Identification of four genes as novel susceptibility loci for early-onset type 2 diabetes mellitus, metabolic syndrome, or hyperuricemia

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Abstract. Given that early-onset type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS), and hyperuricemia have been shown to have strong genetic components, the statistical power of a genetic association study may be increased by focusing on early-onset subjects with these conditions. Although genome-wide association studies have identified various genes and loci significantly associated with T2DM, MetS, and hyperuricemia, genetic variants that contribute to predisposition to these conditions in Japanese subjects remain to be identified definitively. We performed exome-wide association studies (EWASs) for early-onset T2DM, MetS, or hyperuricemia to identify genetic variants that confer susceptibility to these conditions. A total of 8,102 individuals aged ≤65 years were enrolled in the present study. The EWAS for T2DM was performed with 7,407 subjects (1,696 cases, 5,711 controls), that for MetS with 4,215 subjects (2,296 cases, 1,919 controls), and that for hyperuricemia with 7,919 subjects (1,365 cases, 6,554 controls). Single nucleotide polymorphisms (SNPs) were genotyped with Illumina Human Exome-12 DNA Analysis BeadChip or Infinium Exome-24 BeadChip arrays. The relationship of allele frequencies for 31,210, 31,521, or 31,142 SNPs that passed quality control for T2DM, MetS, or hyperuricemia, respectively, was examined with Fisher's exact test. To compensate for multiple comparisons of genotypes with T2DM, MetS, or hyperuricemia, we applied Bonferroni's correction for statistical significance of association. The EWAS of allele frequencies revealed that four, six, or nine SNPs were significantly associated with T2DM ($P<1.60\times10^{-6}$), MetS ($P<1.59\times10^{-6}$), or hyperuricemia ($P<1.61\times10^{-6}$), respectively. Multivariable logistic regression analysis with adjustment for age and sex revealed that three, six, or nine SNPs were significantly related to T2DM (P<0.0031), MetS (P<0.0021), or hyperuricemia (P<0.0014). After examination of the association of identified SNPs to T2DM-, MetS-, or hyperuricemia-related traits, linkage disequilibrium of the SNPs, and results of previous genome-wide association studies, newly identified *ZNF860* and *OR4F6* were the susceptibility loci for T2DM, *OR52E4* and *OR4F6* for MetS, and *HERPUD2* for hyperuricemia. Given that *OR4F6* was significantly associated with both T2DM and MetS, we newly identified four genes (*ZNF860, OR4F6, OR52E4, HERPUD2*) that confer susceptibility to early-onset T2DM, MetS, or hyperuricemia. Determination of genotypes for the SNPs in these genes may prove informative for assessment of the genetic risk for T2DM, MetS, or hyperuricemia.

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Introduction

Diabetes mellitus (DM) has reached epidemic proportions and affects more than 382 million individuals worldwide (1): The number of patients with DM is expected to increase beyond 592 million individuals by 2035 (1). Approximately 90 to 95% of individuals with DM have type 2 DM (T2DM), the characteristics of which can range from predominant insulin deficiency with relatively minor insulin resistance to

predominant insulin resistance with relatively minor insulin deficiency (2). T2DM is a major cause of nephropathy, retinopathy, and neuropathy as well as cardiovascular disease and stroke (3,4). Although obesity resulting from a sedentary lifestyle and overeating is an important risk factor for T2DM, genetic components are involved in the pathogenesis of this condition, given that a positive family history confers a 2.4-fold increased risk for T2DM (5). The heritability of T2DM has been estimated to be 50 to 60% (6).

Genome-wide association studies (GWASs) and meta-analyses have identified >120 susceptibility loci for T2DM (7) in individuals of European (8-13) or African (14) ancestry, in East Asians (15), or in multiple ethnic groups (16,17). Among Japanese, GWASs have identified *KCNQ1*, *UBE2E2*, and *C2CD4A-B* (18-20) as susceptibility genes for T2DM, and a recent meta-analysis identified an additional seven susceptibility loci for this condition (21). Genetic variants that contribute to predisposition to T2DM in Japanese subjects, however, remain to be identified definitively.

Metabolic syndrome (MetS) is a cluster of metabolic traits including abdominal obesity, an increased serum triglycerides, a decreased serum high-density lipoprotein (HDL) cholesterol, high blood pressure (BP), and an increased fasting plasma glucose (FPG) level (22). MetS is a risk factor for atherosclerotic cardiovascular disease, DM (23), and cancer (24). The etiology of MetS is highly complex, with both genetic and environmental factors being thought to play important roles. The heritability of MetS has been estimated to be approximately 50% (25) and traits of this syndrome to be 28 to 48% (26).

GWASs have suggested various loci or genes involved in predisposition to MetS or to traits of this syndrome in individuals of European (27,28) or African (29) ancestry or in Asian Indians (30) or Chinese individuals (31). However, genetic variants that contribute to predisposition to MetS in Japanese individuals remain to be identified definitively.

Circulating uric acid levels are regulated by multiple renal transporters by mediating the excretion or reabsorption of uric acid in the proximal kidney tubules (32). Hyperuricemia is an important risk factor for gout, a common inflammatory arthritis (33), as well as for cardiovascular disease (34) and cancer (35). The heritability of the serum concentration of uric acid has been estimated to be 40% (36), suggesting that genetic variants contribute to regulation of the serum uric acid level by influencing uric acid synthesis, excretion, or reabsorption (36,37).

GWASs have identified single nucleotide polymorphisms (SNPs) significantly associated with the serum uric acid concentration or the prevalence of gout (38-45). A large-scale GWAS in European ancestry populations identified 28 loci that influence the serum concentration of uric acid (46). Although several SNPs have been shown to be associated with gout in Japanese (47,48), genetic variants that contribute to predisposition to hyperuricemia in Japanese remain to be identified definitively.

In a family study of T2DM, a heritability of T2DM was higher in early-onset than late-onset individuals (6). These observations indicate that early-onset T2DM has a strong genetic component (6,49). Similar to T2DM, early-onset forms of MetS (50,51), hyperuricemia, and gout (52,53) have been shown to have strong genetic components. Given that genetic contribution may be greater in early-onset forms of T2DM, MetS, and hyperuricemia than in late-onset forms, statistical power of the genetic association study may be increased by focusing on early-onset subjects with these conditions.

In the present study, we performed exome-wide association studies (EWASs) with the use of human exome array-based genotyping methods to identify genetic variants that confer susceptibility to early-onset T2DM, MetS, or hyperuricemia in Japanese patients. To increase the statistical power of EWASs, early-onset subjects were examined.

Materials and methods

Study subjects. In previous studies, the median age of subjects with T2DM (54), MetS (55), or hyperuricemia (56) was 68, 64, or 62 years, respectively. We thus defined subjects aged ≤65 years as early-onset cases in the present study. A total of 8,102 individuals aged ≤65 years were examined. The subjects were recruited from individuals either who visited outpatient clinics of or were admitted to participating hospitals in Japan (Gifu Prefectural Tajimi Hospital, Tajimi; Gifu Prefectural General Medical Center, Gifu; Japanese Red Cross Nagoya First Hospital, Nagoya; Northern Mie Medical Center Inabe General Hospital, Inabe; Hirosaki University Hospital and Hirosaki Stroke and Rehabilitation Center, Hirosaki) because of various symptoms or for an annual health checkup between October 2002 and March 2014; or who were community-dwelling individuals recruited to a population-based cohort study in Inabe between March 2010 and september 2014 (57).

T2DM was defined according to the criteria of the World Health Organization as described previously (2,58,59). Subjects with T2DM had an FPG level of ≥6.93 mmol/l (126 mg/dl) or a blood hemoglobin A_{1c} content of ≥6.5% or were taking antidiabetes medication. Individuals with T1DM, maturity-onset diabetes of the young, DM associated with mitochondrial diseases or single-gene disorders, pancreatic diseases, or other metabolic or endocrinologic diseases were excluded from the study. Those taking medications that may cause secondary DM were also excluded. The control subjects had an FPG level of <6.05 mmol/l (110 mg/dl), a blood hemoglobin A_{1c} content of <6.2%, and no history of DM or of having taken antidiabetes medication. We thus examined 1,696 subjects with T2DM and 5,711 controls.

Diagnosis of MetS was based on a modified version of the definition proposed by the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity (22). We used cut-off values for waist circumference of ≥90 cm in men or ≥80 cm in women on the basis of a recommendation of the International Diabetes Association (22). A total of 2,296 subjects with MetS thus had three or more of the following five components: i) A waist circumference of ≥90 cm for men or ≥80 cm for women; ii) a serum triglyceride concentration of ≥1.65 mmol/l (150 mg/dl) or drug treatment for elevated triglycerides; iii) a serum HDL-cholesterol concentration of <1.04 mmol/l (40 mg/dl) for men or <1.30 mmol/l (50 mg/dl) for women; iv) a systolic BP of ≥130 mmHg, diastolic BP of ≥85 mmHg, or drug treatment for hypertension; and v) an FPG level of ≥5.50 mmol/l (100 mg/dl) or drug treatment

Table I. Characteristics of subjects with type 2 diabetes mellitus and control individuals.

Characteristic	Control	Type 2 diabetes mellitus	P-value
No. of subjects	5,711	1,696	
Age (years)	50.4±10.2	56.3±7.2	<0.0001
Sex (men/women, %)	52.1/47.9	76.2/23.8	<0.0001
Smoking (%)	40.0	47.5	0.0105
Obesity (%)	28.1	45.2	<0.0001
Body mass index (kg/m^2)	22.9±3.3	24.7±3.9	<0.0001
Hypertension (%)	32.0	71.0	<0.0001
Systolic BP (mmHg)	122±20	140±27	<0.0001
Diastolic BP (mmHg)	75±13	80±15	<0.0001
Fasting plasma glucose (mmol/l)	5.17±0.48	8.95±3.77	<0.0001
Blood hemoglobin A_{1c} (%)	5.48±0.31	7.33±1.87	<0.0001
Dyslipidemia (%)	54.4	80.2	<0.0001
Serum triglycerides (mmol/l)	1.29±0.89	1.82±1.47	<0.0001
Serum HDL-cholesterol (mmol/l)	1.62±0.46	1.32±0.43	<0.0001
Serum LDL-cholesterol (mmol/l)	3.14±0.82	3.15±0.97	0.8052
Chronic kidney disease (%)	10.9	26.8	<0.0001
Serum creatinine (μmol/l)	70.4±63.1	93.1±122.6	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	78.4±18.9	73.2±24.6	<0.0001
Hyperuricemia (%)	14.5	22.9	<0.0001
Serum uric acid (μmol/l)	321±90	340±102	<0.0001

Quantitative data are means ± standard deviations and were compared between subjects with type 2 diabetes mellitus and controls with the unpaired Student's t-test. Categorical data were compared between two groups with Pearson's Chi-square test. Based on Bonferroni's correction, a P-value of <0.0026 (0.05/19) was considered statistically significant. Definitions of diseases: Obesity, body mass index of ≥25 kg/m^2; hypertension, systolic BP of ≥140 mmHg, diastolic BP of ≥90 mmHg, or taking of anti-hypertensive medication; dyslipidemia, serum triglyceride concentration of ≥1.65 mmol/l, serum HDL-cholesterol concentration of <1.04 mmol/l, serum LDL-cholesterol concentration of ≥3.64 mmol/l, or taking of anti-dyslipidemic medication; chronic kidney disease, eGFR of <60 ml min^{-1} 1.73 m^{-2}; hyperuricemia, serum uric acid concentration of >416 μmol/l or taking of uric acid-lowering medication. BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate.

for elevated glucose. History of obesity, dyslipidemia, hypertension, or DM was evaluated with a detailed questionnaire. The control subjects comprised 1,919 individuals who had none of the five traits of MetS.

Hyperuricemia was defined as a serum uric acid concentration of >416 μmol/l (7 mg/dl) or taking of uric acid-lowering medication. Individuals taking drugs that potentially caused secondary hyperuricemia were excluded. The control individuals for the study of hyperuricemia had a serum uric acid concentration of ≤416 μmol/l and had no history of hyperuricemia or gout or of taking uric acid-lowering medication. Thus, we examined 1,365 subjects with hyperuricemia and 6,554 controls.

The 1,040 subjects with both T2DM and MetS as well as 1,884 controls overlapped between the corresponding studies, as did the 375 subjects with both T2DM and hyperuricemia and 4,809 controls as well as the 628 subjects with both MetS and hyperuricemia and 1,771 controls.

EWASs. Venous blood (5-7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), peripheral blood leukocytes were isolated, and genomic DNA was extracted from these cells either with the use of a kit (Genomix from Talent Srl, Trieste, Italy or SMITEST EX-R&D from Medical & Biological Laboratories, Co., Ltd., Nagoya, Japan).

The EWAS for T2DM, MetS, or hyperuricemia included 7,407 individuals (1,696 subjects with T2DM, 5,711 controls), 4,215 individuals (2,296 subjects with MetS, 1,919 controls), or 7,919 individuals (1,365 subjects with hyperuricemia, 6,554 controls), respectively. The EWASs were performed with the use of a Human Exome-12 v1.2 DNA Analysis BeadChip or Infinium Exome-24 v1.0 BeadChip (Illumina, San Diego, CA, USA). These exome arrays include putative functional exonic variants selected from ~12,000 individual exome and whole-genome sequences. The exonic content consisted of ~244,000 SNPs from diverse populations, including European, African, Chinese, and Hispanic individuals (60). SNPs contained in only one exome array (~2.6% of all SNPs) were excluded from analysis. We performed quality control (61) as follows: i) Genotyping data with a call rate of <97% were discarded, with the mean call rate for the remaining data being 99.9%. ii) Sex specification was checked for all the samples, and those for which sex phenotype in the clinical records was inconsistent with genetic sex were discarded. iii) Duplicated samples and cryptic relatedness were checked by calculation

Table II. Characteristics of subjects with metabolic syndrome and control individuals.

Characteristic	Control	Metabolic syndrome	P-value
No. of subjects	1,919	2,296	
Age (years)	47.0±10.7	55.0±8.0	<0.0001
Sex (men/women, %)	42.3/57.7	67.9/32.1	<0.0001
Smoking (%)	35.2	46.1	<0.0001
Waist circumference (cm)	74.0±6.0	88.3±9.0	<0.0001
Body mass index (kg/m^2)	20.8±2.2	26.0±3.7	<0.0001
Systolic BP (mmHg)	109±11	140±25	<0.0001
Diastolic BP (mmHg)	67±9	83±14	<0.0001
Fasting plasma glucose (mmol/l)	4.97±0.36	7.25±3.07	<0.0001
Blood hemoglobin A_{1c} (%)	5.39±0.29	6.58±1.60	<0.0001
Serum triglycerides (mmol/l)	0.82±0.30	2.17±1.46	<0.0001
Serum HDL-cholesterol (mmol/l)	1.86±0.42	1.24±0.37	<0.0001
Serum LDL-cholesterol (mmol/l)	2.95±0.74	3.28±0.96	<0.0001
Chronic kidney disease (%)	15.9	56.6	<0.0001
Serum creatinine (μmol/l)	63.0±18.2	84.4±97.5	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	81.6±17.0	73.3±23.5	<0.0001
Hyperuricemia (%)	5.2	28.0	<0.0001
Serum uric acid (μmol/l)	286±76	356±97	<0.0001

Quantitative data are means ± standard deviations and were compared between subjects with metabolic syndrome and controls with the unpaired Student's t-test. Categorical data were compared between two groups with Pearson's Chi-square test. Base on Bonferroni's correction, a P-value of <0.0029 (0.05/17) was considered statistically significant. BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate. Definitions of chronic kidney disease and hyperuricemia are described in Table I.

of identity by descent, with all the pairs of samples showing a value of >0.1875 being inspected and one sample from each pair excluded. iv) Heterozygosity of SNPs was calculated for all the samples, and those with extremely low or high heterozygosity (>3 standard deviations from the mean) were discarded. v) SNPs in sex chromosomes or mitochondrial DNA were excluded from the analysis, as were non-polymorphic SNPs or SNPs with a minor allele frequency of <1.0%. vi) SNPs whose genotype distributions deviated significantly (P<0.01) from the Hardy-Weinberg equilibrium in control individuals were discarded. vii) Genotype data were examined for population stratification by principal components analysis (62), and population outliers were excluded. Totals of 31,210, 31,521, or 31,142 SNPs passed quality control for the T2DM, MetS, and hyperuricemia studies, respectively, and were subjected to analysis.

Statistical analysis. For analysis of characteristics of the study subjects, quantitative or categorical data were compared between individuals with T2DM, MetS, or hyperuricemia and corresponding controls with the unpaired Student's t-test or Pearson's Chi-square test, respectively. Allele frequencies were estimated by the gene counting method, and Fisher's exact test was used to identify departure from Hardy-Weinberg equilibrium. The relationship of allele frequencies of SNPs to T2DM, MetS, or hyperuricemia in the EWAS was examined using the Fisher's exact test. To compensate for multiple comparisons of allele frequencies with T2DM, MetS, or hyperuricemia, we applied Bonferroni's correction for statistical significance of association. The significance level was set at P<1.60x10^{-6} (0.05/31210), P<1.59x10^{-6} (0.05/31521), or P<1.61x10^{-6} (0.05/31142) for the EWAS of T2DM, MetS, or hyperuricemia, respectively. The inflation factor (λ) was 1.04 for T2DM, 1.05 for MetS, or 1.09 for hyperuricemia. Multivariable logistic regression analysis was performed with T2DM, MetS, hyperuricemia as a dependent variable and independent variables including age, sex (0, woman; 1, man), and genotype of each SNP. Genotypes of each SNP were assessed according to dominant [0, *AA*; 1, *AB* + *BB* (*A*, major allele; *B*, minor allele)], recessive (0, *AA* + *AB*; 1, *BB*), and additive genetic models, and the P-value, OR, and 95% confidence interval were calculated. Additive models comprised additive 1 (0, *AA*; 1, *AB*; 0, *BB*) and additive 2 (0, *AA*; 0, *AB*; 1, *BB*) scenarios, which were analyzed simultaneously with a single statistical model. The relation of genotypes of identified SNPs to T2DM-, MetS-, or hyperuricemia-related traits was examined by one-way analysis of variance (ANOVA). Bonferroni's correction was also applied to other statistical analysis as indicated. Statistical tests were performed with JMP Genomics version 9.0 software (SAS Institute, Cary, NC, USA).

Results

Characteristics of subjects. The characteristics of the 7,407 subjects enrolled in the T2DM study are shown in Table I. Age, the frequency of men, and the prevalence of obesity, hypertension, dyslipidemia, chronic kidney disease (CKD), and hyperuricemia as well as body mass index (BMI),

Table III. Characteristics of subjects with hyperuricemia and control individuals.

Characteristic	Control	Hyperuricemia	P-value
No. of subjects	6,554	1,365	
Age (years)	51.5±9.9	52.9±9.1	<0.0001
Sex (men/women, %)	52.6 /47.4	90.2/9.8	<0.0001
Smoking (%)	38.3	61.6	<0.0001
Obesity (%)	30.0	47.4	<0.0001
Body mass index (kg/m^2)	23.2±3.5	24.8±3.8	<0.0001
Hypertension (%)	38.2	62.0	<0.0001
Systolic BP (mmHg)	126±23	134±24	<0.0001
Diastolic BP (mmHg)	75±14	82±15	<0.0001
Diabetes mellitus (%)	20.8	31.6	<0.0001
Fasting plasma glucose (mmol/l)	6.04±2.38	6.25±2.27	0.0022
Blood hemoglobin A_{1c} (%)	5.96±1.26	6.10±1.28	0.0042
Dyslipidemia (%)	57.9	79.0	<0.0001
Serum triglycerides (mmol/l)	1.34±0.99	1.90±1.38	<0.0001
Serum HDL-cholesterol (mmol/l)	1.57±0.47	1.37±0.42	<0.0001
Serum LDL-cholesterol (mmol/l)	3.15±0.85	3.20±0.93	0.0506
Chronic kidney disease (%)	11.0	33.0	<0.0001
Serum creatinine (μmol/l)	68.7±52.3	109.4±150.3	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	79.2±18.3	67.4±25.8	<0.0001
Serum uric acid (μmol/l)	296±67	455±78	<0.0001

Quantitative data are means ± standard deviations and were compared between subjects with hyperuricemia and controls with the unpaired Student's t-test. Categorical data were compared between two groups with Pearson's Chi-square test. Base on Bonferroni's correction, a P-value of <0.0026 (0.05/19) was considered statistically significant. Definition of diabetes mellitus, fasting plasma glucose level of ≥6.93 mmol/l, blood hemoglobin A_{1c} content of ≥6.5%, or taking of antidiabetes medication. Definitions of other diseases are described in Table I. BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate.

systolic and diastolic BP, and serum concentrations of triglycerides, creatinine, and uric acid were greater, whereas serum concentration of HDL-cholesterol and estimated glomerular filtration rate (eGFR) were lower, in subjects with T2DM than in controls.

Characteristics of the 4,215 subjects enrolled in the MetS study are shown in Table II. Age, the frequency of men, and the prevalence of smoking, CKD, and hyperuricemia as well as BMI, blood hemoglobin A_{1c} content, and serum concentrations of low-density lipoprotein (LDL) cholesterol, creatinine, and uric acid were greater, whereas eGFR were lower, in subjects with MetS than in controls.

Characteristics of the 7,919 subjects enrolled in the hyperuricemia study are shown in Table III. Age, the frequency of men, and the prevalence of smoking, obesity, hypertension, DM, dyslipidemia, and CKD as well as BMI, systolic and diastolic BP, FPG level, and serum concentrations of triglycerides and creatinine were greater, whereas the serum concentration of HDL-cholesterol and eGFR were lower, in subjects with hyperuricemia than in controls.

EWAS for T2DM, MetS, or hyperuricemia. We examined the relationship of allele frequencies of 31,210 SNPs that passed quality control to T2DM with the use of Fisher's exact test. After Bonferroni's correction, four SNPs were significantly ($P<1.60x10^{-6}$) associated with T2DM (Table IV). The relationship of allele frequencies of 31,521 SNPs to MetS was examined with Fisher's exact test. After Bonferroni's correction, six SNPs were significantly ($P<1.59x10^{-6}$) associated with MetS (Table V). The relationship of allele frequencies of 31,142 SNPs to hyperuricemia was also examined with Fisher's exact test. After Bonferroni's correction, nine SNPs were significantly ($P<1.61x10^{-6}$) associated with hyperuricemia (Table VI).

Multivariable logistic regression analysis of the relationship of SNPs to T2DM, MetS, or hyperuricemia. The relationship of the four identified SNPs in the EWAS of T2DM to this condition was further examined by multivariable logistic regression analysis with adjustment for age and sex (Table VII). Three SNPs (rs141569282 of *OR4F6*, rs140232911 of *ZNF860*, rs150552771 of *LPGAT1*) were significantly [P<0.0031 (0.05/16) in at least one genetic model] related to T2DM. The relationship of the six SNPs identified in the EWAS for MetS to this condition was examined by multivariable logistic regression analysis with adjustment for age and sex (Table VIII). All the SNPs were significantly [P<0.0021 (0.05/24)] related to MetS. The relationship of the 9 SNPs identified by the EWAS of hyperuricemia to this condition was also examined by multivariable logistic regression analysis with adjustment for age and sex (Table IX). All SNPs were significantly [P<0.0014 (0.05/36)] related to hyperuricemia.

Table IV. The four SNPs significantly ($P<1.60x10^{-6}$) associated with type 2 diabetes mellitus in the exome-wide association study.

Gene	SNP	Nucleotide substitution[a]	Amino acid substitution	Chromosome	Position	MAF (%)	Allele odds ratio	P-value (allele frequency)
OR4F6	rs141569282	G/A	A117T	15	101806068	1.7	0.29	$2.45x10^{-12}$
ZNF860	rs140232911	C/T	S161L	3	31989561	10.4	3.67	$1.25x10^{-8}$
LPGAT1	rs150552771	T/C	K200E	1	211783358	5.0	20.00	$2.08x10^{-8}$
KRR1	rs17115182	G/A	P43S	12	75508405	7.0	3.57	$8.08x10^{-7}$

Allele frequencies were analyzed with Fisher's exact test. [a]Major allele/minor allele. MAF, minor allele frequency; SNP, single nucleotide polymorphism.

Table V. The six SNPs significantly ($P<1.59x10^{-6}$) associated with metabolic syndrome in the exome-wide association study.

Gene	SNP	Nucleotide substitution[a]	Amino acid substitution	Chromosome	Position	MAF (%)	Allele odds ratio	P-value (allele frequency)
CCDC6	rs1053266	A/C	T470P	10	59792934	28.6	2.33	$3.18x10^{-52}$
APOA5	rs2075291	G/T	G185C	11	116790676	7.3	1.72	$3.94x10^{-11}$
OR4F6	rs141569282	G/A	A117T	15	101806068	1.7	0.34	$1.35x10^{-10}$
HLA-DQB2	rs200716952	C/T	A167T	6	32758997	2.5	0.47	$2.15x10^{-10}$
OR52E4	rs11823828	T/G	F227L	11	5884973	36.6	1.27	$7.99x10^{-7}$
	rs17482753	G/T		8	19975135	12.6	0.73	$1.55x10^{-6}$

Allele frequencies were analyzed with Fisher's exact test. [a]Major allele/minor allele. MAF, minor allele frequency; SNP, single nucleotide polymorphism.

Table VI. The nine SNPs significantly ($P<1.61x10^{-6}$) associated with hyperuricemia in the exome-wide association study.

Gene	SNP	Nucleotide substitution[a]	Amino acid substitution	Chromosome	Position	MAF (%)	Allele odds ratio	P-value (allele frequency)
SLC22A12	rs121907892	G/A	W224*	11	64593747	2.4	0.07	$3.13x10^{-24}$
BRAP	rs3782886	A/G		12	111672685	29.3	0.73	$1.78x10^{-11}$
ACAD10	rs11066015	G/A		12	111730205	27.5	0.73	$7.72x10^{-11}$
HECTD4	rs11066280	T/A		12	112379979	29.0	0.75	$1.36x10^{-9}$
	rs12229654	T/G		12	110976657	22.5	0.74	$1.83x10^{-9}$
SLC2A9	rs3775948	G/C		4	9993558	42.4	0.80	$1.49x10^{-7}$
HERPUD2	rs2305335	T/A	L200H	7	35638368	1.6	2.13	$2.63x10^{-7}$
CCDC63	rs10774610	T/C		12	110902439	23.7	0.78	$1.14x10^{-6}$
CCDC63	rs10849915	T/C		12	110895818	23.6	0.78	$1.23x10^{-6}$

Allele frequencies were analyzed with Fisher's exact test. [a]Major allele/minor allele. MAF, minor allele frequency; SNP, single nucleotide polymorphism. *, stop codon.

Table VII. Association of SNPs to type 2 diabetes mellitus as determined by multivariable logistic regression analysis.

			Dominant			Recessive			Additive 1			Additive 2		
Gene	SNP		P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI
OR4F6	rs141569282	G/A	<0.0001	0.31	0.21-0.47				<0.0001	0.31	0.21-0.47			
ZNF860	rs140232911	C/T	0.0005	2.26	1.43-3.59				0.0005	2.26	1.43-3.59			
LPGAT1	rs150552771	T/C	0.0001	12.09	3.40-43.00				0.0001	12.09	3.40-43.00			

Multivariable logistic regression analysis was performed with adjustment for age and sex. Based on Bonferroni's correction; P-value of <0.0031 (0.05/16) was considered statistically significant. OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism.

Table VIII. Relation of SNPs to metabolic syndrome as determined by multivariable logistic regression analysis.

			Dominant			Recessive			Additive 1			Additive 2		
Gene	SNP		P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI
CCDC6	rs1053266	A/C	<0.0001	1.97	1.70-2.28	<0.0001	2.73	1.96-3.78	<0.0001	1.79	1.53-2.09	<0.0001	3.28	2.36-4.57
APOA5	rs2075291	G/T	<0.0001	1.85	1.51-2.25	0.1335			<0.0001	1.84	1.50-2.25	0.0947		
OR4F6	rs141569282	G/A	<0.0001	0.37	0.25-0.54				<0.0001	0.37	0.25-0.54			
HLA-DQB2	rs200716952	C/T	<0.0001	0.58	0.44-0.76				<0.0001	0.58	0.44-0.76			
OR52E4	rs11823828	T/G	0.0177	1.19	1.03-1.37	<0.0001	1.76	1.43-2.17	0.6181			<0.0001	1.80	1.44-2.24
	rs17482753	G/T	0.0003	0.74	0.63-0.87	0.5086			0.0003	0.74	0.63-0.87	0.3690		

Multivariable logistic regression analysis was performed with adjustment for age and sex. Based on Bonferroni's correction; P-value of <0.0021 (0.05/24) was considered statistically significant. OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism.

Table IX. Association of SNPs to hyperuricemia as determined by multivariable logistic regression analysis.

			Dominant			Recessive			Additive 1			Additive 2		
Gene	SNP		P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI
SLC22A12	rs121907892	G/A	<0.0001	0.06	0.03-0.14	0.9959			<0.0001	0.06	0.03-0.14	0.9959		
BRAP	rs3782886	A/G	<0.0001	0.66	0.58-0.74	<0.0001	0.53	0.42-0.67	<0.0001	0.71	0.62-0.81	<0.0001	0.45	0.36-0.58
ACAD10	rs11066015	G/A	<0.0001	0.66	0.58-0.75	<0.0001	0.51	0.40-0.66	<0.0001	0.72	0.63-0.82	<0.0001	0.45	0.35-0.57
HECTD4	rs11066280	T/A	<0.0001	0.69	0.61-0.78	<0.0001	0.53	0.42-0.67	<0.0001	0.75	0.66-0.86	<0.0001	0.47	0.37-0.60
	rs12229654	T/G	<0.0001	0.69	0.61-0.78	<0.0001	0.47	0.34-0.64	<0.0001	0.74	0.65-0.84	<0.0001	0.42	0.31-0.58
SLC2A9	rs3775948	G/C	<0.0001	0.77	0.68-0.88	<0.0001	0.67	0.57-0.80	0.0109	0.84	0.73-0.96	<0.0001	0.61	0.50-0.73
HERPUD2	rs2305335	T/A	<0.0001	1.89	1.40-2.53	0.4553			<0.0001	1.88	1.40-2.52	0.4345		
CCDC63	rs10774610	T/C	<0.0001	0.71	0.62-0.80	0.0048	0.67	0.51-0.89	<0.0001	0.73	0.64-0.83	0.0003	0.60	0.45-0.79
CCDC63	rs10849915	T/C	<0.0001	0.71	0.62-0.80	0.0036	0.66	0.50-0.87	<0.0001	0.73	0.64-0.83	0.0002	0.59	0.44-0.78

Multivariable logistic regression analysis was performed with adjustment for age and sex. Based on Bonferroni's correction; P-value of <0.0014 (0.05/36) was considered statistically significant. OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism.

Table X. Relation of SNPs identified in the present study to fasting plasma glucose level and blood hemoglobin A_{1c} content.

Gene	SNP		Pasting plasma glucose (mmol/l)		P-value	Blood hemoglobin A_{1c} (%)		P-value
OR4F6	rs141569282	G/A	GG 6.26±2.76	GA 5.35±0.71	**<0.0001**	GG 6.15±1.46	GA 5.57±0.43	**<0.0001**
ZNF860	rs140232911	C/T	CC 6.06±2.46	CT 6.25±1.97	0.6279	CC 5.52±0.73	CT 6.00±1.32	**0.0070**
LPGAT1	rs150552771	T/C	TT 6.05±2.45	TC 6.63±3.88	0.6320	TT 5.35±0.53	TC 5.99±1.31	0.3280

Data are means ± standard deviations and compared among genotypes by one-way analysis of variance. Based on Bonferroni's correction, P-values of <0.0083 (0.05/6) were considered statistically significant and are shown in bold. SNP, single nucleotide polymorphism.

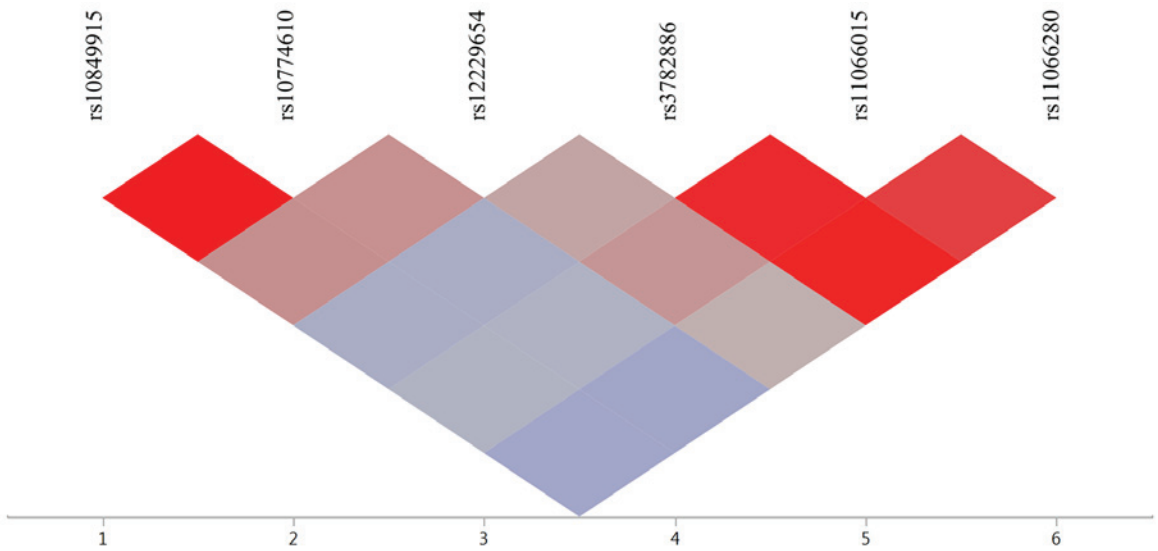

Figure 1. LD map of six SNPs at 12q24.11 to 12q24.13 associated with hyperuricemia. LD was calculated as the square of the correlation coefficient (r^2) and the strength of LD increases according to the color order of blue < gray < red. LD, linkage disequilibrium.

Relationship of SNPs associated with T2DM to FPG level or blood hemoglobin A_{1c} content. We examined the relationship of genotypes for the 3 SNPs associated with T2DM to FPG level or blood hemoglobin A_{1c} content by one-way ANOVA (Table X). A SNP rs141569282 of *OR4F6* was significantly [P<0.0083 (0.05/6)] associated with FPG level and blood hemoglobin A_{1c} content, and rs140232911 of *ZNF860* with blood hemoglobin A_{1c} content.

Relationship of SNPs associated with MetS to each trait of MetS. We examined the relationship of genotypes for the six SNPs associated with MetS to waist circumference, serum concentrations of triglycerides and HDL-cholesterol, systolic and diastolic BP, and FPG level by one-way ANOVA (Table XI). Three SNPs (rs1053266 of *CCDC6*, rs141569282 of *OR4F6*, rs200716952 of *HLA-DQB2*) were significantly (P<0.0014) related to all the traits; rs2075291 of *APOA5* to waist circumference and serum concentrations of triglycerides and HDL-cholesterol; rs11823828 of *OR52E4* to serum concentrations of triglycerides and HDL-cholesterol, systolic BP, and FPG level; and rs17482753 at chromosome 8p21.3 to serum concentrations of triglycerides and HDL-cholesterol.

Relationship of SNPs associated with hyperuricemia to serum concentrations of uric acid. We examined the relationship of the 9 SNPs associated with hyperuricemia to serum concentrations of uric acid by one-way ANOVA (Table XII). Seven SNPs (rs121907892 of *SLC22A12*, rs3782886 of *BRAP*, rs11066015 of *ACAD10*, rs11066280 of *HECTD4*, rs12229654 at chromosome 12q24.1, rs3775948 of *SLC2A9*, rs2305335 of *HERPUD2*) were significantly (P<0.0056) associated with serum concentrations of uric acid.

Linkage disequilibrium (LD) analyses. We examined LD in SNPs associated with MetS or hyperuricemia. For the MetS study, rs11823828 of *OR52E4* and rs2075291 of *APOA5* were not in LD [square of the correlation coefficient (r^2)<0.001]. For the hyperuricemia study, 6 SNPs were located at chromosomal 12q24.11 to 12q24.13. LD plots of these SNPs are shown in Fig. 1. Strong LD was observed between rs10849915 and rs10774610 of *CCDC63* (r^2, 0.991) and among rs3782886 of *BRAP*, rs11066015 of *ACAD10*, and rs11066280 of *HECTD4* (r^2, 0.876 to 0.941).

Relation of genes, chromosomal loci, and SNPs identified in the present study to phenotypes reported by previous GWASs.

Table XI. Relationship of SNPs identified in the present study to each trait of metabolic syndrome.

Gene	SNP		Waist circumference (cm)			P-value
CCDC6	rs1053266	A/C	AA	AC	CC	**<0.0001**
			81.0±10.6	83.8±10.4	87.0±8.6	
APOA5	rs2075291	G/T	GG	GT	TT	**0.0012**
			82.0±10.6	83.7±10.3	82.8±10.3	
OR4F6	rs141569282	G/A	GG	GA		**<0.0001**
			83.2±10.4	79.1±10.8		
HLA-DQB2	rs200716952	C/T	CC	CT		**<0.0001**
			82.5±10.5	79.5±10.7		
OR52E4	rs11823828	T/G	TT	TG	GG	0.0159
			81.8±10.8	81.8±10.8	83.2±10.3	
	rs17482753	G/T	GG	GT	TT	0.0070
			82.5±10.7	81.3±10.3	83.2±10.9	
Gene	SNP		Serum triglycerides (mmol/l)			P-value
CCDC6	rs1053266	A/C	AA	AC	CC	**<0.0001**
			1.45±1.21	1.79±1.46	2.13±1.33	
APOA5	rs2075291	G/T	GG	GT	TT	**<0.0001**
			1.51±1.15	2.06±1.84	2.71±2.63	
OR4F6	rs141569282	G/A	GG	GA		**<0.0001**
			1.71±1.38	1.19±1.00		
HLA-DQB2	rs200716952	C/T	CC	CT		**<0.0001**
			1.62±1.32	1.26±1.11		
OR52E4	rs11823828	T/G	TT	TG	GG	**0.0003**
			1.52±1.22	1.49±1.15	1.74±1.41	
	rs17482753	G/T	GG	GT	TT	**<0.0001**
			1.65±1.32	1.41±1.22	1.55±1.72	
Gene	SNP		Serum HDL-cholesterol (mmol/l)			P-value
CCDC6	rs1053266	A/C	AA	AC	CC	**<0.0001**
			1.59±0.49	1.39±0.48	1.17±0.35	
APOA5	rs2075291	G/T	GG	GT	TT	**<0.0001**
			1.53±0.50	1.38±0.48	1.28±0.43	
OR4F6	rs141569282	G/A	GG	GA		**<0.0001**
			1.44±0.49	1.72±0.42		
HLA-DQB2	rs200716952	C/T	CC	CT		**<0.0001**
			1.49±0.47	1.69±0.47		
OR52E4	rs11823828	T/G	TT	TG	GG	**0.0001**
			1.54±0.49	1.54±0.51	1.44±0.47	
	rs17482753	G/T	GG	GT	TT	**<0.0001**
			1.48±0.49	1.58±0.53	1.62±0.52	
Gene	SNP		Systolic blood pressure (mmHg)			P-value
CCDC6	rs1053266	A/C	AA	AC	CC	**<0.0001**
			122±22	134±28	145±26	
APOA5	rs2075291	G/T	GG	GT	TT	0.0493
			127±25	130±24	132±30	
OR4F6	rs141569282	G/A	GG	GA		**<0.0001**
			131±27	115±16		
HLA-DQB2	rs200716952	C/T	CC	CT		**<0.0001**
			128±26	117±17		
OR52E4	rs11823828	T/G	TT	TG	GG	**<0.0001**
			125±24	126±25	130±25	
	rs17482753	G/T	GG	GT	TT	0.0616
			128±25	126±25	130±23	

Table XI. Continued.

Gene	SNP		Diastolic blood pressure (mmHg)			P-value
CCDC6	rs1053266	A/C	AA	AC	CC	**<0.0001**
			75±14	79±16	82±16	
APOA5	rs2075291	G/T	GG	GT	TT	0.0055
			76±15	78±14	80±13	
OR4F6	rs141569282	G/A	GG	GA		**<0.0001**
			78±15	71±13		
HLA-DQB2	rs200716952	C/T	CC	CT		**0.0002**
			77±15	73±13		
OR52E4	rs11823828	T/G	TT	TG	GG	0.5502
			76±15	76±15	76±14	
	rs17482753	G/T	GG	GT	TT	0.3685
			76±15	76±15	77±14	
Gene	SNP		Fasting plasma glucose (mmol/l)			P-value
CCDC6	rs1053266	A/C	AA	AC	CC	**<0.0001**
			5.90±2.05	6.90±3.33	7.41±3.02	
APOA5	rs2075291	G/T	GG	GT	TT	0.2227
			6.25±2.57	6.46±2.77	6.15±1.77	
OR4F6	rs141569282	G/A	GG	GA		**<0.0001**
			6.53±2.87	5.34±0.76		
HLA-DQB2	rs200716952	C/T	CC	CT		**<0.0001**
			6.36±2.67	5.41±1.05		
OR52E4	rs11823828	T/G	TT	TG	GG	**0.0001**
			6.09±2.32	6.07±2.30	6.57±2.95	
	rs17482753	G/T	GG	GT	TT	0.0048
			6.35±2.63	6.03±2.42	6.55±2.78	

Data are means ± standard deviations and compared among genotypes by one-way analysis of variance. Based on Bonferroni's correction, P-values of <0.0014 (0.05/36) were considered statistically significant and are shown in bold. SNP, single nucleotide polymorphism.

We examined the genes, chromosomal loci, and SNPs identified in the present study to phenotypes previously reported by GWASs available in Genome-Wide Repository of Associations Between SNPs and Phenotypes (GRASP) Search database (https://grasp.nhlbi.nih.gov/Search.aspx) developed by the Information Technology and Applications Center, National Center for Biotechnology Information, National Heart, Lung, and Blood Institute, National Institute of Health (Bethesda, MD, USA).

In the T2DM study, none of the three genes or SNPs was shown to be associated with T2DM or diabetes-related traits in previous GWASs (Table XIII). In the MetS study, *HLA-DQB2* was shown to be related to T1DM and plasma total cholesterol; rs17482753 at chromosome 8p21.3 to plasma concentrations of triglycerides and HDL-cholesterol; *CCDC6* to serum uric acid level; and *APOA5* to plasma concentrations of triglycerides, HDL-cholesterol, and LDL-cholesterol (Table XIV). In the hyperuricemia study, *SLC2A9*, *SLC22A12*, and *BRAP* were shown to be related to serum uric acid concentrations; rs12229654 at 12q24.1 to plasma HDL-cholesterol; *ACAD10* to plasma LDL-cholesterol and T1DM; and *HECTD4* to plasma HDL-cholesterol and LDL-cholesterol and FPG level (Table XV).

Discussion

T2DM, MetS, and hyperuricemia are important public health problems because of the high prevalence of these conditions as well as risk factors for more serious conditions such as cardiovascular disease, cancer, or gout (1,3,4,23,24,33-35). Identification of genetic variants that confer susceptibility to T2DM, MetS, and hyperuricemia are thus clinically important to prevent these conditions. We have now performed EWASs for T2DM, MetS, and hyperuricemia in early-onset subjects with these conditions who likely had greater genetic components compared with late-onset individuals.

In the T2DM study, rs150552771 of *LPGAT1*, rs140232911 of *ZNF860*, and rs141569282 of *OR4F6* were significantly associated with early-onset T2DM. None of these genes was shown to be associated with T2DM or diabetes-related traits in the previous GWASs. Given that rs150552771 of *LPGAT1* was not related to FPG level or blood hemoglobin A_{1c} content, *LPGAT1* was removed from new susceptibility locus, even though this discrepancy may be attributable to the effect of medical treatment for T2DM. We have thus newly identified *ZNF860* and *OR4F6* as susceptibility loci for T2DM. A SNP rs141569282 of *OR4F6* was significantly related to FPG level

Table XII. Relationship of SNPs identified in the present study to the serum concentration of uric acid.

Gene	SNP		Serum uric acid (μmol/l)			P-value
SLC22A12	rs121907892	G/A	GG	GA	AA	**<0.0001**
			333±91	231±82	52±19	
BRAP	rs3782886	A/G	AA	AG	GG	**0.0002**
			332±97	324±91	318±88	
ACAD10	rs11066015	G/A	GG	GA	AA	**0.0004**
			332±97	324±91	319±88	
HECTD4	rs11066280	T/A	TT	TA	AA	**0.0006**
			332±96	325±92	318±87	
	rs12229654	T/G	TT	TG	GG	**0.0002**
			331±96	326±91	312±84	
SLC2A9	rs3775948	G/C	GG	GC	CC	**<0.0001**
			335±94	328±92	312±96	
HERPUD2	rs2305335	T/A	TT	TA	AA	**<0.0001**
			327±93	358±116	407±88	
CCDC63	rs10774610	T/C	TT	TC	CC	0.0067
			330±96	325±91	318±92	
CCDC63	rs10849915	T/C	TT	TC	CC	0.0083
			330±96	325±91	318±92	

Data were means ± standard deviations and compared among genotypes by one-way analysis of variance. Based on Bonferroni's correction, P-values of <0.0056 (0.05/9) were considered statistically significant and are shown in bold. SNP, single nucleotide polymorphism.

Table XIII. Relationship of genes and SNPs associated with type 2 diabetes mellitus in the present study to previously reported diabetes-related phenotypes.

Gene	SNP	Chromosome	Position	Previously reported phenotypes
LPGAT1	rs150552771	1	211783358	None
ZNF860	rs140232911	3	31989561	None
OR4F6	rs141569282	15	101806068	None

Data were obtained from Genome-wide repository of associations between SNPs and phenotypes (GRASP) search database (https://grasp.nhlbi.nih.gov/Search.aspx) with a P-value of $<1.0\text{x}10^{-6}$. SNP, single nucleotide polymorphism.

and blood hemoglobin A_{1c} content with the minor *A* allele being related to decreases in these parameters, while rs140232911 of *ZNF860* was related to blood hemoglobin A_{1c} content with the minor *T* allele being related to an increase in this parameter. Analyses of these traits as well as logistic regression analysis indicate that the *A* allele of rs141569282 in *OR4F6* is protective against T2DM, whereas the *T* allele of rs140232911 in *ZNF860* is a risk factor for this condition.

In the MetS study, six SNPs in five genes and one chromosomal locus were significantly associated with early-onset MetS. Of these genes and locus, *HLA-DQB2* (63), rs17482753 at 8p21.3 (63), and *APOA5* (63) were previously shown to be related to lipid profiles; and *CCDC6* to serum uric acid level (46). *OR52E4* or *OR4F6* has not been shown to be associated with MetS or MetS-related traits in the previous GWASs. A SNP rs11823828 of *OR52E4* was significantly related to serum concentrations of triglycerides and HDL-cholesterol, systolic BP, and FPG level; and rs141569282 of *OR4F6* to all traits. We have thus newly identified *OR52E4* and *OR4F6* as susceptibility loci for MetS. The minor *G* allele of rs11823828 in *OR52E4* was significantly related to increased serum triglycerides, decreased serum HDL-cholesterol, increased systolic BP, and increased FPG level, whereas the minor *A* allele of rs141569282 in *OR4F6* was related to reduced waist circumference, decreased serum triglycerides, increased serum HDL-cholesterol, decreased systolic and diastolic BP, and reduced FPG level. Analyses of these traits and logistic regression analysis indicate that the *G* allele of rs11823828 in *OR52E4* represents a risk factor for MetS, whereas the *A* allele of rs141569282 in *OR4F6* is protective against this condition.

In the hyperuricemia study, 9 SNPs in seven genes and one chromosomal locus were significantly associated with early-onset hyperuricemia. Of these genes and locus, *SLC2A9* (46), *SLC22A12* (46), and *BRAP* (46) were previously shown to be related to serum uric acid level; and rs12229654 at 12q24.1 (64), *ACAD10* (63), and *HECTD4* (63) to lipid profiles. *HERPUD2* or *CCDC63* has not been shown to be associated with hyperuricemia, gout, or serum uric acid level

Table XIV. Relationship of genes, chromosomal locus, and SNPs associated with metabolic syndrome in the present study to previously reported metabolic disease-related phenotypes.

Gene/chr. locus	SNP	Chr.	Position	Previously reported phenotypes
HLA-DQB2	rs200716952	6	32758997	Type 1 diabetes (17554300, 17632545), total cholesterol (20686565)
8p21.3	rs17482753	8	19975135	Triglycerides (20686565, 23063622, 19060906, 18193043, 21943158, 18179892, 19913121, 17463246), HDL-cholesterol (20686565, 23063622, 19060906, 21943158, 20031538, 20370913, 20339536, 19913121)
CCDC6	rs1053266	10	59792934	Serum urate (23263486)
OR52E4	rs11823828	11	5884973	None
APOA5	rs2075291	11	116790676	Triglycerides (20686565, 23063622, 22629316, 19060906, 21943158, 19913121, 18193043, 19802338, 23505323, 23236364, 21386085, 19197348), HDL-cholesterol (23063622, 22629316, 20686565, 21386085, 19913121, 23236364), LDL-cholesterol (20686565, 19913121), total cholesterol (20686565, 23063622, 20339536, 18179892)
OR4F6	rs141569282	15	101806068	None

Data were obtained from Genome-wide repository of associations between SNPs and phenotypes (GRASP) search database (https://grasp.nhlbi.nih.gov/Search.aspx) with a P-value of $<1.0x10^{-6}$. Numbers in parentheses are PubMed IDs. SNP, single nucleotide polymorphism; Chr., chromosome.

Table XV. Relationship of genes, chromosomal locus, and SNPs associated with hyperuricemia in the present study to previously reported metabolic disease-related phenotypes.

Gene/chr. locus	SNP	Chr.	Position	Previously reported phenotypes
SLC2A9	rs3775948	4	9993558	Serum urate (23263486)
HERPUD2	rs2305335	7	35638368	None
SLC22A12	rs121907892	11	64593747	Serum urate (20139978, 23263486, 21768215, 20884846, 19503597)
CCDC63	rs10849915	12	110895818	None
	rs10774610	12	110902439	None
12q24.1	rs12229654	12	110976657	HDL-cholesterol (21909109)
BRAP	rs3782886	12	111672685	Serum urate (23263486)
ACAD10	rs11066015	12	111730205	LDL-cholesterol (20686565), type 1 diabetes (17554300)
HECTD4	rs11066280	12	112379979	HDL-cholesterol (21572416, 21909109, 22751097), LDL-cholesterol (21572416, 20686565), fasting blood glucose (23575436)

Data were obtained from Genome-wide repository of associations between SNPs and phenotypes (GRASP) search database (https://grasp.nhlbi.nih.gov/Search.aspx) with a P-value of $<1.0x10^{-6}$. Numbers in parentheses are PubMed IDs. SNP, single nucleotide polymorphism; Chr., chromosome.

in the previous GWASs. A SNP rs2305335 of *HERPUD2* was significantly related to the serum uric acid level, whereas two SNPs of *CCDC63* were not related to this parameter. Therefore *CCDC63* was removed from new loci. We have thus newly identified *HERPUD2* as a susceptibility locus for hyperuricemia. The minor *A* allele of rs2305335 in *HERPUD2* was significantly related to increased serum uric acid. Examination of this trait and logistic regression analysis indicate that the *A* allele of rs2305335 in *HERPUD2* represents a risk factor or hyperuricemia. Furthermore, *OR4F6* was significantly associated with both T2DM and MetS. We thus newly identified four genes (*ZNF860, OR4F6, OR52E4, HERPUD2*) that confer susceptibility to early-onset T2DM, MetS, or hyperuricemia.

We previously showed that four, five, or three SNPs were related to T2DM ($P<1.44x10^{-4}$), MetS ($P<0.05$), or hyperuricemia ($P<0.05$) determined by multivariable logistic regression

analysis with adjustment for age and sex after the initial EWAS screening of allele frequencies in both early-onset and late-onset individuals with these conditions (54-56). The relationship of two of four SNPs [rs138313632 ($P=1.11x10^{-7}$), rs139012426 ($P=4.29x10^{-5}$)] to T2DM were replicated ($P<0.05$) in the present study. The relationship of two of five SNPs [rs1007732 (P=0.0405), rs7350481 ($P=3.17x10^{-5}$)] to MetS were replicated in the present study. The relationship of two of three SNPs [rs115445569 (P=0.0205), rs60854092 (P=0.0490)] to hyperuricemia were replicated in the present study. These results suggest that genetic variants associated with T2DM, MetS, or hyperuricemia differ, in part, between early-onset and late-onset individuals with these conditions.

There are several limitations to our study: i) Given that the results were not replicated, their validation will be necessary in independent study populations or in other ethnic groups. ii) It is possible that SNPs identified in the present study are in LD with other genetic variants in the same gene or in other nearby genes that are actually responsible for the development of T2DM, MetS, or hyperuricemia. iii) The functional relevance of identified SNPs to the pathogenesis of T2DM, MetS, or hyperuricemia remains to be elucidated.

In conclusion, we have newly identified four genes (*ZNF860, OR4F6, OR52E4, HERPUD2*) that confer susceptibility to early-onset T2DM, MetS, or hyperuricemia. Determination of genotypes for the SNPs in these genes may prove informative for assessment of the genetic risk for T2DM, MetS, or hyperuricemia in Japanese.

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Availability of data and materials

All data underlying the findings described in the article are available upon request from the corresponding author.

Authors' contributions

YYam contributed to conception and design of the study; to acquisition, analysis, and interpretation of the data; and to drafting of the manuscript. KK, MO, HH and TF each contributed to acquisition of the data and to revision of the manuscript. YYas, IT and JS contributed to analysis and interpretation of the data as well as to revision of the manuscript.

Ethics approval and consent to participate

The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine, Hirosaki University Graduate School of Medicine, and participating hospitals (Gifu Prefectural Tajimi Hospital, Gifu Prefectural General Medical Center, Japanese Red Cross Nagoya First Hospital, Northern Mie Medical Center Inabe General Hospital, and Hirosaki Stroke and Rehabilitation Center). Written informed consent was obtained from all subjects.

Consent for publication

All authors approved submission of the final version of the article for publication.

Competing interests

The authors declare that they have no competing interests.

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