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ORIGINAL ARTICLE

Assessment of plasma glucose cutoff values to predict the development of type 2 diabetes in a Japanese sample: the Funagata Study

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Abstract The lower cutoff limit used to define impaired fasting glucose (IFG) varies between countries/organizations and is usually 100 or 110 mg/dl. Therefore, we evaluated the cutoff value for IFG that predicted the development of type 2 diabetes among the participants of the Funagata Study, a Japanese population-based, longitudinal study. Overall, 3,413 individuals (age 56.2 ± 12.1 years) without diabetes at baseline and who attended follow-up examinations were included in this analysis. Diabetes was diagnosed based on 75-g oral glucose tolerance tests according to the 1998 World Health Organization criteria. Follow-up visits were completed in 2007 (mean follow-up 147 months). The development of diabetes was used as the endpoint. During the follow-up period, 156 individuals developed diabetes. Life-table method analysis showed significantly decreased diabetes-free survival in individuals with fasting plasma glucose (FPG) ≥ 96 mg/dl ($p = 0.002$). Cox's proportional hazard model analyses showed a high risk for the development of diabetes in individuals with FPG ≥ 101 mg/dl. The hazard ratio for patients with an FPG of 101–105 mg/dl was 5.50 (95% confidence interval (CI) 2.12–14.25; $p < 0.001$). The 5-year incidence of diabetes was also substantially increased in individuals with FPG ≥ 101 mg/dl. The odds ratio for patients with FPG 101–105 mg/dl was 10.9 (95% CI 2.6–46.0; $p < 0.001$). Receiver operating characteristic curve analysis showed an optimal FPG cutoff value of 100 mg/dl. Based on these results, the optimal FPG cutoff value used to define IFG in Japanese individuals should be 100 mg/dl rather than 110 mg/dl.

Keywords Impaired fasting glucose (IFG) · Cohort study · Cutoff values

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Introduction

Type 2 diabetes is a major health problem associated with severe vascular complications such as blindness, end-stage renal disease, and cardiovascular diseases. Furthermore, the prevalence of diabetes is increasing rapidly worldwide [1]. Therefore, in addition to treating diabetes, preventing or delaying its onset is a significant clinical concern. As several clinical trials have demonstrated that lifestyle or pharmacological interventions can prevent or delay the onset of diabetes in individuals with impaired glucose tolerance (IGT), a high-risk condition for the development of diabetes [2–4], identifying high-risk individuals seems essential. Individuals whose glucose levels are higher than normal but do not meet the criteria for diabetes are considered to be at high risk of developing diabetes or having prediabetes [5]. Prediabetes is defined as the presence of impaired fasting glucose (IFG) or IGT based on fasting plasma glucose (FPG) levels and 2-h plasma glucose (2hPPG) levels, respectively, determined by 75-g oral glucose tolerance tests (OGTT) [5]. However, although the criteria for IGT are the same worldwide, those for IFG are not [5–8]. For example, the American Diabetes Association

(ADA) defines IFG as FPG levels of 100–125 mg/dl, whereas the World Health Organization (WHO) and other diabetes organizations, including the Japan Diabetes Society (JDS), define IFG as FPG levels of 110–125 mg/dl [5–8]. Therefore, we conducted a retrospective analysis of the Funagata study to evaluate the optimal cutoff values of FPG and 2hPPG for IFG and IGT to predict the onset of diabetes in Japanese individuals.

Materials and methods

Study participants

The Funagata Study is a population-based, longitudinal study conducted in Funagata, an agricultural area located about 400 km north of Tokyo, Japan [9, 10]. Starting in 1990, all individuals >35 years of age residing in the town were registered for the study. Each participant's glucose tolerance was evaluated by an OGTT at the initial health-care examination (i.e., baseline examination). Those who were not diabetic underwent OGTTs at follow-up examinations every 5 years until they were found to be diabetic or until the follow-up period ended. Participants who attended examinations in 1993 ($n = 163$) attended the follow-up examinations at various intervals. Series of examinations were held in 1990–1993, 1995–1997, 2000–2002, and 2005–2007, and the participants in each series composed cohorts 1–4, respectively (2,814, 2,251, 1,962, and 1,447 individuals, respectively; some participants were included in multiple cohorts). The primary study cohort included all participants in cohorts 1–3 ($n = 2,538$, 579, and 296, respectively) who were not diabetic at the baseline examination and who attended at least one follow-up examination ($n = 3,413$; males/females 1,516/1,897; mean ± standard deviation (SD), age 56.2 ± 12.1 years). In 1995, 4,183 people living in Funagata were >35 years; therefore, the participation rate was 81.6% ($n = 3,413/4,183$). Follow-up was completed at the examinations conducted in July 2007, giving a maximum follow-up of 179 months.

Individuals were stratified based on FPG and 2hPPG as follows: FPG ≤ 80 (reference group), 81–85, 86–90, 91–95, 96–100, 101–105, 106–110, 111–115, 116–120, and 121–125 mg/dl; 2hPPG ≤ 99 (reference group), 100–109, 110–119, 120–129, 130–139, 140–149, 150–159, 160–179, and 180–199 mg/dl. Participants with FPG > 126 mg/dl and 2hPPG ≥ 200 mg/dl were defined as having diabetes and were excluded from this analysis. The endpoint was the onset of diabetes diagnosed using an OGTT according to the 1998 WHO criteria [11]. Participants identified as having diabetes by public health nurses through contacts with outpatient clinics were defined without undergoing an OGTT. Participants who moved away were identified by residence transfer documents. Overall, 197 participants died and 44 moved away during the follow-up period. During the study period, the participants received no interventions except for ordinary advice for health promotion. The median and mean follow-up times were 176 and 147 months, respectively. The clinical characteristics of the study groups at baseline are shown in Table 1.

Table 1 Baseline characteristics of the study cohort

Trait	Statistics
Number	3,413
Age (years)	56.2 ± 12.1
Sex (male/female)	1,516/1,897
Height (cm)	155.2 ± 8.8
Body weight (kg)	57.0 ± 9.8
Body mass index (kg/m^2)	23.6 ± 3.2
Waist circumference (cm)[a]	79.0 ± 9.4
Hip circumference (cm)[a]	91.8 ± 6.0
Waist-to-hip ratio[a]	0.860 ± 0.075
Fasting plasma glucose (mg/dl)	92.2 ± 9.8
2-h plasma glucose (mg/dl)	107.9 ± 29.2

[a] Data were not obtained from some individuals ($n = 2,514$)

As a secondary study cohort, 1,390, 1,210, and 916 individuals from cohorts 1–3, respectively, who were not diabetic at the corresponding examination and who attended the 5-year follow-up examinations were combined ($n = 3,516$; men/women 1,496/2,020; age 58.2 ± 10.4 years) to examine the risk of developing diabetes during the 5-year follow-up. Unlike individuals in the primary study cohort, individuals included in two or three cohorts ($n = 1,034$) were included in this secondary study cohort multiple times. This study was approved by the Ethics Committee of Yamagata University School of Medicine, Japan, and informed consent to participate was obtained from all participants.

Statistical analysis

Clinical characteristics are presented as means ± SD. Survival curves of each group stratified based on FPG and the 2hPPG were constructed by the life-table method and compared using the log-rank test. Multivariate Cox's proportional hazard model was used to calculate the age- and sex-adjusted hazard ratios (HRs) for each FPG and 2hPPG category compared with the corresponding reference group. The 5-year cumulative incidence of diabetes for each FPG and 2hPPG category was calculated as the number of individuals who had diabetes at the 5-year follow-up divided by the sum of the duration of follow-up for each participant. Thus, the incidence was expressed as the

number of cases of diabetes per 1,000 persons per year (person–years) and was compared by multiple logistic regression analysis to assess the significance of differences between each category, with adjustment for age and sex. All analyses described above were conducted using StatView-J Version 5.0 for Macintosh (SAS Institute Inc, Cary, NC, USA). Receiver operator characteristic (ROC) curves were also plotted to determine the optimal FPG and 2hPPG cutoff values to predict the development of diabetes at the 5-year follow-up. ROC curves were plotted using GraphPad Prism Version 4.00 for Macintosh (GraphPad Software, San Diego, CA, USA). In all analyses, $p < 0.05$ was considered statistically significant.

Results

Evaluating FPG values to predict the risk of developing diabetes during the follow-up

During the follow-up period, 156 individuals developed diabetes. Survival-curve analysis showed significantly lower diabetes-free survivals (i.e., a higher incidence of diabetes) in individuals with FPG $\geq$ 96 mg/dl or 2hPPG $\geq$ 130 mg/dl than in the corresponding reference groups. The p values for individuals with FPG 96–100 mg/dl and 2hPPG 130–139 mg/dl were 0.002 and 0.016, respectively. The diabetes-free survival rates decreased further and became more significant ($p < 0.001$) in individuals with FPG $\geq$ 100 mg/dl or 2hPPG $\geq$ 140 mg/dl. Similarly, as shown in Fig. 1, Cox's proportional hazard model analyses, with adjustment for age and sex, showed that the risk of developing diabetes was increased in individuals with FPG $\geq$ 96 mg/dl or 2hPPG $\geq$ 130 mg/dl. HRs for individuals with FPG 96–100 mg/dl and those with 2hPPG 130–139 mg/dl were 3.88 (95% confidence interval (CI) 1.51–9.96; $p = 0.005$) and 2.26 (95% CI 1.19–4.29; $p = 0.013$), respectively. The magnitude and significance of these HRs were increased further in individuals with FPG $\geq$ 100 mg/dl and in those with 2hPPG $\geq$ 140 mg/dl. The HRs for individuals with FPG 101–105 mg/dl and those with 2hPPG 140–149 mg/dl were 5.50 (95% CI 2.12–14.25; $p < 0.001$) and 5.13 (95% CI 2.90–9.08; $p < 0.001$), respectively. These results indicate that FPG $\geq$ 96 mg/dl and 2hPPG $\geq$ 130 mg/dl pose significant risk for developing diabetes, and that this risk increases further at FPG $\geq$ 101 mg/dl and 2hPPG $\geq$ 140 mg/dl in this Japanese population.

Evaluating FPG values to predict the risk of developing diabetes by the 5-year follow-up

As shown in Fig. 2, the 5-year incidence of diabetes was significantly higher in individuals with FPG $\geq$ 96 mg/dl and in those with 2hPPG $\geq$ 130 mg/dl compared with the corresponding reference groups. The age- and sex-adjusted odds ratios (ORs) for individuals with FPG 96–100 mg/dl and individuals with 2hPPG 130–139 mg/dl were 4.6 (95% CI 1.1–19.7; $p = 0.042$) and 2.8 (95% CI 1.3–6.2; $p = 0.011$), respectively. The incidence and ORs increased further in individuals with FPG $\geq$ 100 mg/dl or 2hPPG $\geq$ 140 mg/dl, with age- and sex-adjusted ORs for individuals with FPG 100–105 mg/dl and 2hPPG 140–149 mg/dl being 10.9 (95% CI 2.6–46.0; $p < 0.001$) and 8.3 (95% CI 4.2–16.4; $p < 0.001$), respectively.

ROC curves were then plotted to determine the cutoff values of FPG and 2hPPG in relation to the incidence of diabetes at the 5-year follow-up examinations. In these analyses, FPG 100 mg/dl (sensitivity 66.4%; specificity 81.5%) and 2hPPG 135 mg/dl (62.0 and 85.9%, respectively) yielded the greatest sensitivity and specificity (Fig. 3). The cutoff values with sensitivity $\geq$ 80% were 95

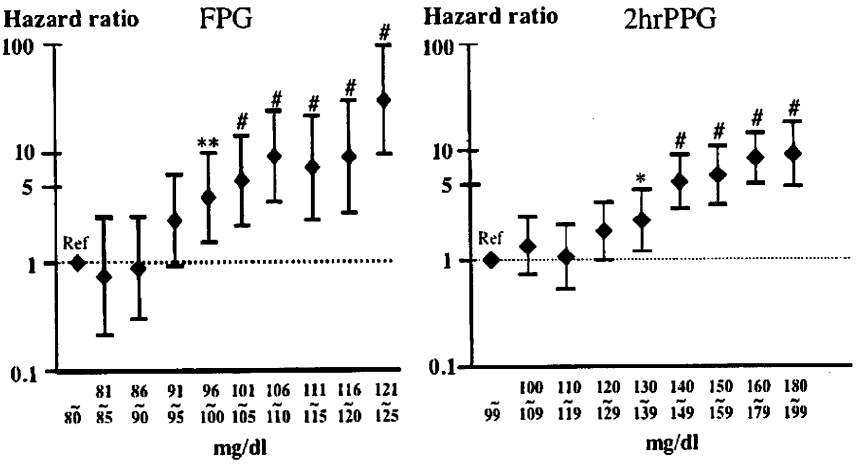

Fig. 1 Risk of diabetes in the primary cohort with individuals stratified according to fasting (FPG) and 2-h postload (2hPPG) plasma glucose levels after a 75-g oral glucose tolerance test (OGTT). The age- and sex-adjusted hazard ratios (HRs) of each group to the reference group (*Ref*) were assessed by multivariate Cox's proportional hazard models. *Bars* represent 95% confidence intervals. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{\#}p < 0.001$

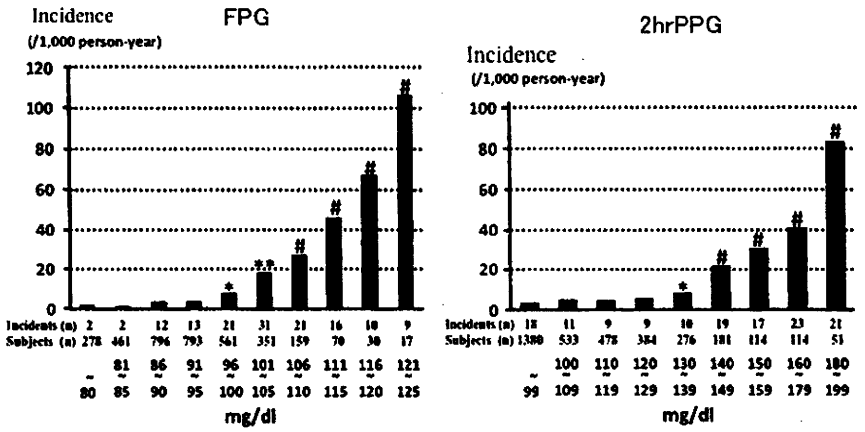

Fig. 2 Risk of developing diabetes at 5-year follow-up examinations of the secondary cohort with individuals stratified according to fasting (FPG) and 2-h postload (2hPPG) plasma glucose levels after a 75-g oral glucose tolerance test (OGTT). The cumulative incidence of diabetes is expressed as number of new cases per 1,000 persons per year (person-years) and is compared by multiple logistic regression analysis with adjustment for age and sex. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{\#}p < 0.001$

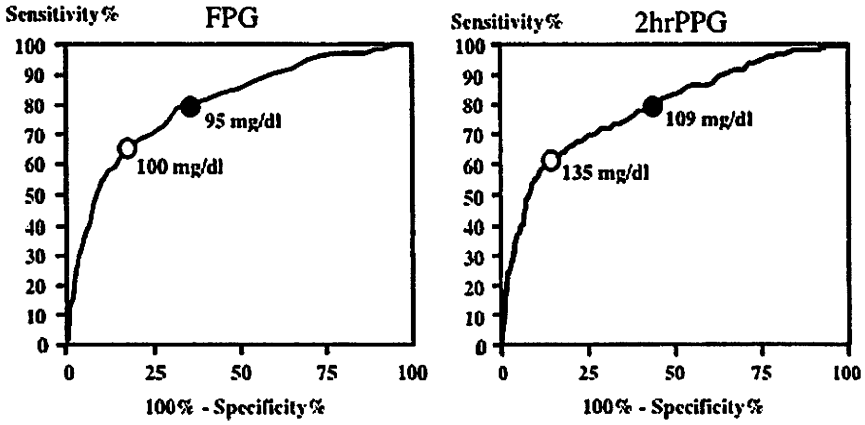

Fig. 3 Receiver operator characteristic (ROC) curves to determine optimal cutoff values of fasting (FPG) and 2-h postload (2hPPG) plasma glucose levels after a 75-g oral glucose tolerance test as predictors for developing diabetes by the 5-year follow-up examinations. *Open circle* cutoff values yielding the greatest sensitivity and specificity. *Filled circle* cutoff values yielding ≥80% sensitivity

and 109 mg/dl for FPG and 2hPPG, respectively, although the corresponding specificities decreased substantially (64.4% and 54.5%, respectively). The areas under the ROC curves for FPG and 2hPPG were 0.804 (95% CI 0.764–0.844) and 0.792 (95% CI 0.750–0.835), respectively.

Discussion

Results of the primary study cohort analysis showed that the risk of developing diabetes was significantly increased in individuals with FPG ≥ 96 mg/dl and in those with 2hPPG ≥ 130 mg/dl. These risks, quantified by HRs, were increased by more than fivefold in individuals with FPG ≥ 101 mg/dl or 2hPPG ≥ 140 mg/dl compared with individuals with FPG ≤ 80 mg/dl and those with 2hPPG ≤ 99 mg/dl, respectively. Similarly, the secondary study cohort analysis, conducted to determine the 5-year risk of developing diabetes, also showed that the risk increased significantly in individuals with FPG ≥ 96 mg/dl and in those with 2hPPG ≥ 130 mg/dl, and that the risks quantified by ORs were increased by more than fivefold in individuals with FPG ≥ 101 mg/dl or 2hPPG ≥ 140 mg/dl compared with individuals with FPG ≤ 80 mg/dl or 2hPPG ≤ 99 mg/dl, respectively. Taken together, these results indicate that FPG ≥ 96 mg/dl and 2hPPG ≥ 130 mg/dl are significant risk factors for developing diabetes and that these risks increase further in individuals with FPG ≥ 101 mg/dl and 2hPPG ≥ 140 mg/dl in this Japanese population.

As described above, prediabetes is a condition that poses a relatively high risk for developing diabetes. Therefore, the FPG and 2hPPG cutoff values that seemed to define the prediabetic conditions IFG and IGT were 96–100 and 130–139 mg/dl, respectively. However, the risks of developing diabetes among individuals with FPG 96–100 mg/dl or 2hPPG 130–139 mg/dl were not much higher than those of the corresponding reference groups. However, the risks of developing diabetes were substantially higher in individuals with FPG 101–105 mg/dl and 2hPPG 140–149 mg/dl. Therefore, PG levels of 100 and 140 mg/dl seem to be appropriate cutoff values to define

IFG and IGT, respectively. Furthermore, ROC analysis showed that the optimal cutoff values for FPG and 2hPPG to predict the risk of developing diabetes by the 5-year follow-up were 100 and 135 mg/dl, respectively. Taken together, these findings suggest that the cutoff value to define IFG should be lowered to 100 mg/dl from 110 mg/dl, which is the current cutoff value defined by the JDS and WHO. Meanwhile, results reported here provide further evidence supporting the current cutoff value to define IGT.

The current IFG cutoff value of 110 mg/dl used in Japan is the same as that recommended by the WHO [6, 8]. However, no data have definitively shown that this is an appropriate cutoff value to predict diabetes development in Japanese individuals. In fact, several studies have reported that the optimal cutoff value for IFG is approximately 100 mg/dl in Japanese individuals [12–14], which is consistent with our findings. However, it must be acknowledged that in the previous studies, diabetes was defined by self-report and FPG values alone. Thus, glucose tolerance or diabetes may have been misclassified, and some individuals who were diabetic at baseline and at the follow-up may have been misclassified as nondiabetic, and vice versa, which might have led to inaccurate results. The results reported here seem to be more accurate than those of previous studies because we evaluated FPG and 2hPG using OGTTs to evaluate glucose tolerance and diabetes. We previously reported that 96.5 mg/dl was the optimal cutoff value for FPG to predict the risk of developing diabetes by the 5-year follow-up based on an ROC analysis of individuals in cohort 2 ($n = 1{,}189$) [15]. In the study presented here, we used individuals from cohorts 1–3, which increased the number of eligible individuals ($n = 3{,}413$) and also extended the follow-up time (mean follow-up 147 months). Therefore, this study not only validates the previous reports, but extends their findings by providing more reliable and conclusive results.

The cutoff values for IFG and/or IGT may differ between men and women. Although we analyzed the cutoff values separately for each sex, we found no significant differences between sexes in the cutoff values. Notably, the 5-year incidence of diabetes was significantly increased in both sexes in individuals with FPG $\geq$ 101 mg/dl or 2hPPG $\geq$ 140 mg/dl compared with the corresponding reference groups. The age-adjusted ORs for individuals with FPG 101–105 mg/dl were 10.4 (95% CI 1.4–79.4; $p = 0.024$) and 10.6 (95% CI 1.4–82.8; $p = 0.024$) for men and women, respectively. The corresponding ORs for individuals with 2hPPG 140–149 mg/dl were 9.2 (95% CI 3.4–24.6; $p < 0.001$) and 7.3 (95% CI 2.8–18.7; $p < 0.001$). Similar findings have been reported elsewhere [14]. Therefore, the cutoff values do not seem to be dependent on sex.

As diabetes is a heterogeneous disorder of glucose metabolism characterized by insulin resistance and pancreatic β-cell dysfunction, many environmental and genetic factors are involved in its pathophysiology. However, in this study, we only included age and sex as possible confounding factors to examine the cutoff values for PG levels to predict diabetes development. Thus, the lack of adjustment for other factors, particularly genetic information such as family history of diabetes, is a limitation of the study. We evaluated the cutoff values based only on the risk of developing diabetes; other factors, such as the risk of cardiovascular diseases, were not considered, giving rise to another limitation. Furthermore, we did not consider the implications of lowering the cutoff value for IFG and the resulting increase in the prevalence of IFG on public health, or the benefits and disadvantages of labeling individuals with slightly elevated FPG (e.g., 100–109 mg/dl) as having IFG. These factors must be considered in order to draw a definitive conclusion in terms of cutoff values for IFG.

In conclusion, results of this study indicate that the cutoff value for IFG as a predictor of type 2 diabetes should be decreased from 110 to 100 mg/dl.

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Conflict of interest The authors declare that they have no conflict of interest.

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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

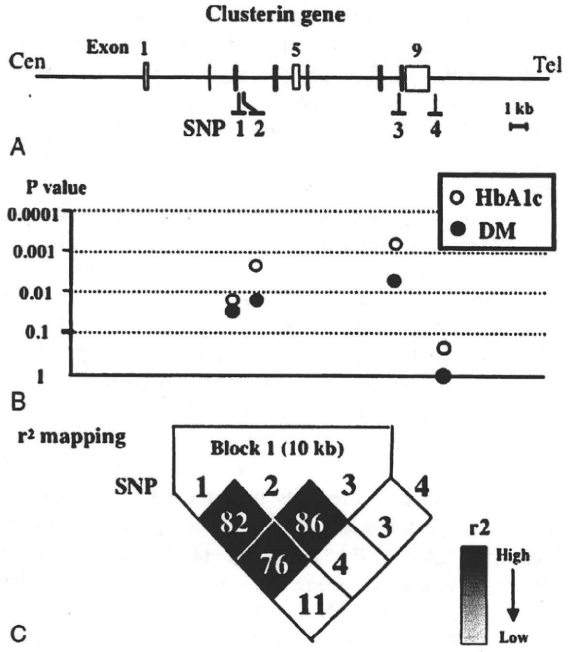

Fig. 1. Association mapping of the clusterin gene polymorphisms (SNPs). A, The positions of the SNPs examined (SNPs 1-4 [rs ID]: 1532278, 1532277, 2279590, and 2279591, respectively) are schematically shown. The box represents the exon. Cen indicates centromeric; Tel, telomeric. B, *P* values of the 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.

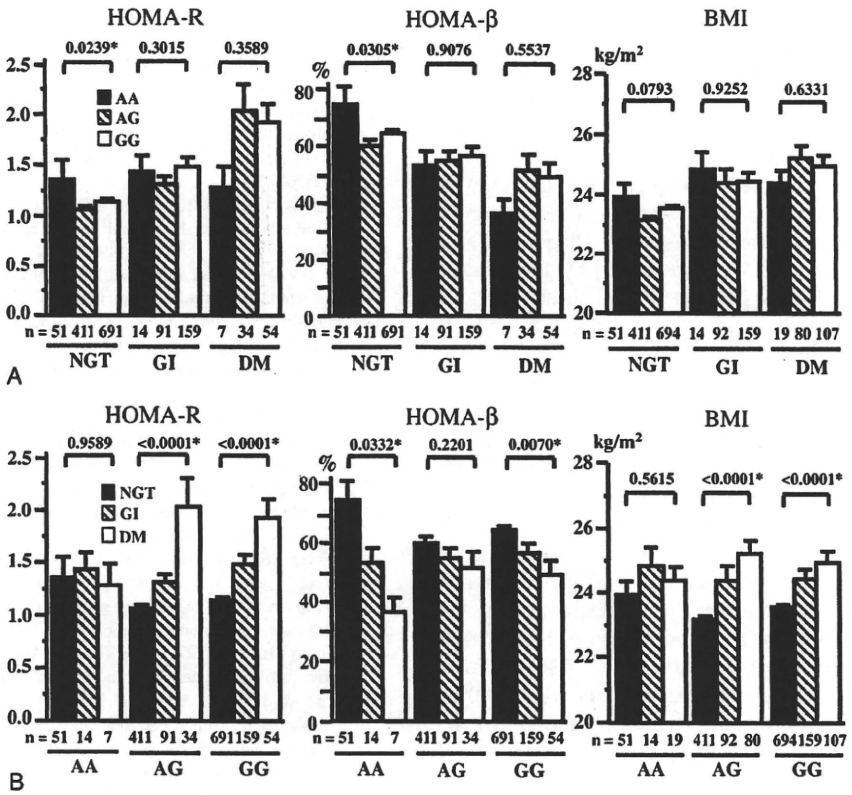

Fig. 2. Effect of the clusterin gene polymorphism rs2279590 on the insulin resistance and secretion indexes, HOMA-R and HOMA-β, and BMI. A, The trait values according to the genotype groups in each category of the glucose tolerance, namely, NGT, GI, and diabetes. B, The trait values according to the 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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Hemoglobin A1c in predicting progression to diabetes☆

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ABSTRACT

The predictive value of hemoglobin A1c (HbA1c) in comparison to fasting plasma glucose (FPG) is evaluated for 5-year incident diabetes (DM), as HbA1c may be more practical than FPG in the screening for DM in the future. Of 1189 non-DM subjects aged 35–89 years old from the Funagata Study, 57 subjects (4.8%) had developed DM on the WHO criteria at 5-year follow-up. The odds ratio (95% confidence interval: CI) for a one standard deviation increase in FPG/HbA1c was 3.40 (2.44–4.74)/3.49 (2.42–5.02). The area under the receiver operating characteristic curve for FPG/HbA1c was 0.786 (95% CI: 0.719–0.853)/0.785 (0.714–0.855). The HbA1c corresponding to FPG 5.56 mmol/l was HbA1c 5.3%. There was no statistical difference in sensitivity between FPG 5.56 mmol/l and HbA1c 5.3% (61.4% vs. 56.1%), while specificity was higher in HbA1c 5.3% than FPG 5.56 mmol/l (87.8% vs. 82.5%, p-value < 0.001). 0.001). The fraction of incident case from those with baseline IGT was similar between the groups, however the fraction of people above the cut-off was significantly lower in HbA1c 5.3% than FPG 5.56 mmol/l (14.3% vs. 19.6%, p-value < 0.001). HbA1c is similar to FPG to evaluate DM risk, and HbA1c could be practical and efficient to select subjects for intervention.

1. Introduction

The prevalence of type 2 diabetes (T2DM) is increasing rapidly worldwide, and emerging as a serious health issue [1]. Recent clinical trials have demonstrated that lifestyle or pharmacological interventions in subjects with impaired glucose tolerance (IGT) can delay or prevent T2DM [2–4]. More recent epidemiological study [5] and clinical trial [6] have shown that aggressive glycemic control should be started as early as possible to delay or prevent serious diabetes-related complications in subjects with DM. Thus, high-risk subjects for T2DM should be identified at early stage of the disease for intensive interventions.

In Japan, people with possible (hemoglobin A1c [HbA1c] 5.6–6.0%) and probable (HbA1c ≥6.1% and under treatment of diabetes) DM increased from 16.2 million in 2002 to 22.1 million in 2007 among the general population over 20 years old, representing an average 7.3% increase in rate per year [7]. The high-risk approach where either FPG or HbA1c is

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Abbreviations: ADA, American Diabetes Association; CI, confidence intervals; BMI, body mass index; DM, diabetes mellitus; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; IGT, impaired glucose tolerance; JDS, Japan Diabetes Society; OGTT, oral glucose tolerance test; OR, odds ratio; ROC, receiver operating characteristic; Wc, Waist circumference; WHO, World Health Organization; 2 h PG, 2 h plasma glucose.

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incorporated into the general health check targeted future lifestyle-related diseases including DM has been launched in 2008 [8]. Although 2 h plasma (2 h PG) on an oral glucose tolerance test (OGTT) is a better predictor of DM than FPG [9,10], an OGTT is abandoned at opportunistic screening for DM. The simple and inexpensive substitutes would be required at primary health care. To date, both HbA1c and FPG are significant predictors of DM in some studies [11,12]. However, these studies used the American Diabetes Association (ADA) criteria [13] for the diagnosis of DM and the impact of HbA1c on incident DM based on 2 h PG was not taken into account. Thus, the aim of the current study was to assess the predictabilities of baseline FPG and HbA1c for DM based on the World Health Organization (WHO) criteria [14] at 5-year follow-up, by comparing baseline 2 h PG on an OGTT. Moreover, the cut-off points on baseline HbA1c were examined with respect to the prediction of DM at 5-year follow-up.

2. Subjects and methods

Funagata Study has been described previously [15]. Briefly, the Funaga Study is a population-based study conducted in an agricultural area 400 km north of Tokyo to clarify the risk factors, related conditions, and consequence of type 2 DM. The baseline data from the 2nd survey performed between 18th June1995 and 6th July 1997 consisted of 2154 subjects aged 35–89 years (participation rate: 48.4%). Of those, 1189 subjects without DM on the 1999-WHO criteria [14] were repeatedly performed an OGTT at the 3rd survey conducted between 16th June 2000 and 7th June 2002.

In both baseline and 5-year follow-up, blood samples were drawn from the antecubital vein after overnight fasting for measurement of FPG and lipids (enzymatic and direct methods) followed by an 75 g OGTT (Trelan-G®, Shimizu Pharmaceutical, Shimizu) in subjects without a treatment of DM. HbA1c was measured after the calibration standardized of the Japan Diabetes Society (JDS) [16,17] and the JDS assigned HbA1c values, which is 0.3% lower than the National Glycoprotein Standardization Program assigned values [18], were used in the present study. Intra-assay coefficient of variation for HbA1c was 1.0% at values 5.2% and 10.5%. Waist circumference (Wc) was measured at the navel level at the end of expiration under normal breathing in a standing position. Systolic and diastolic blood pressures were measured in the sitting position after a 5 min rest using a mercury sphygmomanometer. All participants were questioned about their smoking and alcohol habits.

2.1. Statistical analyses

McNemar's test was used to compare proportions between dependent samples. The 5-year cumulative incidence of DM was calculated as the number of subjects who developed DM at 5-year follow-up divided by the sum of duration of follow-up for each subject, in the three glucose categories for FPG, 2 h PG and HbA1c, respectively, as follows: FPG <5.05, 5.05–5.55, 5.56–6.99 mmol/l, 2 h PG <5.60, 5.60–7.79, 7.80-11.09 mmol/l, and HbA1c <5.0, 5.0–5.2, ≥5.3%. The FPG 5.56 mmol/l and 2 h PG 7.80 mmol/l were chosen, as they are defined as the lower limit of abnormal glucose metabolism in non-DM glucose range [14]. The HbA1c 5.3% was chosen, as it corresponds to FPG 5.56 mmol/l in the receiver operating characteristic (ROC) curve analysis [19] described below. The below these cut-offs, subjects were equally divided into cited group for FPG, 2 h PG and HbA1c, respectively.

Odds ratios (ORs) for the presence of DM at 5-year follow-up were estimated by using logistic regression analysis and reported with their 95% confidence intervals (CIs). The model adjusted for age (continuous), sex (categorical), Wc (continuous), FPG, 2 h PG or HbA1c (categorical) was made and tested by one by one for following explanatory variables: systolic blood pressure (continuous), cholesterol (continuous), triglyceride (continuous), high density lipoprotein cholesterol (continuous), smoking status (categorical, none/past smoker/current smoker), alcohol habits (categorical, none/drink occasionally/drink regularly) and family history of DM (categorical, none/present in first degree relatives). A variable of family history of DM, which came out to be significant in the former model, was fitted in a final model with age, sex, Wc and variables for FPG, 2 h PG or HbA1c. The subsequent logistic regression model, in which a continuous variable for a one standard deviation increase in FPG (0.58 mmol/l), 2 h PG (1.83 mmol/l) or HbA1c (0.4%) was entered, was fitted to see which of the glucose index has the strongest impact on the development of DM.

2.1.1. *Performance of three glucose indices as screening tests for DM at 5-year follow-up*

The ability of baseline FPG, 2 h PG and HbA1c to predict the incidence of DM at 5-year follow-up was determined by computing sensitivity and specificity and plotting them in a ROC curve [19]. The optimal cut-off maximizing sum of sensitivity plus specificity was explored for each glucose indicator. The sensitivity, specificity, positive predictive value (PPV) and false negative predictive value (NPV) for DM at 5-year follow-up and the proportion of subjects above the cut-off were calculated at baseline FPG 5.56 mmol/l and 2 h PG 7.80 mmol/l. The same calculation was made for HbA1c 5.1%, 5.2%, 5.3% and 5.4%.

The study was approved by the Institutional Review Board of Yamagata University and the informed consent to participate was obtained from all participants. All statistical analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL, USA). A p-value < 0.05 was considered as statistically significance.

3. Results

During a 5-year follow-up period, 34 men (6.8% [95% CI: 4.6–9.0]) and 23 women (3.3% [2.0–4.7]) developed DM. The overall cumulative 5-year incidence density of DM was 12.1 (95% CI: 8.9–15.2) per 1000 person years of follow-up for men and women combined (Table 1).

3.1. *Incidence density and risk prediction of DM at 5-year follow-up from baseline FPG, 2 h PG, or HbA1c*

The 5-year cumulative incidence density and the multivariate ORs of DM at 5-year follow-up were significantly higher in subjects with the highest glucose category than the lowest

Table 1 – Incidence density and adjusted odds ratios for the presence of DM at 5-year follow-up according to baseline glucose categories.

	Number of subjects (%)	Number of incident case (incident case from IGT)	Incidence density of DM 1000 person-years (95% CI)	[a] Adjusted ORs for DM (95% CI)
Fasting plasma glucose (mmol/l)				
<5.05	507 (42%)	8 (1)	4.0 (1.2–6.7)	1.00
5.05–5.55	449 (38%)	14 (9)	7.9 (3.8–12.0)	1.72 (0.71–4.19)
5.56–6.99	233 (20%)	35 (25)	37.8 (25.5–50.1)	7.53 (3.35–16.93)
2 h plasma glucose (mmol/l)				
<5.60	512 (43%)	6 (0)	3.0 (0.6–5.3)	1.00
5.60–7.79	541 (46%)	16 (0)	7.5 (3.8–11.1)	2.38 (0.91–6.26)
7.80–11.09 (IGT)	136 (11%)	35 (35)	64.8 (44.1–85.6)	20.64 (8.13–52.37)
HbA1c (%)				
<5.0	559 (47%)	8 (2)	3.6 (1.1–6.1)	1.00
5.0–5.2	460 (39%)	17 (7)	9.3 (4.9–13.7)	2.14 (0.91–5.05)
≥5.3	170 (14%)	32 (26)	47.4 (31.4–63.4)	10.06 (4.44–22.79)
Total				
	1189 (100%)	57 (35)	12.1 (9.0–15.2)	

[a] Adjusting for age, sex, waist circumference, and family history of DM.

glucose category for FPG, 2 h PG and HbA1c (Table 1). There was no difference in the 5-year cumulative incidence density between three glucose indicators for each of the lowest, middle and the highest glucose category.

Modeling with continuous FPG, 2 h PG or HbA1c, the risk for DM at 5-year follow-up related to a one standard deviation increase in FPG, 2 h PG and HbA1c were 3.40 (2.44–4.74), 4.76 (3.30–6.86) and 3.49 (2.42–5.02), respectively.

3.2. *ROC curve analyses predicting DM from baseline FPG, 2 h PG, or HbA1c*

The area under the ROC curve for DM at 5-year follow-up was not statistically different across three glucose indicators: 0.830 (0.767–0.893) for 2 h PG, 0.786 (0.719–0.853) for FPG and 0.785 (0.714–0.855) for HbA1c (Fig. 1). The optimal cut-offs for FPG, 2 h PG and HbA1c were 5.36 mmol/l, 7.52 mmol/l and 5.1%, respectively. The HbA1c 5.3% gave the same sum of sensitivity plus specificity as FPG 5.56 mmol/l/l.

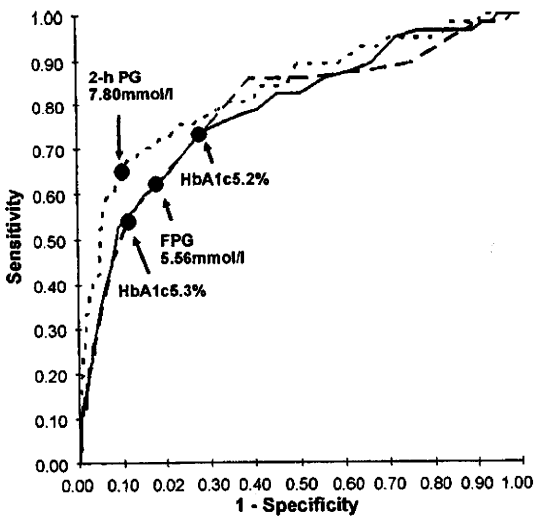

Fig. 1 – Receiver operating characteristic curves for incident diabetes at 5-year follow-up: baseline FPG (solid line), 2 h PG (dotted line) and HbA1c (solid and dotted line) among 1189 non-diabetes subjects at baseline.

3.3. *Performance as the screening test for future DM at various Pre-DM glucose cut-offs*

There was no statistical difference in sensitivity and 100-PPV between FPG 5.56 mmol/l, 2 h PG 7.80 mmol/l, HbA1c 5.2% and HbA1c 5.3%. The specificity was the highest in 2 h PG 7.80 mmol/l, the second highest in HbA1c 5.3%, followed by FPG 5.56 mmol/l, and the lowest in HbA1c 5.2% (all p-values <0.01). There was a precise reverse order in the proportion of subjects above the cut-off (all p-values <0.05).

The distribution of incident case of DM from subjects with baseline IGT was almost similar between the categories for baseline FPG and baseline HbA1c (Table 1). The proportion of incident case of DM from subjects with baseline IGT was significantly higher in those with baseline HbA1c 5.2% (89%, 31/35)(p-values <0.001) than that in those with baseline FPG 5.56 mmol/l (71%, 25/35) or baseline HbA1c 5.3% (74%, 26/35).

4. Discussion

The FPG is an established predictor of DM and considered as a relevant screening test for DM in the future [9–12]. However, blood sampling at fasting state in the morning is oftentimes difficult to perform in general population. Our study has shown that HbA1c has a similar ability to FPG for evaluating future DM risk and for detecting incident cases of DM, especially from the group of subjects with IGT at baseline. Obtained data also demonstrated that 2 h PG on an OGTT had a slightly better predictability for future DM than FPG or HbA1c, which is partly accordance with European reports [9,10]. However, its use as an initial screening test is unrealistic. In the screening at non-fasting state, HbA1c could be practically

and efficiently used to identify subjects at high-risk for DM who should be targeted for intensive prevention intervention.

The 2 h PG depends on insulin secretary capacity of pancreatic beta cells, peripheral insulin sensitivity, and hepatic glucose output and uptake whereas FPG largely depends on hepatic glucose production. While HbA1c reflects glucose metabolism over the past 1–2 months [16], can be converted into the estimated average glucose levels [20], has smaller variability than FPG and 2 h PG [21], and is closely correlated with post-load glucose in its low range and correlated with FPG in its high range [22]. Thus, HbA1c could cover a wider range of pathophysiological processes of DM than FPG. In our study, HbA1c showed almost the same overall predictability for DM in the future as FPG. In some previous studies, HbA1c seemed to be inferior to FPG with respect to the risk prediction and detection [11,12]. This might partly be due to the application of ADA criteria for the diagnosis of DM [11,12]. In our data, 70% of new cases of DM was identified by isolated 2 h PG (data not shown) and these subjects would not be identified as DM by the ADA criteria. In our country, HbA1c ≥6.5% has been used as a supportive test for the diagnosis of DM for past 10 years [23]. The International Expert Committee appointed by the ADA, the European Diabetes Association for the Study of Diabetes, and the International Diabetes Federation has recommended diagnosing DM by using HbA1c, since June 2009 [24]. Moreover, HbA1c has been provided a treatment target for patients with DM in many organizations including JDS [23]. Thus, HbA1c could be used in different stages of the diseases: screening, diagnosis and treatment. Meanwhile, HbA1c measurement by enzymatic method (Arkray, Kyoto) has become possible at a reasonable cost [25]. This satisfactorily correlates with HbA1c measurement by the HPLC method, does not need standardization, and is more economical than it measurement by HPLC method. This might be a rationale for recommending HbA1c in evaluating future DM risk.

Recently, we have shown that FPG ≥5.56 mmol/l is the better predictor than metabolic syndrome or a constellation of cardiovascular risk factors except for FPG ≥5.56 mmol/l regardless of abdominal adiposity in the Funagata Study [26]. The same trend was obtained when HbA1c ≥5.3% replaced FPG ≥5.56 mmol/l (data not shown). This highlighted glucose itself as the screening test for DM in the future. In our data, HbA1c 5.3% corresponded to FPG 5.56 mmol/l for predicting DM (Fig. 1 and Table 2) and both cut-offs identified similar risk of DM (Table 1) and had equal detection rate of DM, especially from the group of subjects with baseline IGT (Tables 1 and 2). On the other hand, the proportion of people above the cut-off was significantly lower in HbA1c 5.3% than FPG 5.56 mmol/l. Thus, HbA1c 5.3% rather than FPG 5.56 mmol/l might be efficient to identify those targeted for intensive intervention. Since the decision of the screening cut-off is tentative, the cut-off for HbA1c applied in Japan of 5.2% [8] might be too low in our study subjects. Since HbA1c 5.2% could identify significantly more incident cases from those with IGT than FPG 5.56 mmol/l or HbA1c 5.3%, the use of HbA1c 5.2% would make markedly high proportion of subjects (= one third of the entire screened population) who would be followed by intensive intervention.

There are limitations in our study. First, despite concerted efforts to maximize follow-up, the participation rate at 5-year of follow-up was 60%, which, although comparable to other studies of this nature, could potentially bias our results. When comparing baseline characteristics between those who did and did not participate in follow-up, the participants were younger and were healthier than non-participants (data not shown). This is in line with the frequent observation of "healthy participants' effect", which has also been reported in other studies [27]. This would lead to an underestimation of the true cumulative incidence in the general population, and thus our results are conservative. Second, the study population is approximately 10-years older than the representative sample of the Japanese general population [7], and this may have influenced our results. The relevance of Japanese cut-off of 5.2% for HbA1c to screen subjects requiring health guidance in the screening program [8] should be further examined in other Japanese studies. Third, FPG and 2 h PG in this population were assessed only once at both baseline and follow-up. The inter- and intra-coefficients of variations in glucose values may have caused some random misclassification in glucose categories [21], and thereby influenced our results. Fourth, the total number of incident cases is too small to obtain conclusive cut-off discriminating risks and performance as the screening between different strata. Fifth, we did not run sex-stratified analysis due to limited number of incident cases but did adjustment by sex. Since the crude proportion of incident case in men was double-folds higher than women, the overall predictabilities of DM based on ROC curve analysis did not differ between sexes for each glucose indicators or not differ across three glucose indicators in both

Table 2 – Performance (%) [95% confidence interval] of cut-offs on three glucose indicators for predicting DM at 5-year follow-up.

Variables	Cut-offs	Number (%)	% Sensitivity	% Specificity	100-Positive predictive value (%)	100-Negative predictive value (%)
FPG	5.56 mmol/l	233 (19.6)	61.4 [48.8–74.0]	82.5 [80.3–84.7]	85.0 [80.4–89.6]	2.3 [1.4–3.3]
2 h PG	7.80 mmol/l	136 (11.4)	61.4 [48.8–74.0]	91.1 [89.4–92.7]	74.3 [66.9–81.6]	2.1 [1.2–3.0]
HbA1c	5.1%	490 (41.2)	86.0 [76.9–95.0]	61.0 [58.2–63.9]	90.0 [87.3–92.7]	1.1 [0.4–1.9]
	5.2%	360 (30.3)	73.7 [62.3–85.1]	71.9 [69.3–74.5]	88.3 [85.0–91.6]	1.8 [0.9–2.7]
	5.3%	170 (14.3)	56.1 [43.3–69.0]	87.8 [85.9–89.7]	81.2 [75.3–87.1]	2.5 [1.5–3.4]
	5.4%	113 (9.5)	45.6 [32.7–58.5]	92.3 [69.3–74.5]	77.0 [69.2–84.8]	2.9 [1.9–3.9]

FPG: fasting plasma glucose, 2 h PG: 2 h plasma glucose.

sexes (data not shown). Sixth, the application of micro- and macro-vascular complication as the hard end point was not unable in the current study. However, notwithstanding the limitations, our study has notable strengths, being population-based, consisting of both men and women, having FPG and 2 h PG to enable rigorous biochemical diagnosis of DM based on either FPG or 2 h PG criteria and a well-phenotyped sample at baseline and follow-up.

In conclusion, HbA1c can be practically used to screen high-risk of future DM in a general Japanese population. It could also effectively be used in association with IGT who could be targeted for intensive prevention intervention.

Conflicts of interest

The authors declare that they have no conflict of interest.

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