Impact of Kir6.2 E23K Polymorphism on the Development of Type 2 Diabetes in a General Japanese Population

The Hisayama Study

Yasufumi Doi,[1] Michiaki Kubo,[1,3] Toshiharu Ninomiya,[2] Koji Yonemoto,[2] Masanori Iwase,[1] Hisatomi Arima,[2] Jun Hata,[2] Yumihiro Tanizaki,[1] Mitsuo Iida,[1] and Yutaka Kiyohara[2]

OBJECTIVE—The association between the E23K polymorphism of ATP-sensitive K^+ channel subunit Kir6.2 and diabetes has been reported in Caucasians but not in Asians. We examined this issue in follow-up and cross-sectional studies in a general Japanese population.

RESEARCH DESIGN AND METHODS—In a 14-year follow-up study of 976 subjects aged 40–79 years with normal glucose tolerance (NGT), we investigated the impact of the E23K polymorphism on change of glucose tolerance status using a 75-g oral glucose tolerance test. Additionally, we confirmed this association in a cross-sectional survey of 2,862 subjects.

RESULTS—In the follow-up study, the frequencies of the K/K genotype or K-allele were significantly higher in subjects with conversion from NGT to diabetes than in those in whom NGT was maintained (genotypes, $P = 0.01$; alleles, $P = 0.008$). In multivariate analysis, the risk for progression to diabetes was significantly higher in subjects with the E/K (odds ratio 2.10 [95% CI 1.16–3.83]) and K/K (2.40 [1.01–5.70], P for trend = 0.01) genotypes than in those with the E/E genotype after adjustment for confounding factors, namely, age, sex, fasting plasma glucose, family history of diabetes, BMI, physical activity, current drinking, and current smoking. In the cross-sectional study, the frequencies of the K/K genotype or K-allele were also significantly higher in those with diabetes than in those with NGT (genotypes, $P = 0.006$; alleles, $P = 0.001$).

CONCLUSIONS—Our findings suggest that the Kir6.2 E23K polymorphism is an independent genetic risk factor for diabetes in the general Japanese population. ***Diabetes*** **56:2829–2833, 2007**

From the [1]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; the [2]Department of Environmental Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; and the [3]Laboratory for Genotyping, SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Yokohama, Japan.

Address correspondence and reprint requests to Yasufumi Doi, MD, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: doi@intmed2.med.kyushu-u.ac.jp.

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IFG, impaired fasting glycemia; IGT, impaired glucose tolerance; K_{ATP} channel, ATP-sensitive K^+ channel; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PAR, population-attributable risk.

Variants in genes encoding key components of insulin secretion pathways may confer a susceptibility to type 2 diabetes. Among candidate genes for such variants, the ATP-sensitive K^+ channel (K_{ATP} channel) genes play an essential role in glucose-stimulated insulin secretion (1). The K_{ATP} channel in pancreatic β-cells is composed of an ATP-sensitive and pore-forming inwardly rectifying K^+ channel (Kir6.2) subunit (2) and a regulatory sulfonylurea receptor 1 subunit (3). A single nucleotide polymorphism at codon 23 of the *KCNJ11* gene (rs5219) results in a glutamic acid to lysine substitution (E23K) in the NH_2-terminal tail of Kir6.2 (4–6), and this polymorphism may cause modest reductions in ATP sensitivity and insulin secretion (7). Although there is general consensus that this polymorphism is a risk factor for type 2 diabetes in Caucasian populations (8–12), very few studies have examined its effect in Asian populations. Recently, one study reported that the E23K polymorphism was not significantly related to diabetes in Japanese (13). The aim of the present article is to assess the association of this polymorphism with diabetes in a defined Japanese population after accounting for comprehensive risk factors for diabetes.

RESEARCH DESIGN AND METHODS

Follow-up study group. In 1988, a screening survey for the present study was performed in the town of Hisayama, a suburb of Fukuoka, Japan (14). The age and occupational distributions for Hisayama were almost identical to those of Japan as a whole based on data from the national census. Of all residents aged 40–79 years in 1988, 2,587 participated in the baseline survey (participation rate 80.2%). After excluding 82 subjects who had already had breakfast, 15 who ceased taking a 75-g oral glucose tolerance test (OGTT) due to nausea or general fatigue during the ingestion of glucose, and 10 who were on insulin therapy, 2,480 subjects completed the OGTT. Of these, 1,561 subjects with normal glucose tolerance (NGT) were enrolled in the baseline examination. After the initial screening, glucose tolerance levels were measured again in 2002. The genotype data of the E23K polymorphism and glucose tolerance levels were successfully obtained for a total of 976 subjects (383 men and 593 women), and these were the subjects selected for the 14-year follow-up study.

Cross-sectional study group. A diabetes survey similar to that done in 1988 was performed in 2002. Of all residents aged 40 years or over, 3,328 underwent the examination (participation rate 77.6%). Among the participating residents, 3,196 subjects agreed to the genetic analysis. Of these, a total of 2,851 subjects completed the OGTT after excluding 234 who refused the OGTT, 76 who had already eaten breakfast, and 35 who were on insulin therapy. Among those who did not undergo the OGTT, the following 78 subjects were categorized as having diabetes and included in the cross-sectional study: 21 with fasting plasma glucose ≥7.0 mmol/l, 6 with plasma glucose ≥11.1 mmol/l after eating breakfast, 16 who were taking oral hypoglycemic agents, and 35 on insulin

TABLE 1
Clinical characteristics of subjects according to the Kir6.2 E23K polymorphism in NGT subjects in 1988 and total subjects in 2002

	NGT subjects at baseline from the follow-up study in 1988				Total subjects in the cross-sectional study in 2002			
	E/E	E/K	K/K	*P* for trend	E/E	E/K	K/K	*P* for trend
n	414	442	120		1,197	1,296	369	
Age (years)	53 ± 8	52 ± 8	52 ± 8	0.48	60 ± 12	60 ± 12	61 ± 12	0.56
Men (%)	38.7	39.4	40.8	0.67	44.6	44.4	43.1	0.26
Fasting plasma glucose (mmol/l)	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.4	0.76	6.1 ± 1.4	6.2 ± 1.4	6.3 ± 1.4	0.03
2-h postload glucose (mmol/l)	5.9 ± 1.1	5.9 ± 1.1	6.1 ± 1.0	0.29	7.9 ± 3.8	8.3 ± 3.8	8.5 ± 3.8	0.002
Fasting insulin (pmol/l)	32.9 (14.2–76.2)	31.2 (13.6–71.1)	31.5 (14.5–68.6)	0.11	39.7 (12.8–123.5)	39.1 (12.6–121.5)	40.0 (12.9–124.5)	0.92
2-h postload insulin (pmol/l)	157.1 (42.0–587.9)	144.1 (37.1–559.9)	140.0 (40.6–482.7)	0.03	241.9 (57.7–1,013.6)	238.9 (57.0–1001.8)	230.2 (54.9–965.4)	0.30
HOMA-IR	1.3 (0.5–3.1)	1.2 (0.5–2.9)	1.2 (0.5–2.9)	0.11	1.8 (0.5–6.4)	1.7 (0.5–6.4)	1.8 (0.5–6.6)	0.72
Family history of diabetes (%)	6.1	9.6	8.4	0.14	14.9	15.8	15.3	0.61
BMI (kg/m^2)	23.0 ± 2.8	22.9 ± 2.8	22.5 ± 2.8	0.09	23.3 ± 3.3	23.2 ± 3.3	23.2 ± 3.3	0.65
Regular exercise (%)	9.0	10.9	15.8	0.04	10.9	10.2	11.6	0.90
Current drinking (%)	29.0	31.5	35.8	0.15	46.0	45.3	46.6	0.97
Current smoking (%)	19.6	21.3	33.3	0.006	22.5	22.1	24.5	0.35

Data are means ± SD, percentages, or geometric mean (range). Fasting insulin, 2-h postload insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) values are geometric mean and value by 1 SD computed on the log-transformed variable and the mean and SD values converted to the original scale. Age and percent of men are not adjusted.

therapy. Consequently, 2,929 subjects whose glucose tolerance levels were determined underwent analysis of the E23K polymorphism. Of these, the genotype data were successfully obtained from a total of 2,862 subjects (1,268 men and 1,594 women), for a genotyping success rate of 97.7%.

Genotyping. Genotyping of the Kir6.2 E23K polymorphism was done by a TaqMan assay at HuBit Genomix (Tokyo, Japan). The TaqMan genotyping reaction was amplified on a GeneAmp PCR System 9700, and fluorescence was detected on an ABIPRISM 7900HT Sequence Detection System. We confirmed the genotyping results of 376 randomly selected subjects by the direct sequencing method at the RIKEN Institute (Yokohama, Japan). Consequently, the concordance rate was 100% in the 369 subjects who were successfully genotyped by both the TaqMan and direct sequencing methods. The distributions of the E23K polymorphism were in Hardy-Weinberg equilibrium.

Clinical evaluation. In both the 1988 and 2002 surveys, the study subjects underwent the OGTT between 8:00 and 10:30 A.M. after at least a 12-h overnight fast. The plasma glucose levels were determined by the glucose-oxidase method and serum insulin by a radioimmunoassay in 1988 and a chemiluminescent enzyme immunoassay in 2002. Plasma glucose levels were classified according to World Health Organization criteria (15). In the 2002 survey, the subjects who were taking antidiabetic medicine or had a glucose concentration of ≥11.1 mmol/l after eating breakfast were categorized as having diabetes. Homeostasis model assessment of insulin resistance was calculated from fasting plasma glucose and serum insulin (16). BMI was calculated from the height and weight. Diabetes in first- or second-degree relatives was taken to indicate a family history of diabetes. Alcohol intake and smoking habits were classified as either currently habitual or not. Subjects engaging in sports at least three times per week during their leisure time were classified into a regular exercise group.

Statistical analysis. The distributions of the E23K polymorphism were analyzed according to glucose tolerance levels by χ^2 test. Adjusted odds ratios (ORs) and 95% CIs were calculated by logistic regression model. The population-attributable risk (PAR) percentage and its 95% CI were estimated according to the method used in the previous report (17). If assuming a significance level of $P < 0.05$ and 80% power, it is estimated that the OR for the risk of diabetes between the E/E and K/K genotypes becomes significant at the difference of 1.37-fold or greater in our cross-sectional survey. In a meta-analysis of the cross-sectional studies (10), it has been reported that the OR for the risk of diabetes in the K/K genotype was 1.49, significantly higher than that for the E/E genotype. Thus, the sample size of our study seems to be enough for the detection of the significant association.

This study was conducted with the approval of the ethics committee of the Faculty of Medicine, Kyushu University, and written informed consent was obtained from all study subjects.

RESULTS

Table 1 shows the clinical characteristics of NGT subjects in 1988 and total subjects in 2002 according to Kir6.2 E23K genotypes.

The frequencies of the E23K polymorphism based on change of glucose tolerance status are shown in Table 2. The distributions of the E23K polymorphism in subjects with conversion from NGT to impaired fasting glycemia (IFG) or impaired glucose tolerance (IGT) were not significantly different from those in whom NGT was maintained over the 14-year follow-up. The frequencies of the E/K and K/K genotypes or K-allele, however, were significantly higher in individuals with progression to diabetes than in those in whom NGT was maintained (genotypes, $P = 0.01$; alleles, $P = 0.008$).

The age- and sex-adjusted or multivariate-adjusted ORs of the E23K polymorphism for the development of diabetes were analyzed between subjects with conversion from NGT to diabetes during the follow-up period and subjects with NGT maintained (Table 3). The risk for progression from NGT to diabetes was more than twofold higher in the E/K and K/K genotypes than in the E/E genotype after age and sex adjustment. This association remained significant even after adjustment for confounding factors at baseline, i.e., age, sex, fasting plasma glucose, family history of diabetes, BMI, physical activity, current drinking, and current smoking (E/K genotype, adjusted OR 2.10 [95% CI

TABLE 2
Genotype and allele frequencies of the Kir6.2 E23K polymorphism according to change of glucose tolerance status in the follow-up study, 1988–2002

	Change of glucose tolerance status			
	NGT → NGT	NGT → IFG	NGT → IGT	NGT → diabetes
E23K genotype				
E/E	259 (0.44)	41 (0.46)	95 (0.42)	19 (0.26)
E/K	260 (0.44)	35 (0.39)	104 (0.46)	43 (0.59)
K/K	66 (0.11)	14 (0.16)	29 (0.13)	11 (0.15)
P vs. NGT		0.41	0.74	0.01
E23K allele				
E	778 (0.66)	117 (0.65)	294 (0.64)	81 (0.55)
K	392 (0.34)	63 (0.35)	162 (0.36)	65 (0.45)
P vs. NGT		0.69	0.43	0.008

Data are *n* (frequency).

1.16–3.83]; K/K genotype, 2.40, [1.01–5.70]; *P* for trend = 0.01). A similar result was revealed in the allele frequency model; the risk for conversion to diabetes was higher in subjects with the minor K-allele than in those with the major E-allele (1.58 [1.09–2.30], $P = 0.01$). The likelihood ratio in the multivariate-adjusted model that included the above-mentioned confounding risk factors and the E23K polymorphism was significantly higher than that in the model with only the confounding factors (genotypes, $P = 0.02$; alleles, $P = 0.01$). Furthermore, the multivariate-adjusted OR of the dominant model in which the E/K and K/K genotypes were combined was 2.16 (95% CI 1.21–3.85) compared with the E/E genotype. When estimating the PAR percentage, 40.1% (10.8–62.2) of the progression to diabetes among NGT subjects was attributable to these dominant genotypes.

Furthermore, we investigated the association between the E23K polymorphism and the risk of diabetes in the total subject group in 2002 (Table 4). The genotype and allele frequencies of the E23K polymorphism in subjects with IFG and IGT were not significantly different from those in the subjects with NGT, while the frequencies of the K/K genotype or K-allele were significantly higher in those with diabetes than in those with NGT (genotypes, $P = 0.006$; alleles, $P = 0.001$).

DISCUSSION

In our follow-up and large cross-sectional studies, we showed a positive association between the Kir6.2 E23K polymorphism and type 2 diabetes. These associations remained significant even after adjusting for other confounding factors. The likelihood ratio was significantly higher in the multivariate-adjusted model with the polymorphism than in that without. These findings suggest that the E23K polymorphism is a significant predictor of future diabetes in the general Japanese population.

Several case-control studies indicated that the E23K polymorphism is a risk factor for type 2 diabetes (8–12), but this association was not observed in cohort studies—two Finnish prospective studies showed that the E23K polymorphism had no effect on the development of diabetes (18,19). The present study is the first to indicate an association between the E23K polymorphism and diabetes using a follow-up design. Our study group consisted of exclusively Japanese subjects, with no population stratifications (20), and their glucose tolerance levels were determined in principle using an OGTT. This study design provided us an opportunity to precisely examine the ability of the E23K polymorphism to predict diabetes.

In our follow-up study, the PAR percentage for progression to diabetes was 40.1% in the NGT subjects, which was higher than that reported in previous studies of this gene (8,12). This difference seems to be attributable to the fact that we used control subjects in whom NGT was maintained, as confirmed by the OGTT, over a 14-year interval. However, since our result was based on a small number of subjects, it is better to confirm this value in other large populations.

The present study is the first to show a significant association between the E23K polymorphism and diabetes in Japanese. Recently, it was reported that this polymorphism was not associated with diabetes in Japanese indi-

TABLE 3
Age- and sex-adjusted or multivariate-adjusted ORs and 95% CIs for the progression to diabetes from NGT by the Kir6.2 E23K genotype and allele in the follow-up study, 1988–2002

	E23K genotype				E23K allele		
	E/E	E/K	K/K	*P* value for trend	E	K	*P*
Subjects at risk (*n*)	278	303	77		859	457	
Cases of diabetes (*n*)	19	43	11		81	65	
Age- and sex-adjusted OR (95% CI)	1 (referent)	2.25 (1.28–3.97)	2.27 (1.03–5.02)	0.008	1 (referent)	1.59 (1.12–2.26)	0.009
Multivariate-adjusted OR (95% CI)	1 (referent)	2.10 (1.16–3.83)	2.40 (1.01–5.70)	0.01	1 (referent)	1.58 (1.09–2.30)	0.01

Multivariate adjustment was made for age, sex, fasting plasma glucose, family history of diabetes, BMI, physical activity, current drinking, and current smoking.

TABLE 4
Genotype and allele frequencies of the Kir6.2 E23K polymorphism according to glucose tolerance status in the cross-sectional study, 2002

	Glucose tolerance status			
	NGT	IFG	IGT	Diabetes
E23K genotype				
E/E	617 (0.43)	124 (0.44)	254 (0.42)	202 (0.37)
E/K	655 (0.46)	117 (0.42)	261 (0.44)	263 (0.48)
K/K	161 (0.11)	39 (0.14)	84 (0.14)	85 (0.15)
P vs. NGT		0.31	0.20	0.006
E23K allele				
E	1,889 (0.66)	365 (0.65)	769 (0.64)	667 (0.60)
K	977 (0.34)	195 (0.35)	429 (0.37)	433 (0.39)
P vs. NGT		0.73	0.29	0.001

Data are *n* (frequency).

viduals (13). In that study, subjects were recruited from university hospitals and the control subjects defined as subjects with A1C levels <5.6%. However, a group of subjects with A1C <5.6% could include individuals with diabetes, as well as IFG and IGT (21). Actually, the frequency of the minor K-allele in the control subjects of that report was significantly higher than that in our NGT subjects, although the K-allele frequencies in the respective groups of diabetic subjects were similar. The different criteria used to define the phenotype are partly responsible for these different outcomes.

Some limitations of our study must be mentioned. First, there is an overlap of subjects used in both the cross-sectional and follow-up analyses—34% of the subjects in the cross-sectional analysis were also enrolled in the follow-up analysis, and thus our results are not really replicated. Further investigations will be needed to confirm our results in other Asian populations. Second, a 75-g OGTT has low reproducibility (22). Some of the participants might have been categorized into different glucose tolerance levels after repeat testing. Nonetheless, any misclassification would be expected to weaken rather than strengthen the association found in this study. Thus, the true association may be stronger than that shown in our results. Third, subjects with type 1 diabetes may have been included in our study population. In a clinical study, 3–4% of the group of nonobese Japanese diabetic patients were positive for the GAD antibody (23). Immune abnormality in pancreatic β-cells is considered to lead to diabetes independently of the E23K polymorphism, suggesting that subjects with type 1 diabetes are distributed equally among the genotypes. Thus, this limitation does not seem to invalidate the association of the E23K polymorphism with the risk of type 2 diabetes.

In conclusion, we confirmed the association between the E23K polymorphism of the K_{ATP} channel subunit Kir6.2 and susceptibility to type 2 diabetes in a follow-up study and a large cross-sectional study in a general Japanese population. Considering that populations throughout the world have a high frequency of the E23K polymorphism, this polymorphism may be a pathogenic gene for diabetes worldwide.

REFERENCES

1. Ashcroft FM, Rorsman P: ATP-sensitive K^+ channels: a link between β-cell metabolism and insulin secretion. *Biochem Soc Trans* 18:109–111, 1990
2. Seino S, Inagaki N, Namba N, Gonoi T: Molecular biology of the β-cell ATP-sensitive K^+ channel. *Diabetes Rev* 4:177–190, 1996
3. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP 4th, Boyd AE 3rd, Gonzalez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA: Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 268:423–426, 1995
4. Sakura H, Wat N, Horton V, Millns H, Turner RC, Ashcroft FM: Sequence variations in the human Kir6.2 gene, a subunit of the β-cell ATP-sensitive K-channel: no association with NIDDM in while Caucasian subjects or evidence of abnormal function when expressed in vitro. *Diabetologia* 39:1233–1236, 1996
5. Inoue H, Ferrer J, Warren-Perry M, Zhang Y, Millns H, Turner RC, Elbein SC, Hampe CL, Suarez BK, Inagaki N, Seino S, Permutt MA: Sequence variants in the pancreatic islet β-cell inwardly rectifying K^+ channel Kir6.2 (Bir) gene: identification and lack of role in Caucasian patients with NIDDM. *Diabetes* 46:502–507, 1997
6. Hansen L, Echwald SM, Hansen T, Urhammer SA, Clausen JO, Pedersen O: Amino acid polymorphisms in the ATP-regulatable inward rectifier Kir6.2 and their relationships to glucose- and tolbutamide-induced insulin secretion, the insulin sensitivity index, and NIDDM. *Diabetes* 46:508–512, 1997
7. Schwanstecher C, Meyer U, Schwanstecher M: Kir6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic β-cell ATP-sensitive K^+ channels. *Diabetes* 51:875–879, 2002
8. Schwanstecher C, Schwanstecher M: Nucleotide sensitivity of pancreatic ATP-sensitive potassium channels and type 2 diabetes. *Diabetes* 51 (Suppl. 3):S358–S362, 2002
9. Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, Frayling TM: Large-scale association studies of variants in genes encoding the pancreatic β-cell K_{ATP} channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) confirm that the *KCNJ11* E23K variant is associated with type 2 diabetes. *Diabetes* 52:568–572, 2003
10. Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, Glumer C, Thorsteinsson B, Borch-Johnsen K, Hansen T, Pedersen O: The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 52:573–577, 2003
11. Florez JC, Burtt N, de Bakker PI, Almgren P, Tuomi T, Holmkvist J, Gaudet D, Hudson TJ, Schaffner SF, Daly MJ, Hirschhorn JN, Groop L, Altshuler D: Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive K^+ channel gene region. *Diabetes* 53:1360–1368, 2004
12. Hansen SK, Nielsen EM, Ek J, Andersen G, Glumer C, Carstensen B, Mouritzen P, Drivsholm T, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O: Analysis of separate and combined effects of common variation in *KCNJ11* and *PPARG* on risk of type 2 diabetes. *J Clin Endocrinol Metab* 90:3629–3637, 2005
13. Yokoi N, Kanamori M, Horikawa Y, Takeda J, Sanke T, Furuta H, Nanjo K, Mori H, Kasuga M, Hara K, Kadowaki T, Tanizawa Y, Oka Y, Iwami Y, Ohgawara H, Yamada Y, Seino Y, Yano H, Cox NJ, Seino S: Association studies of variants in the genes involved in pancreatic β-cell function in type 2 diabetes in Japanese subjects. *Diabetes* 55:2379–2386, 2006
14. Ohmura T, Ueda K, Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Nomiyama K, Ohmori S, Yoshitake T, Shinkawa A, Hasuo Y, Fujishima M: Prevalence of type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in the Japanese general population: the Hisayama Study. *Diabetologia* 36:1198–1203, 1993
15. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
17. Daly LE: Confidence limits made easy: interval estimation using a substitution method. *Am J Epidemiol* 147:783–790, 1998
18. Laukkanen O, Pihlajamaki J, Lindstrom J, Eriksson J, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Tuomilehto J, Uusitupa M, Laakso M: Polymorphisms of the SUR1 (*ABCC8*) and Kir6.2 (*KCNJ11*) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. *J Clin Endocrinol Metab* 89:6286–6290, 2004
19. Lyssenko V, Anevski D, Almgren P, Sjogren M, Svensson M, Orho-Melander M, Tuomi T, Groop L: Genetic prediction of type 2 diabetes. *PloS Medicine* 2:1299–1308, 2005
20. Kubo M, Hata J, Ninomiya T, Matsuda K, Yonemoto K, Nakano T, Matsushita T, Yamazaki K, Ohnishi Y, Saito S, Kitazono T, Ibayashi S,

Sueishi K, Iida M, Nakamura Y, Kiyohara Y: A nonsynonymous SNP in PRKCH (protein kinase C η) increases the risk of cerebral infarction. *Nat Genet* 39:529–533, 2007
21. Ko GT, Chan JC, Yeung VT, Chow CC, Tsang LW, Li JK, So WY, Wai HP, Cockram CS: Combined use of a fasting plasma glucose concentration and HbA1c or fructosamine predicts the likelihood of having diabetes in high-risk subjects. *Diabetes Care* 21:1221–1225, 1998
22. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
23. Bando Y, Ushiogi Y, Toya D, Tanaka N, Fujisawa M: Antibodies to glutamic acid decarboxylase (GAD) in non-obese Japanese diabetics without insulin therapy: a comparison of two commercial RIA kits based on recombinant and pig brain GAD. *Diabetes Res Clin Pract* 41:25–33, 1998