs17163078	5	129024984	ADAMTS19	0	T>C	0.010	196/4/0	31/4/0	165/0/0	1.15E-05	5.99(4.72-7.63)	0.9985	185163
21	rs2552043	2	36498366	AC009414.1	28165	G>C	0.021	187/8/0	28/6/0	159/2/0	1.18E-05	4.63(2.00-10.64)	7.95E-04	96
22	rs468280	21	21162674	C1QBPP	-30232	A>T	0.045	183/18/0	26/10/0	157/8/0	1.27E-05	3.44(1.76-6.71)	4.33E-04	54
23	rs6856730	4	123837948	NUDT6	0	A>G	0.042	185/15/1	26/10/0	159/5/1	1.50E-05	3.65(1.87-7.14)	1.34E-03	170
23	rs2125450	4	123851312	SPATA5	0	G>C	0.042	185/15/1	26/10/0	159/5/1	1.50E-05	3.65(1.87-7.14)	1.34E-03	170
25	rs12588764	14	47925920	MDGA2	0	A>G	0.462	58/94/43	3/16/17	55/78/26	1.52E-05	2.65(1.61-4.39)	7.25E-05	15
26	rs7034206	9	77217424	RORB	0	A>G	0.209	125/65/9	13/16/6	112/49/3	1.56E-05	2.53(1.57-4.08)	1.18E-04	17
27	rs7427130	3	60119817	FHIT	0	G>A	0.026	185/10/0	29/7/0	156/3/0	1.61E-05	4.10(1.88-8.93)	1.63E-03	211
28	rs12507568	4	123874300	SPATA6	0	T>G	0.043	184/15/1	26/10/0	158/5/1	1.62E-05	3.64(1.86-7.09)	1.40E-03	180
29	rs4887003	15	78370775	SH2D7	0	T>C	0.010	195/4/0	32/4/0	163/0/0	1.71E-05	5.78(4.57-7.35)	9.99E-01	185163
30	rs545845	11	100937890	PGR	0	G>A	0.015	195/6/0	31/5/0	164/1/0	2.20E-05	4.93(1.98-12.20)	1.90E-03	250

31	rs2416070	14	47882355	MDGA2	0	C>T	0.403	73/93/34	5/17/14	68/76/20	2.88E-05	2.48(1.54-3.98)	8.59E-05	16
32	rs2840546	1	38804339	AL513479.1	-57992	T>G	0.040	185/16/0	27/9/0	158/7/0	3.06E-05	3.45(1.72-6.94)	6.17E-04	81
33	rs9302387	16	20478724	ACSM2A	0	T>G	0.126	151/46/2	16/19/0	135/27/2	3.12E-05	2.59(1.53-4.39)	2.04E-04	24
34	rs9681146	3	60122926	FHIT	0	G>A	0.028	184/11/0	27/7/0	157/4/0	3.22E-05	3.95(1.81-8.62)	1.67E-03	216
34	rs17075874	13	22671341	AL137248.1	0	G>A	0.060	177/22/1	25/10/1	152/12/0	3.22E-05	3.13(1.69-5.81)	1.46E-04	21
36	rs7376617	4	6236339	JAKMIP1	-34021	A>G	0.445	58/107/36	21/13/2	37/94/34	4.06E-05	0.39(0.22-0.66)	1.51E-04	22
37	rs4454609	10	13304301	PHYH	15495	T>A	0.403	75/90/36	6/15/15	69/75/21	4.15E-05	2.47(1.53-3.98)	3.04E-04	31
38	rs17141149	6	5679538	FARS2	0	C>T	0.012	197/3/1	32/3/1	165/0/0	4.46E-05	5.92(4.67-7.52)	9.98E-01	184286
39	rs2779732	9	130022540	GARNL3	0	G>A	0.276	103/82/14	11/15/9	92/67/5	4.47E-05	2.34(1.46-3.73)	4.35E-04	57
40	rs6448144	4	22316708	AC093735.2	-34365	C>T	0.027	190/11/0	29/7/0	161/4/0	4.74E-05	3.83(1.75-8.33)	1.66E-03	215
41	rs9881474	3	60121220	FHIT	0	C>G	0.027	190/11/0	29/7/0	161/4/0	4.74E-05	3.83(1.75-8.33)	2.21E-03	312
42	rs17062315	3	60122023	FHIT	0	T>C	0.027	190/11/0	29/7/0	161/4/0	4.74E-05	3.83(1.75-8.33)	2.21E-03	312
43	rs7638267	3	60120995	FHIT	0	C>T	0.028	189/11/0	29/7/0	160/4/0	5.06E-05	3.80(1.75-8.33)	2.23E-03	319
44	rs262430	11	36044370	LDLRAD3	0	C>A	0.259	109/80/12	12/16/8	97/64/4	5.14E-05	2.29(1.44-3.65)	1.25E-04	20
45	rs11105851	12	91263782	AC078873.1	-68651	G>A	0.035	186/14/0	27/8/0	159/6/0	5.17E-05	3.56(1.70-7.41)	4.23E-04	51
46	rs7378331	4	6236372	JAKMIP1	-34054	A>T	0.443	59/106/36	21/13/2	38/93/34	5.39E-05	0.39(0.23-0.67)	1.70E-04	23
47	rs10494694	1	194053429	RP11-563D10.1	-65174	A>T	0.065	175/26/0	24/12/0	151/14/0	5.69E-05	2.89(1.56-5.38)	8.76E-04	104
48	rs10869421	9	77174230	RORB	0	C>T	0.241	118/69/14	12/17/7	106/52/7	5.84E-05	2.38(1.49-3.79)	2.08E-04	26
49	rs11144010	9	77174810	RORB	0	G>T	0.241	118/69/14	12/17/7	106/52/7	5.84E-05	2.38(1.49-3.79)	2.08E-04	26
50	rs955433	9	77177142	RORB	0	T>C	0.241	118/69/14	12/17/7	106/52/7	5.84E-05	2.38(1.49-3.79)	2.08E-04	26
51	rs7205704	16	27400893	IL21R	-12793	T>C	0.445	61/99/39	3/18/14	58/81/25	7.69E-05	2.39(1.46-3.92)	3.12E-04	32
52	rs2229434	19	7142921	INSR	0	G>A	0.050	181/20/0	26/10/0	155/10/0	8.01E-05	3.08(1.58-6.02)	3.32E-04	33
53	rs2229430	19	7142843	INSR	0	G>C	0.050	180/20/0	26/10/0	154/10/0	8.62E-05	3.07(1.57-5.99)	3.42E-04	34
54	rs2134253	16	55763874	SLC6A2	24690	C>T	0.185	133/60/7	14/19/3	119/41/4	9.43E-05	2.34(1.44-3.80)	4.40E-04	59

SNP indicates dbSNP ID; Chr, chromosome; Position, human reference sequence GRCh37; Distance, distance from the closest gene; MAF, minor allele frequency; *P* (trend), *P* value derived from the Cochrane–Armitage trend test; *P* (logistic), *P* value derived from logistic regression analysis of the additive models with age and sex as covariates. Total, Affected, and Nonaffected indicate the number of subjects in major homo/hetero/ minor homo. Allelic Risk Ratio (RR) (95% confidence interval) is associated with the minor allele. The top 16 SNPs met requirement for association based on the FDR-corrected genome-wide significance level ($P < 4.29 \times 10^{-6}$) using

the Cochrane–Armitage trend test.

Figure 1
Click here to download high resolution image

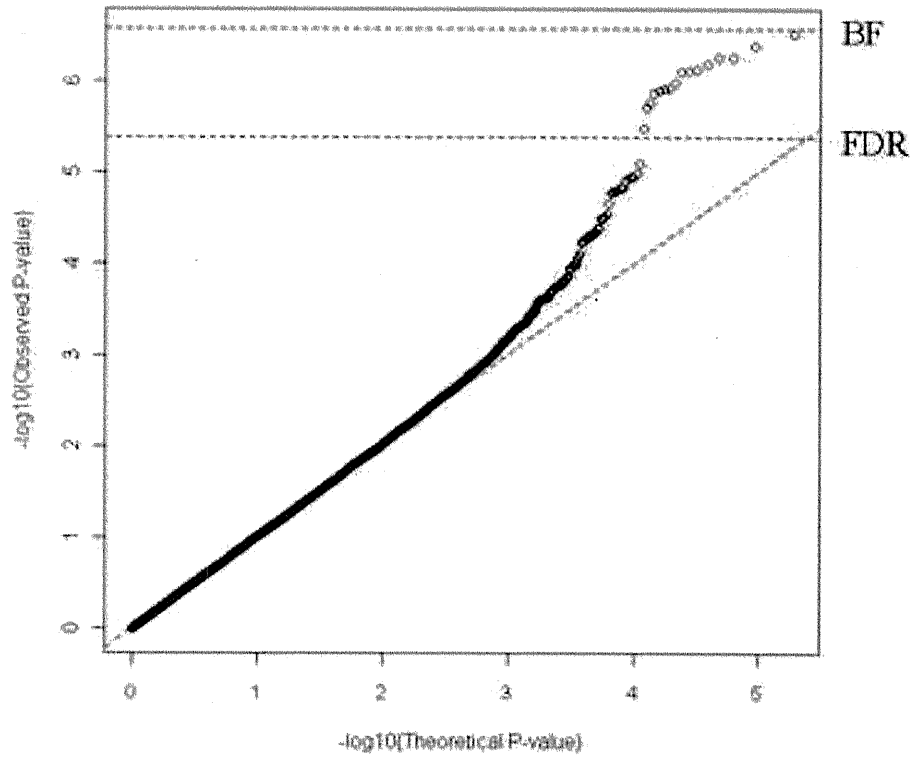

Figure 1 Kurose et al.

Figure 2
Click here to download high resolution image

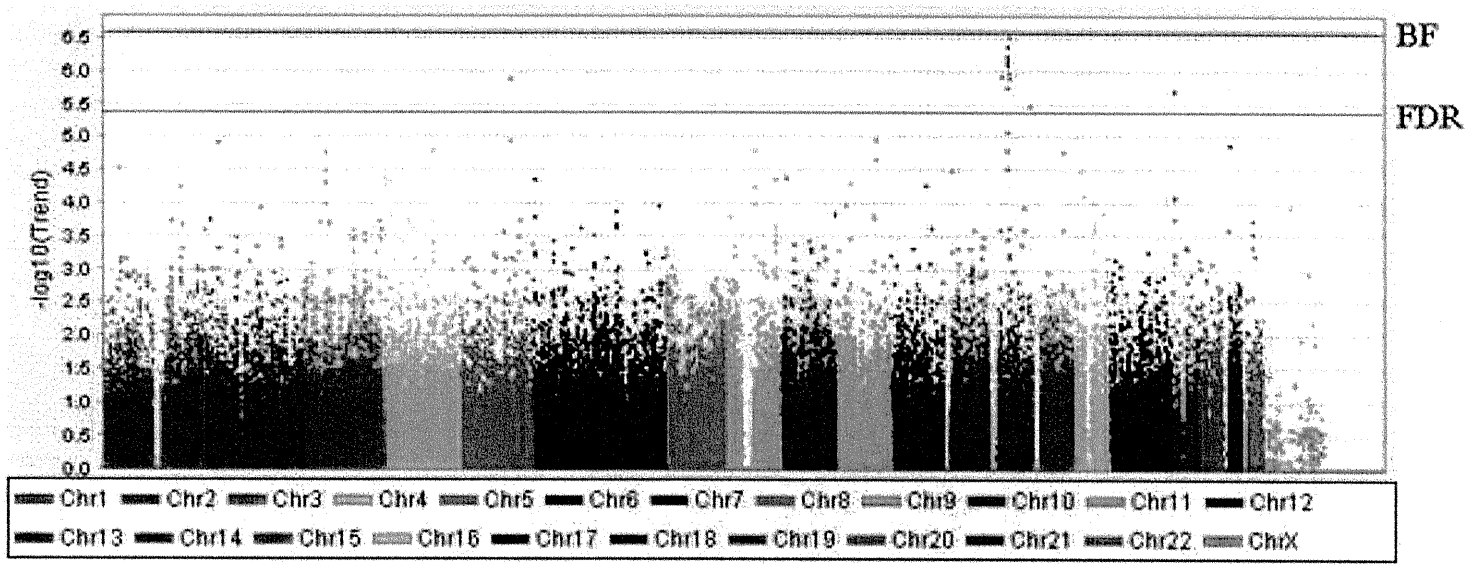

Figure 2 Kurose et al.

Figure 3
Click here to download high resolution image

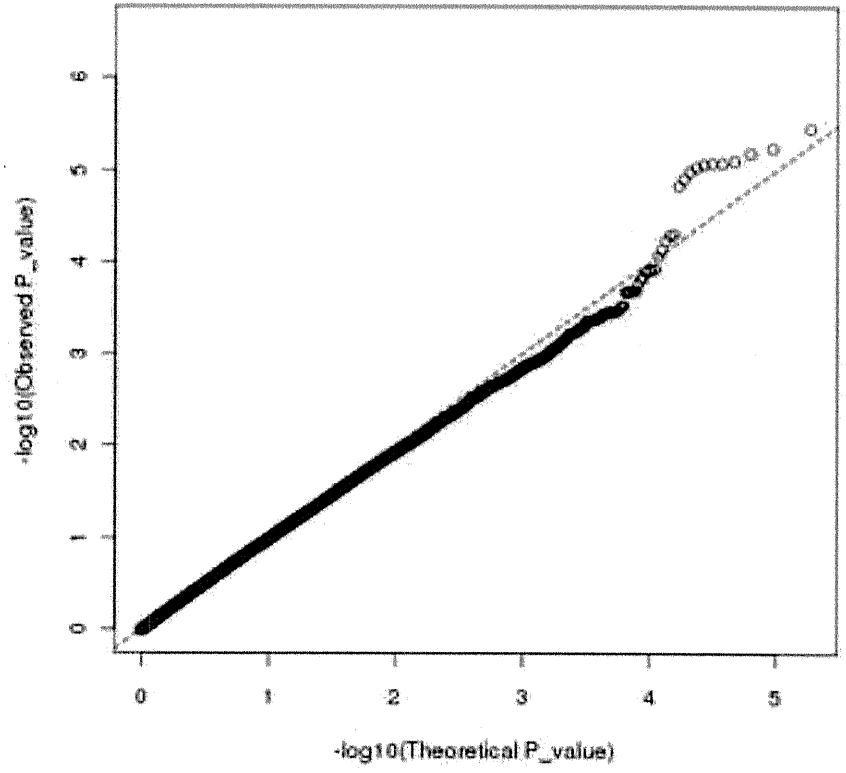

Figure 3 Kurose et al.

Figure 4
Click here to download high resolution image

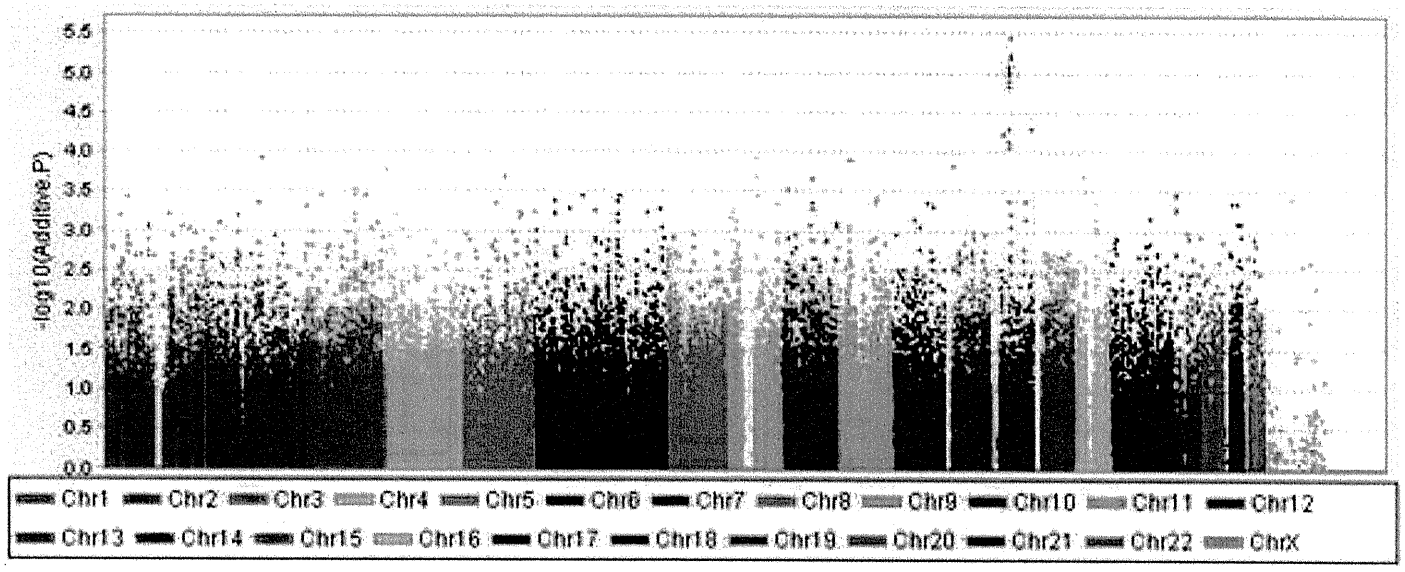

Figure 4 Kurose et al.