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Association of the clusterin gene polymorphisms with type 2 diabetes mellitus

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Abstract

The association of the clusterin (CLU) gene polymorphism (single nucleotide polymorphisms [SNPs] 1-4: rs1532278, rs1532277, rs2279590, and rs2279591, respectively) with type 2 diabetes mellitus was examined using a population of the Funagata study (n [male-female] = 1631 [741:884]; age, 62.0 ± 12.1 years), a Japanese community-based study. Single nucleotide polymorphisms 1 to 3 were significantly associated with hemoglobin A_{1c} levels (P = .0154, .0021, and .0006, respectively) and diabetes (.0310, .0170, and .0021, respectively). A case-control association study of SNP 3 with diabetes by multiple logistic regression analysis showed a significant association of genotype AA (the at-risk genotype) with an odds ratio (OR) of 2.33 (P = .0039) independently of age and sex. The association was marginally validated by a study with another Japanese community-based sample, the Takahata Study (n [male-female] = 2.948 [1333:1615]; age, 63.0 ± 10.2 years) (OR, 1.59; P = .0595; χ^2 P = .0264). When the 2 samples were combined, the association became more significant (OR, 1.75; P = .0025). In subjects with the non-at-risk genotypes, the insulin resistance index—homeostasis model assessment of insulin resistance (HOMA-R)—increased significantly (P < .0001) and the insulin secretion index—HOMA-β—appeared to decrease (P = .1803 and .0097, respectively, for the genotypes AG and GG) as the glucose tolerance progressed toward diabetes (normal glucose tolerance to glucose intolerance to diabetes). However, in subjects with the at-risk genotype, HOMA-R and HOMA-β showed a significant increase already in the subjects with normal glucose tolerance (P = .0239 and .0305, respectively); and as the glucose tolerance progressed toward diabetes, HOMA-R stayed approximately the same, whereas HOMA-β decreased significantly (P = .0332). The CLU gene was associated with diabetes, probably through an increase in insulin resistance primarily and through an impairment of insulin secretion secondarily.

1. Introduction

Type 2 diabetes mellitus (diabetes) is a heterogeneous disorder of glucose metabolism characterized by both insulin resistance and pancreatic β-cell dysfunction. Oxidative stress, which occurs because of overproduction of reactive oxygen species (ROS) that exceeds the cell's antioxidant capacity, leads to both insulin resistance and pancreatic β-cell dysfunction and thus seems to play a major role in the pathophysiology leading to the progression of diabetes [1-3]. In this regard, genes functionally involved in the processes downstream from oxidative stress or, namely, in oxidative injury seem to be candidate genes susceptible for diabetes.

Clusterin (CLU) is a 449–amino acid disulfide-linked heterodimeric glycoprotein composed of α and β subunits and generated by a single cleavage in the single-chain precursor protein [4-6]. There are 2 isoforms of CLU: the cytoprotective secreted CLU (sCLU) and the pro–death factor nuclear CLU (nCLU) [4-7]. CLU is expressed in most human tissues [4,7-9]; and sCLU has been implicated in

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several physiologic processes and many pathologic conditions, including aging, atherosclerosis, tumorigenesis, and diabetes, all of which are characterized by increases in ROS [7,10-14]. The expression of the CLU gene has been shown to be up-regulated in the conditions reported above and regulated by a variety of stimuli, including cytokines, growth factors, heat shock, radiation, and oxidants, which may promote the production of ROS as well [7,10-14]. sCLU seems to have 2 major functions: an apolipoprotein function at the high-density lipoprotein (HDL) particle (thus, also called *apolipoprotein J*) and a small heat shock protein–like chaperon function [7,10]. The latter is a function known to protect cells from the deleterious effects of ROS [7,10,15-17]. Therefore, impaired function of sCLU may lead to an impairment of protection against oxidative stress and, subsequently, to insulin resistance and/or pancreatic β-cell dysfunction and, eventually, to diabetes.

However, to date, no association of the CLU gene polymorphisms with diabetes has been reported. We thus examined the association of the CLU gene polymorphisms with diabetes in large population-based Japanese samples.

2. Materials and methods

2.1. Subjects

The subjects (N = 1631) who participated in the Funagata study in 2001, 2002, and 2005 were enrolled in the present study. The Funagata study was a population-based study held in an agricultural area located about 400 km north of Tokyo. The details of the study have been reported previously [18].

The present study was approved by the Ethics Committee of the Yamagata University School of Medicine, and informed consent was obtained from all the participants. The clinical characteristics of the study population are shown in Table 1. Those on medication for diabetes were diagnosed as diabetic. The diabetic conditions of all the other subjects were classified according to the 1998 World Health Organization criteria using both the fasting plasma glucose (FPG) and 2-hour plasma glucose levels [19] because a 75-g oral glucose tolerance test was conducted in all of them. Subjects known to have type 1 diabetes mellitus were excluded. The numbers of subjects with normal glucose tolerance (NGT), glucose intolerance (GI), and diabetes were 1159, 265, and 207, respectively.

Differences in clinical characteristics, such as insulin resistance and secretion indexes assessed by homeostasis model assessment using FPG and serum insulin (FI) levels (HOMA-R and HOMA-β, respectively), and body mass index (BMI) among subjects with NGT, GI, and diabetes in each genotype group of the CLU gene polymorphism rs2279590 were examined. Conversely, these differences were also examined among the genotype groups in subjects with NGT, GI, and diabetes. The HOMA-R and HOMA-β were assessed using the formulas [FPG (in milligrams per deciliter) × FI (in microunits per milliliter)]/405 and (360 × FI)/(FPG − 63), respectively. To evaluate FI and HOMA indexes precisely, subjects on medication for diabetes and with FPG levels of at least 140 mg/dL were excluded from the analysis of differences; and thus, the numbers of subjects with each genotype of SNP 3 (AA, AG, and GG) used for this analysis were 72, 536, and 904, respectively.

Hypertension was defined as blood pressure of at least 140/90 mm Hg or being on treatment for hypertension. *Hyperlipidemia* was defined as total cholesterol of at least 240 mg/dL, triglycerides of at least 150 mg/dL, or being on treatment for hyperlipidemia.

For validation, other study subjects (the Takahata sample) (n = 2948; mean age ± SD, 63.0 ± 10.2 years; sex ratio [male-female], 1333:1615) from the Takahata study, which is another distinct population-based cross-sectional epidemiological study of Japanese older than 35 years [20], were used for a case-control association study with diabetes. In the Takahata study, only FPG criteria were used to classify diabetic conditions: the numbers of subjects with normal fasting glucose, impaired fasting glucose, and diabetes were 2563, 155, and 230, respectively.

2.2. Genotyping

Four single nucleotide polymorphisms (SNPs) of the CLU gene (SNPs 1-4: rs1532278 and rs1532277 in intron 3, rs2279590 in intron 7, and rs2279591 in the 3′ flanking region) (Fig. 1A) were analyzed. Single nucleotide polymorphisms were selected from the JSNP database (http://snp.ims.u-tokyo.ac.jp/) as those common among the Japanese. The CLU gene is organized into 9 exons and spans about 16.5 kilobases (kb) [6]. Genes next to the CLU gene are SCAR3 (scavenger receptor class A, member 3) (about 18 kb apart) and EPHX2 (epoxide hydrolase 2, cytoplasmic) (about 50 kb apart) in the 5′ and 3′ regions, respectively. Genomic DNA was extracted from peripheral blood leukocytes. The genotyping was conducted with a fluorogenic polymerase chain reaction as described previously [21]. Linkage disequilibrium (LD) between each pair of SNPs was assessed with the software Haploview (http://www.broad.mit.edu/mpg/haploview/) using pairwise combinations with an r^2 value greater than 0.1. The study population was divided into 3 groups according to the genotype of SNP 3: AA (n = 84), AG (n = 585), and GG (n = 962). The mean age ± SD and sex ratio (male-female) of the groups (AA, AG, and GG) were 60.9 ± 12.2 and 40:44, 62.8 ± 12.0 and 279:306, and 61.6 ± 12.1 and 428:534, respectively. No statistical differences in age and sex ratio were observed among the groups.

2.3. Statistical analysis

Data are given as the means ± SD. A quantitative association between the genotypes and the trait values (parametric) and a case-control association between the genotypes and the frequencies of the condition (nonparametric) were analyzed by analysis of variance (ANOVA) and χ^2 tests, respectively. The independent association of the

Table 1
Clusterin genotype differences in clinical characteristics of the Funagata sample

Trait	Total	Genotype (clusterin: rs2279590)			*P* for ANOVA
		AA	AG	GG	
n (male-female)	1631 (747:884)	84 (40:44)	585 (279:306)	962 (428:534)	.4448
Age (y)	62.0 ± 12.1	60.9 ± 12.2	62.8 ± 12.0	61.6 ± 12.1	.1413
Height (cm)	155.3 ± 9.3	156.3 ± 9.2	155.4 ± 9.1	155.2 ± 9.4	.5556
Body weight (kg)	57.6 ± 10.7	59.3 ± 10.1	57.2 ± 10.5	57.7 ± 10.9	.2091
BMI (kg/m^2)	23.8 ± 3.5	24.2 ± 2.9	23.6 ± 3.7	23.8 ± 3.4	.2585
Fat (%)	25.2 ± 7.7	26.2 ± 7.0	25.0 ± 8.4	25.3 ± 7.3	.3906
Waist circumference (cm)	78.6 ± 9.5	79.6 ± 7.9	78.3 ± 9.6	78.7 ± 9.5	.4413
FPG (mg/dL)[a]	96.2 ± 15.8	97.0 ± 15.1	96.1 ± 16.4	96.2 ± 15.4	.9006
2-h plasma glucose (mg/dL)[a]	122.3 ± 46.8	127.4 ± 50.8	124.7 ± 53.2	120.4 ± 45.7	.1639
Postprandial plasma glucose (mg/dL)	126.5 ± 53.5	141.2 ± 70.6	128.9 ± 57.1	123.7 ± 49.1	.0058*
HbA_{1c} (%)	5.23 ± 0.76	5.52 ± 1.41	5.20 ± 0.64	5.21 ± 0.74	.0014*
Fasting serum insulin (μU/mL)[b]	5.14 ± 3.31	5.69 ± 4.35	4.91 ± 3.02	5.23 ± 3.37	.0702
HOMA-R[b]	1.23 ± 0.85	1.37 ± 1.13	1.17 ± 0.79	1.25 ± 0.86	.0786
HOMA-β[b]	61.1 ± 39.4	66.7 ± 42.1	58.7 ± 36.3	62.1 ± 40.8	.1396
Systolic blood pressure (mm Hg)	130.0 ± 17.8	130.5 ± 19.2	129.8 ± 17.4	130.1 ± 17.9	.9170
Diastolic blood pressure (mm Hg)	76.5 ± 10.5	76.4 ± 12.5	76.2 ± 10.3	76.6 ± 10.5	.7287
Total cholesterol (mg/dL)	200.9 ± 32.7	205.1 ± 28.7	200.9 ± 33.3	200.5 ± 32.7	.4606
Triglyceride (mg/dL)	120.2 ± 132.3	119.1 ± 60.1	120.2 ± 100.7	120.4 ± 152.4	.9960
HDL cholesterol (mg/dL)	59.0 ± 14.6	59.7 ± 14.7	59.0 ± 14.7	59.0 ± 14.5	.9110
Adiponectin (mg/dL)[c]	11.0 ± 6.1	10.5 ± 7.1	11.0 ± 6.0	11.0 ± 6.0	.8345
Hypertension, n (%)	705 (43.2)	34 (40.5)	241 (41.2)	430(44.7)	.3424
Hyperlipidemia, n (%)	495 (30.3)	24 (28.6)	181 (30.9)	290 (30.1)	.8864
Diabetes, n (%)	207(12.7)	19(22.6)	81(13.8)	107(11.1)	.0058*

[a] Data were not obtained from some of the subjects, most of which were known to be diabetic before the examination (n: AA, AG, and GG: 74, 550, and 919).
[b] The subjects who were on treatment for diabetes and whose FPG levels were 140 mg/dL or more were excluded (n: AA, AG, and GG: 72, 536, and 904).
[c] Data were not obtained from some of the subjects (n: AA, AG, and GG: 50, 359, and 538).
* $P < .01$.

CLU gene polymorphisms from age and sex was examined by analysis of covariance and multiple logistic regression analysis for parametric and nonparametric data, respectively. $P < .05$ was accepted as significant.

3. Results

3.1. Traits of the study sample

As described previously, the study sample was composed of 1159 NGT (age ± SD, 60.0 ± 12.1 years; sex ratio [male-female], 492:667), 265 GI (66.0 ± 10.5, 147:118), and 207 diabetic subjects (67.7 ± 10.8, 108:99). The diabetic subjects were the most obese (BMI: 23.4 ± 3.3, 24.5 ± 3.9, and 25.0 ± 3.5 for NGT, GI, and diabetes, respectively; $P < .0001$) and had the highest serum total cholesterol (199.5 ± 31.9, 203.7 ± 35.0, and 205.2 ± 33.7 mg/dL, respectively; $P = .0209$) and triglyceride levels (111.9 ± 130.1, 138.3 ± 149.2, and 144.0 ± 115.7 mg/dL, respectively; $P = .0003$) and the lowest serum HDL cholesterol levels (59.9 ± 14.3, 56.7 ± 14.1, and 57.3 ± 16.1 mg/dL, respectively; $P = .0011$) among the groups. The systolic blood pressures (127.7 ± 17.8, 135.7 ± 16.2, and 135.7 ± 17.1 mm Hg, respectively; $P < .0011$) and HOMA-R (1.13 ± 0.77, 1.42 ± 0.87, and 1.91 ± 1.26, respectively; $P < .0011$) were the highest in the diabetic subjects, whereas HOMA-β was the lowest (63.3% ± 39.6%, 56.0% ± 39.3%, and 49.2% ± 33.0%, respectively; $P = .0002$).

3.2. Association of the CLU gene polymorphisms with diabetes

We first examined the associations of SNPs 1 to 4 with diabetes as well as with hemoglobin A_{1c} (HbA_{1c})levels, which we used as a surrogate marker for diabetes. As shown in Fig. 1B, SNPs 1 to 3 showed a significant association with diabetes (vs NGT; $P = .0310$, .0170, and .0051, respectively) and the HbA_{1c} levels (age- and sex- adjusted $P = .0154$, .0021, and .0006, respectively), whereas SNP 4 was not associated with diabetes and the HbA_{1c} levels ($P = .940$ and .2734, respectively).

The analysis to estimate an LD block structure using the genotype data of these 4 SNPs revealed one LD block composed of SNPs 1 to 3 (Fig. 1C). Therefore, SNPs 1 to 3 appeared to be in tight linkage. The LD block had 8 haplotypes, among which only 2 (haplotypes 1 and 2: the composition of the alleles of SNPs 1 to 3 was T, T, and A and C, C, and G, respectively) were determined to have frequencies greater than 5% (0.223 and 0.720, respectively). Haplotype 1 was composed of the at-risk alleles of SNPs 1 to 3 for diabetes and thus seemed to be an at-risk haplotype for diabetes. However, the large number of haplotypes observed appeared to reduce the statistical power. The differences in the frequencies of diabetic subjects among the diplotype groups reached significant levels ($P = .0071$) only when the diplotype groups were combined into 4 groups (diplotypes

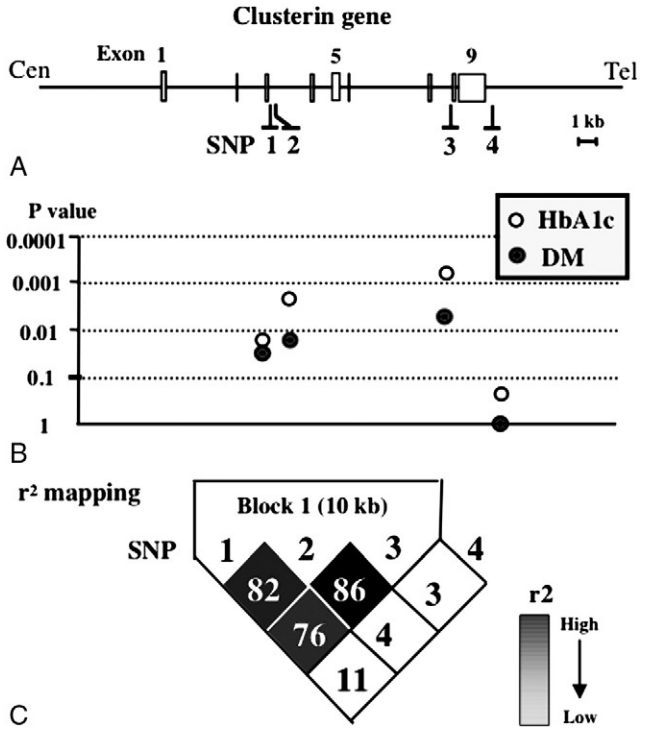

Fig. 1. Association mapping of the clusterin gene polymorphisms (SNPs). A, The positions of the SNPs examined (SNPs 1-4 [rs ID]: 1532278, 1532277, 2279590, and 2279591, respectively) are schematically shown. The box represents the exon. Cen indicates centromeric; Tel, telomeric. B, *P* values of the SNPs for the quantitative association study with the HbA_{1c} levels and for the case-control association study for diabetes are shown. C, Linkage disequilibrium plot of the region generated by Haploview (http://www.broad.mit.edu/mpg/haploview/), showing r^2 values. The r^2 value for any 2 SNPs is presented in the box representing their intersection.

11, 12, 22, and others). Therefore, we then focused on the association of SNP 3, which showed the strongest association with diabetes and the HbA_{1c} levels among the SNPs examined and thus seemed to represent the association of the LD block with diabetes.

We examined whether any other clinical traits were different among the genotype groups divided by SNP 3 because such clinical traits may be confounding factors for the association. As shown in Table 1, except for the frequencies of diabetic subjects and clinical traits related to diabetes, such as HbA_{1c} and postprandial plasma glucose levels, no other clinical traits differed significantly among the genotype groups; and thus, the association of SNP 3 with diabetes seems to be independent of the factors examined here.

3.3. Impact of SNP 3 of the CLU gene on the association with diabetes

As shown in Table 2, the frequencies of diabetic subjects were significantly higher in subjects with the genotype AA of SNP 3 of the CLU gene than in the others ($P = .0051$). Multiple logistic regression analysis showed that the genotype AA was significantly associated with diabetes independently of age and sex, with odds ratios (ORs) of 2.50 ($P = .0023$) (vs the genotype GG) and 2.33 ($P = .0039$) (vs the genotypes AG + GG).

The difference in the frequencies of diabetic subjects among the genotype groups was further validated in the Takahata sample ($P = .0264$). However, in the Takahata sample, a multiple logistic regression analysis could not show a significant association of the genotype AA with diabetes, although the *P* value for the association ($P = .0595$) (the genotypes AA vs AG + GG) was very close to the significant levels. When the additional sample for validation (the Takahata sample) was combined with the study sample (the Funagata sample), the associations of the genotype AA with diabetes were still significant, with ORs of 1.70 ($P = .0052$) (vs the genotype GG) and 1.75 ($P = .0025$) (vs the genotypes AG + GG).

3.4. Effect of SNP 3 of the CLU gene on insulin resistance and secretion

We next examined the effect of SNP 3 on the insulin resistance and secretion indexes, HOMA-R and HOMA-β, as shown in Fig. 2. In subjects with the genotypes AG and GG, HOMA-R increased significantly (*P* for trend and ANOVA < .0001, each) and HOMA-β appeared to decrease (*P* for trend and ANOVA = .1803 and .2201 and .0097 and .0070, respectively, for the genotypes AG and GG) as the glucose tolerance progressed toward diabetes (NGT to GI to diabetes). However, in subjects with the genotype AA, HOMA-R, which was significantly higher among the genotype groups in subjects with NGT ($P = .0239$), remained similar even as the glucose tolerance progressed toward diabetes (*P* for trend and ANOVA = .8675 and .9589, respectively). On the other hand, HOMA-β, which was also significantly higher among the genotype groups in subjects with NGT ($P = .0305$), similarly to HOMA-R, decreased significantly as the glucose tolerance progressed toward diabetes in subjects with the genotype AA (*P* for trend and ANOVA = .0237 and .0332, respectively); and the decrease appeared to be substantial (HOMA-β for NGT, GI, and diabetes was 74.4 ± 46.5, 53.6 ± 18.9, and 36.5 ± 14.0, respectively). Differences in BMI among subjects with NGT, GI, and diabetes in each genotype group were also examined because such differences in BMI, which seem to be a surrogate marker for inappropriate lifestyles leading to diabetes, may have some influence on the observed relationship between the genotypes and the clinical traits, namely, HOMA-R and HOMA-β. Differences in BMI were very similar to those observed in HOMA-R: BMI increased in subjects with the genotypes AG and GG (*P* for trend and ANOVA < .0001, for both genotypes) but remained similar in subjects with the genotype AA (*P* for trend and ANOVA = .5718 and .5615, respectively) as the glucose tolerance progressed toward diabetes. Body mass index was not significantly different among the genotype groups in subjects with NGT ($P = .0753$).

4. Discussion

The association of SNP 3 of the CLU gene with diabetes was clearly shown by both quantitative and case-control association analyses in a relatively large population-based Japanese sample (the Funagata sample) (Tables 1 and 2, Fig. 1). In this study, diabetic condition was classified by both FPG and 2-hour plasma glucose and thus accurately determined. Therefore, this study seemed to have increased statistical power to determine genetic factors that predispose individuals to diabetes. On the other hand, the analysis with another sample set of Japanese used for validation (the Takahata sample) appeared to have substantially reduced statistical power because the diabetic condition of the subjects of the Takahata sample was classified only by FPG criteria; and thus, the status of glucose tolerance was very likely to have been incorrectly defined. However, the analysis for validation showed a significant association: although not all analyses reached a significant level, the differences in the frequencies of diabetic subjects among the genotype groups of SNP 3 were significant (Table 2). Together, these results strongly indicated the association of SNP 3 of the CLU gene with diabetes.

In general, when an increase in insulin resistance occurs mostly as a consequence of inappropriate lifestyles, a compensatory increase in insulin secretion occurs concomitantly to maintain plasma glucose levels in the reference range. As this compensatory increase in insulin secretion declines, impairment of glucose tolerance progresses. As expected, in subjects with the genotypes AG and GG, insulin resistance assessed by HOMA-R increased with concomitant increases in BMI, which is a surrogate marker for inappropriate lifestyle, whereas insulin secretion assessed by HOMA-β appeared to decrease as glucose tolerance progressed toward diabetes. However, in subjects with the genotype AA, the at-risk genotype, HOMA-R and HOMA-β were already increased in the subjects with NGT. Neither HOMA-R nor BMI increased, whereas HOMA-β decreased substantially, as glucose tolerance progressed toward diabetes. Namely, in subjects with the at-risk genotype, insulin resistance did not change but insulin secretion decreased substantially as the glucose tolerance progressed toward diabetes. These facts may indicate that increased insulin resistance is a primary phenotype related to the genotype AA and that a subsequent decrease in insulin secretion, which is compensatorily increased even in subjects with NGT, is secondarily responsible for the progression of glucose tolerance toward diabetes. In addition, in subjects with the at-risk genotype, inappropriate lifestyles may not have a substantial influence on the development of diabetes;

Table 2
Association of the clusterin gene polymorphism (rs2279590) with diabetes

Genotypes	Phenotypes (n(%))		Chi-square	Multiple logistic analysis#	
	DM	NGT	*P*	OR (95% CI)	*P*
Funagata (n = 1,366)					
GG	107 (51.7)	696 (60.1)	.0051	1	Ref
AG	81 (39.1)	412 (35.5)		1.19 (0.86-1.65)	.2938
AA	19 (9.2)	51 (4.4)		2.50 (1.39-4.52)	.0023
AA vs AG+GGt	–	–		2.33 (1.31-4.15)	.0039
Takahata (n = 2.793)					
GG	134 (58.3)	1385 (54.0)	.0264	1	Ref
AG	75 (32.6)	1027 (40.1)		0.77 (0.57-1.04)	.0840
AA	21 (9.1)	151 (5.9)		1.44 (0.88-2.36)	.1510
AA vs AG+GG	–	–		1.59 (0.98-2.58)	.0595
Combined (n = 4,159)					
GG	241 (55.1)	2081 (55.9)	.0059	1	Ref
AG	156 (35.7)	1439 (38.7)		0.93 (0.75-1.15)	.5080
AA	40 (9.2)	202 (5.4)		1.70 (1.17-2.46)	.0052
AA vs AG+GG	–	–		1.75 (1.22-2.51)	.0025

Adjusted for age and sex.

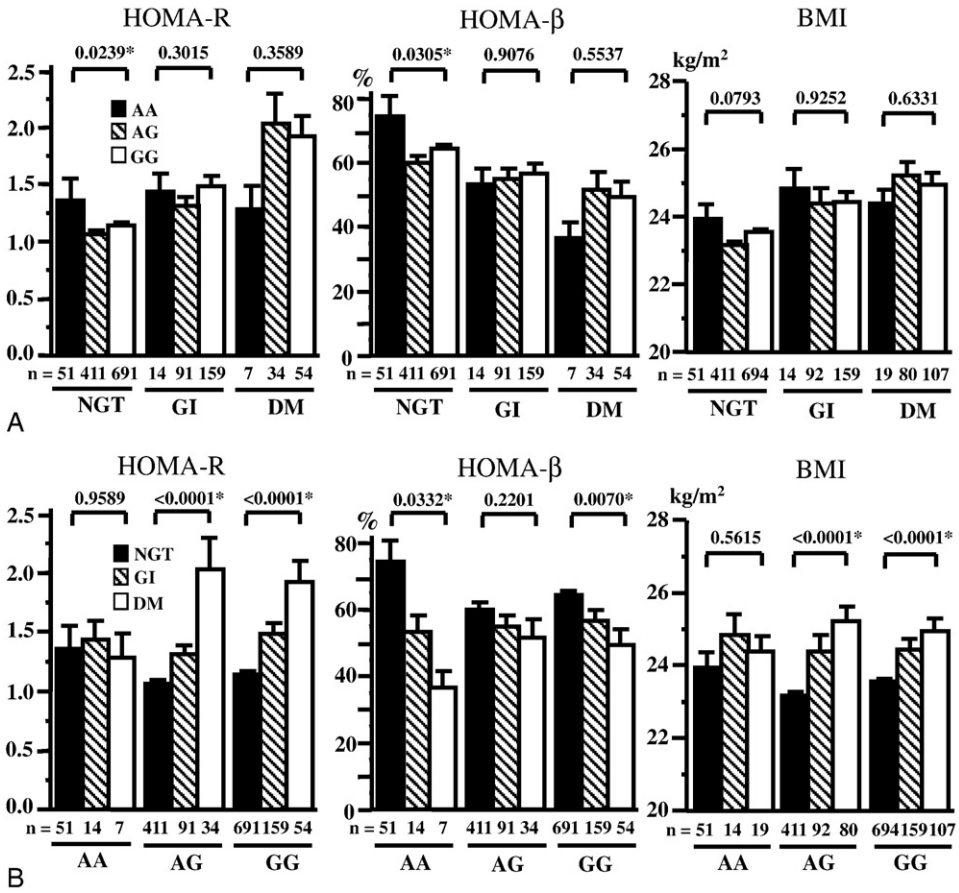

Fig. 2. Effect of the clusterin gene polymorphism rs2279590 on the insulin resistance and secretion indexes, HOMA-R and HOMA-*β*, and BMI. A, The trait values according to the genotype groups in each category of the glucose tolerance, namely, NGT, GI, and diabetes. B, The trait values according to the category of the glucose tolerance in each genotype group of the polymorphism. Bars above the columns represent standard error. *P* values for ANOVA are shown above the corresponding column. **P* < .05. The number of subjects of each group is shown below the columns. DM indicates diabetes.

rather, the genotype may have a major influence, probably through an increase in insulin resistance and a subsequent substantial decrease in insulin secretion.

As described previously, sCLU has a protective function against oxidative injury [4-7]; and thus, in this regard, an impaired function of sCLU may be involved in the pathophysiology leading to insulin resistance and pancreatic *β*-cell dysfunction. The facts that both insulin resistance and pancreatic *β*-cell dysfunction were observed in the subjects with the at-risk genotype seemed to be in accordance with the hypothesis mentioned above. Pancreatic -cells are known to be particularly sensitive to ROS because of the low activities of enzymes in free-radical quenching, such as catalase, glutathione peroxidase, and superoxide dismutase [1,2]. Therefore, even a modest increase in ROS production subsequent to a modest increase in plasma glucose can impair pancreatic *β*-cell function, especially in subjects with the at-risk genotype, who may have impaired protective effects of sCLU on oxidative injury. Furthermore, sCLU has been reported to be a growth factor–like molecule involved in pancreatic *β*-cell neogenesis from pancreatic stem cells [22,23]. Therefore, impaired function of sCLU may also impair the function to maintain the number of pancreatic *β*-cells. However, whether or not the at-risk genotype is associated with an impaired function and, if so, what kind of function is responsible will have to be clarified.

Previous genomewide association studies with analyses of as many as 500000 SNPs revealed several genes to be strongly associated with diabetes [24,25]. The association of the CLU gene with diabetes was not extracted in these previous studies: only 1 SNP (rs 9331931) of the CLU gene was examined, and it was not found to be associated with diabetes (*P* = .139) in those studies (http://www.wtccc.org.uk; http://www.broad.mit.edu/diabetes). The latest release (#24) of the phased haplotypes for the CEU (European) population of the HapMap database showed an LD block of about 13 kb, which was composed of 8 SNPs spanning from introns 1 to 7 of the CLU gene. Single nucleotide polymorphism rs9331931 was in this region of the LD block but was not accepted as a component of the LD block, whereas SNPs 1 and 3, which were found to be associated with diabetes in the present study, were accepted as components. Therefore, SNP rs9331931 might not fully represent the LD block for the association with diabetes as SNPs 1 and 3 might. Furthermore, although SNP rs9331931 has a minor allele frequency of 0.2 to 0.3 in European

populations, the minor allele frequency of the SNP rs9331931 in the Japanese population is reported to be null (http://www.ncbi.nlm.nih.gov/SNP/). Therefore, the association of the SNP rs9331931 could not be examined among the Japanese. The latest release (#24) of the phased haplotypes for the combined JPT (Japanese) + CHB (Chinese) population of the HapMap database also showed an LD block of about 12 kb, which was composed of 5 SNPs including SNPs 1 and 3 used in this study. Therefore, SNPs used in this study seemed to represent the LD block among the Japanese; and thus, the results of these previous genomewide association studies do not seem to conflict with the present results.

The study was a population-based study; and thus, although the number of subjects was large, the number of diabetic subjects was relatively low compared with that of control subjects. The relatively low number of diabetic subjects seemed to reduce statistical power to detect the differences in the frequencies of the diabetic subjects among the genotype groups and thus seemed to be a limitation. However, the statistical power estimated using the software Sampsize (http://sampsize.sourceforge.net/iface/index.html) did not seem to be very low. The study population had about 80% and 50% power to detect minimal ORs of 2.30 and 1.75, respectively, at a level of significance of .05. The Takahata sample for validation had about 65% and 50% power to detect minimal ORs of 1.75 and 1.60, respectively; and when these 2 samples were combined, the statistical power became 85% to detect a minimal OR of 1.75. Even in the analysis that had the lowest statistical power among the association studies (the Takahata sample), the association was found to be significant in some analysis (χ^2 analysis), strengthening the association of the CLU gene with diabetes. Therefore, statistical power did not seem to be a substantial limitation.

Gene × gene and gene × environment interactions might affect the susceptibility for diabetes together with the CLU gene polymorphisms. Namely, these interactions might affect the prevalence of diabetes, as the prevalence of diabetic subjects differed even in the samples used (12.7% and 7.8% for the study sample and the Takahata sample). However, these interactions were not considered in the present study; and thus, the possibilities of interactions of the CLU gene polymorphisms with other genetic and/or environmental factors remain to be clarified.

In conclusion, the CLU gene was associated with diabetes. This seems to warrant further examination to determine whether or not CLU has functional relevance leading to the increase in insulin resistance and subsequent impairment of insulin secretion observed in subjects with the at-risk genotype.

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