Brief Genetics Report

The E23K Variant of Kir6.2 Associates With Impaired Post–OGTT Serum Insulin Response and Increased Risk of Type 2 Diabetes

Eva-Maria D. Nielsen,[1] Lars Hansen,[1,2] Bendix Carstensen,[1] Søren M. Echwald,[1] Thomas Drivsholm,[3] Charlotte Glümer,[1] Birger Thorsteinsson,[4] Knut Borch-Johnsen,[1] Torben Hansen,[1] and Oluf Pedersen[1,5]

The E23K polymorphism of the pancreatic β-cell ATP-sensitive K^+ (K_{ATP}) channel subunit Kir6.2 (*KCNJ11*) is associated with type 2 diabetes in whites, and a recent in vitro study of the E23K variant suggests that the association to diabetes might be explained by a slight inhibition of serum insulin release. In a study comprising 519 unrelated glucose-tolerant subjects, we addressed the question as to whether the E23K variant was related to reduced serum insulin release during an oral glucose tolerance test (OGTT). Furthermore, the polymorphism was examined in a case-control study comprising 803 type 2 diabetic patients and 862 glucose-tolerant control subjects. The E23K variant was associated with significant reductions in the insulinogenic index ($P = 0.022$) and serum insulin levels under the response curve during an OGTT (0–120 min) ($P = 0.014$) as well as with an increase in BMI ($P = 0.013$). In the present study, the association of the E23K polymorphism with type 2 diabetes was not significant ($P = 0.26$). However, the K23K genotype significantly associated with type 2 diabetes in a meta-analysis of white case and control subjects ($n = 2,824$, odds ratio [OR] 1.49, $P = 0.00022$). In conclusion, the widespread E23K polymorphism may have a diabetogenic effect by impairing glucose-induced insulin release and increasing BMI.
***Diabetes* 52:573–577, 2003**

The complex regulation of glucose-induced insulin secretion from the pancreatic β-cells involves the level of pancreatic β-cell glucose metabolism and the electrical activity controlled by the various plasma membrane ion channels (1). Among these, the activity of the ATP-sensitive K^+ (K_{ATP}) channel is critical because the channel links glucose metabolism to electrical activity of the cells (2). Classical plasma membrane K_{ATP} channels are complexes of two structurally unrelated subunits, a regulatory sulfonylurea receptor (SUR) subunit belonging to the ATP-binding cassette transporter superfamily (3) and an ATP-sensitive and pore-forming inwardly rectifying K^+ channel (Kir6.X) subunit that belongs to the Kir6.0 subfamily of the inward rectifier family (4).

Various combinations of Kir6.X and SUR are possible. The pancreatic β-cell K_{ATP} channels comprise Kir6.2 and SUR1; cardiac and skeletal muscle K_{ATP} channels consist of Kir6.2 and SUR2A, whereas the K_{ATP} channels in the vascular smooth muscle are composed of either Kir6.1 or Kir6.2 and SUR2B (5). The genes encoding human Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) are positioned adjacent to one another on chromosome 11p15.1 with only 4.5 kb separating the 3′ end of *ABCC8* and the 5′ end of *KCNJ11* (6).

Impaired β-cell K_{ATP} channel function due to mutations of the *ABCC8* or *KCNJ11* gene is responsible for the autosomal recessive disorder familial persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (5,7,8). In contrast to the loss-of-function mutations associated with PHHI, transgenic mice expressing gain-of-function mutations in *KCNJ11*, and therefore over-active K_{ATP} channels, in the β-cells develop neonatal hypoinsulinemic diabetes and ketoacidosis due to loss of ATP sensitivity (9).

As a candidate gene for type 2 diabetes in humans, a nonsynonymous E23K variant in the NH_2-terminal tail of Kir6.2 was identified (10–12). The variant did not associate with type 2 diabetes or with changes in glucose- or tolbutamide-induced serum insulin responses during an intravenous glucose tolerance test in vivo (10–14). However, in French whites (15) and in the U.K. Prospective Diabetes Study (UKPDS) cohort (16), K23K homozygozity was more frequent among type 2 diabetic patients than among control subjects. These studies together with recently published in vitro experiments showing that the E23K variant leads to an over-active Kir6.2 channel with a decreased sensitivity toward ATP (by 1.4- and 2.2-fold in a heterozygous E23K and homozygous K23K model, respectively) (17), suggest that decreased insulin release may be

From the [1]Steno Diabetes Center and Hagedorn Research Institute, Gentofte, Copenhagen, Denmark; [2]Clinical Genetics, Novo Nordisk, Bagsvaerd, Denmark; the [3]Centre for Preventive Medicine, Glostrup University Hospital, Glostrup, Denmark; the [4]Department of Internal Medicine F, Hilleroed Hospital, Hilleroed, Denmark; and [5]Faculty of Health Science, University of Aarhus, Aarhus, Denmark.

Address correspondence and reprint requests to Lars Hansen, MD, Clinical Genetics, Novo Nordisk, Krogshoejvej 53A, DK-2880 Bagsvaerd, Denmark. E-mail: larh@novonordisk.com.

Received for publication 20 September 2002 and accepted in revised form 14 November 2002.

AUC, area under the curve; IGT, impaired glucose tolerance; K_{ATP}, ATP-sensitive K^+; OGTT, oral glucose tolerance test; OHA, oral hypoglycemic agents; OR, odds ratio; PHHI, persistent hyperinsulinemic hypoglycemia of infancy; SUR, sulfonylurea receptor; UKPDS, U.K. Prospective Diabetes Study.

TABLE 1
Clinical and biochemical characteristics of 519 unrelated glucose-tolerant whites classified according to E23K Kir6.2 genotype

	E/E	E/K	K/K	*P* (KK vs. EX)	*P* (EE vs. XK)
n (men/women)	198 (93/105)	246 (111/135)	75 (38/37)		
Age (years)	57 ± 10	56 ± 10	58 ± 10		
BMI (kg/m^2)	25.3 ± 3.2	26.1 ± 3.8	26.4 ± 4.6	0.24	0.013
Waist (cm)	85.9 ± 10.8	86.4 ± 12.0	88.6 ± 11.0	0.51	0.22
Waist-to-hip ratio	0.89 ± 0.11	0.87 ± 0.10	0.89 ± 0.09	0.91	0.006
Fasting plasma glucose (mmol/l)	5.0 ± 0.4	5.1 ± 0.4	5.1 ± 0.5	0.67	0.16
Fasting serum insulin (pmol/l)	39 ± 19	39 ± 17	40 ± 27	0.40	0.26
Insulinogenic index	31.0 ± 22.1	27.6 ± 14.6	27.6 ± 16.3	0.35	0.022
AUC 0–120 min for serum insulin	24,164 ± 14,756	22,033 ± 12,271	22,379 ± 11,515	0.57	0.014

Data are means ± SD.

one possible explanation for the association between E23K and type 2 diabetes.

The aim of the present study was to investigate whether the Kir6.2 E23K variant is associated with impairments in the serum insulin response during an oral glucose tolerance test (OGTT) in glucose-tolerant subjects and to reinvestigate whether the variant is associated with type 2 diabetes among whites (present case-control study and meta-analysis).

The E23K polymorphism was investigated in a genotype-phenotype correlation study comprising a sample of 519 unrelated glucose-tolerant subjects (Table 1). E23K and K23K carriers had significant reductions in post-OGTT serum insulin levels, as estimated from the insulinogenic index ($P = 0.022$, adjusted for BMI) as well as the area under the curve (AUC) 0–120 min for serum insulin levels during an OGTT ($P = 0.014$, adjusted for BMI) (Table 1).

To establish whether the E23K polymorphism was associated with type 2 diabetes, the polymorphism was investigated in a case-control study comprising 803 cases and 862 control subjects. The allele frequency of the E23K polymorphism among type 2 diabetic patients was 40.5% (95% CI 38.1–42.9) and 38.1% (36.9–39.3) among glucose-tolerant control subjects ($P = 0.36$) (Table 2). The E23K polymorphism was in Hardy-Weinberg equilibrium. It was not possible to discriminate between dominant, codominant, and recessive models in the Danish study. The estimated relative risk in the recessive model was 1.20 (0.87–1.68, $P = 0.26$).

A meta-analysis on published data (11,15,16) of the E23K polymorphism in whites was subsequently performed. Three published studies comprising 268 and 182 British whites (10,11) and 133 Danish whites (12), respectively, were omitted from the meta-analysis because they are included in the UKPDS cohort (16) and the present Danish study. Furthermore, a study by Altshuler et al. (14) could not be included in the meta-analysis because genotype data were not available. Genotype frequencies for the E23K variant of the case-control studies included in the meta-analysis are listed in Table 3.

In the meta-analysis, a reduction to a recessive model was the only admissible model and it was possible to describe the recessive effect with a common odds ratio (OR) (see RESEARCH DESIGN AND METHODS, Statistical methods). The common estimates of the recessive effect, i.e., the OR for the homozygous carriers of the K-allele versus carriers of the E-allele was 1.49 (95% CI 1.20–1.83, $P = 0.00022$) (Fig. 1).

In the present genotype-phenotype study, the E23K variant was associated with a reduction in estimates of glucose-induced serum insulin levels in middle-aged glucose-tolerant subjects. This finding is in accordance with the recent in vitro finding that the E23K variant is associated with a reduced ATP sensitivity of the Kir6.2/SUR1 channel complex (17).

Because the penetrance of a single allele on a physiological trait like post-OGTT serum insulin levels is likely to be higher in nondiabetic subjects than in patients with a complex metabolic disorder like type 2 diabetes, which is characterized by changes in both insulin secretion and insulin sensitivity, we hypothesize as shown in the meta-analysis that the dominant impact of the E23K polymorphism on post-OGTT serum insulin release is transformed into a recessive effect on susceptibility to type 2 diabetes. Although a similar trend was shown, the present case-control study in Danish whites (OR 1.20) could not directly confirm previous studies in British (16) and French (15) populations. This discrepancy might be related to differences in approaches for sampling cases and control subjects (16). This slight inconsistency of data is reflected in genome-wide linkage studies of type 2 diabetic siblings where *KCNJ11* has been identified as a susceptibility

TABLE 2
Genotype and allele frequencies of the Kir6.2 E23K variant in Danish type 2 diabetic patients and glucose-tolerant control subjects

E23K genotype	Type 2 diabetic patients (513 men/290 women)	Control subjects (242 men/620 women)	*P*
E/E	287 (36)	330 (39)	
E/K	382 (47)	408 (47)	
K/K	134 (17)	124 (14)	0.53*
Allele frequency (%)	40.5 (38.1–42.9)	38.1 (36.9–39.3)	0.36

Data are number of subjects with each genotype (% of each group) and allele frequencies of the minor allele in % (95% CI). The *P* values compare genotype distribution (*) and allele frequencies between type 2 diabetic patients and glucose-tolerant control subjects.

TABLE 3
Genotype frequencies of the Kir6.2 E23K variant in the case-control studies reported from U.K. (16), France (15), Utah (11) and the present Danish study.

Genotype	Study	Type 2 diabetic patients (n = 1,473)	Glucose-tolerant subjects (n = 1,351)
E/E	UKPDS	133 (37)	125 (41)
	Fr	53 (28)	45 (40)
	UT	52 (44)	21 (31)
	DK	287 (36)	330 (39)
E/K	UKPDS	161 (45)	152 (49)
	Fr	87 (45)	53 (46)
	UT	55 (46)	44 (65)
	DK	382 (47)	408 (47)
K/K	UKPDS	66 (18)	30 (10)
	Fr	51 (27)	16 (14)
	UT	12 (10)	3 (4)
	DK	134 (17)	124 (14)

Data are number of subjects with each genotype (% of each group). DK, present Danish study; Fr, France; UT, Utah.

locus in French whites but not in English whites or Americans of European ancestry (18–20). Such characteristics are shared with the other common type 2 diabetes susceptibility codon 12 variant in the peroxisome proliferator–activated receptor-γ gene (14,21).

At a glance, our findings appear to be contradicted by recently published data on the impact of the E23K variant on post-OGTT serum insulin and glucagon release (22) and serum insulin secretion during a 3 h hyperglycemic clamp (10 mmol/l) (23) in a combined group of subjects with both normal glucose tolerance and impaired glucose tolerance (IGT). Both Tschritter et al. (22) and 't Hart et al. (23) report no effect of the E23K variant on insulin release when glucose is administered intravenously, thus indicating that the effect of the E23K polymorphism on insulin release might be dependent on the route of glucose administration (orally versus intravenously). However, Tschritter et al. (22) showed that the E23K variant is associated with a diminished suppression of glucagon secretion in response to both intravenous and orally administered glucose. In the present study we did not measure serum glucagon levels, but changes in peripheral glucagon may reflect intra-islet hormone homeostasis consistent with the hypothesis that insulin is a physiological regulator of serum glucagon responses to hyper- and hypoglycemia (24,25). In this context the data from Tschritter et al. (22) suggest that the E23K variant perturbs the normal intra-islet β- and α-cell interrelationship secondary to β-cell dysfunction, and impaired insulin secretion cannot be excluded. It is hypothesized that a combined effect from a likewise E23K-dependent impairment in glucose-induced GLP-1 secretion from the intestinal L-cells (26) diminishes incretin effect, reduces insulin secretion, and at the same time impairs the GLP-1–induced glucagon suppression during an oral glucose load.

However, two important discrepancies remain between the two OGTT studies. First, the effect of the E23K variant on glucagon secretion as reported by Tschritter et al. (22) might be contributed by the IGT carriers of E23K, since IGT is associated with impaired insulin-induced suppression of glucagon secretion (27). It is possible that the inclusion of IGT subjects may have also increased the variation on the estimates of insulin secretion and thus concealed the effect of the E23K variant in the study samples. Second, the P value of 0.021 for the dominant model for the insulinogenic index in the present study would have been 0.081 had the study sample size been 298, as in the study of Tschritter et al. (22). Similarly, the P value of 0.015 for AUC 0–120 min for serum insulin levels would have been 0.064. Thus, effects of the magnitude we found would not be detectable in a study sample as that applied by Tschritter et al. (22). Finally, the finding that the E23K variant is associated with a significant increase in BMI (Table 1) suggests that in addition to an effect on insulin release this polymorphism may be diabetogenic through obesity-related mechanisms. We hypothesize that the impact on BMI is secondary to E23K-induced functional changes of the glucose-sensing hypothalamic neurons that express Kir6.2 together with various SUR isoforms. Quantitative as well as qualitative changes in these neurons have been demonstrated in a rodent model of diet-induced obesity and type 2 diabetes (28). In conclusion, the widespread E23K polymorphism may have a diabetogenic effect by impairing glucose-induced insulin release and increasing BMI.

RESEARCH DESIGN AND METHODS

Subjects. The association study was performed as a case-control study in a group of unrelated type 2 diabetic patients recruited from North Zealand in Denmark at the outpatient clinic of Steno Diabetes Center and at the Department of Internal Medicine F at Hilleroed Hospital during 1992–2001 and in two groups of unrelated glucose-tolerant control subjects. In the group of type 2 diabetic patients the age was 59 ± 10 years (mean ± SD), age of clinical diagnosis 53 ± 11 years, BMI 29.5 ± 5.2 kg/m^2, and HbA_{1c} 8.1 ± 1.6%. The patients were treated with diet alone (20%), with oral hypoglycemic agents (OHA) (60%), with insulin (15%), or with insulin in combination with OHA (5%). The first group of glucose-tolerant subjects was sampled during 1994–1997 from the Central Population Register or at the Copenhagen County Center for Preventive Medicine (29). In this group of glucose-tolerant subjects the age was 57 ± 10 years and BMI 25.8 ± 3.7 kg/m^2. The second group of glucose-tolerant subjects is a population-based sample of unrelated glucose-tolerant subjects examined during 1999–2001 at the Copenhagen County

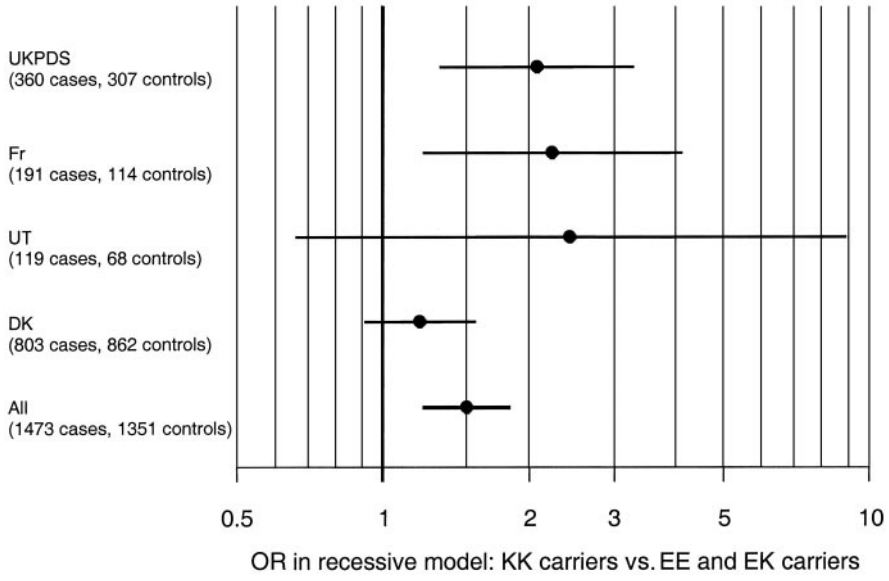

FIG. 1. Parameter estimates from the recessive models for the effects of the Kir6.2 E23K variant based on studies reported from the U.K. (UKPDS) (16), France (FR) (15), Utah (UT) (11), and the present Danish study (DK). The pooled estimates at the bottom are from a stratified analysis controlling for study. No account is taken of possible sex- and age-confounding factors.

Center for Preventive Medicine (30). In this group of glucose-tolerant subjects the age was 57 ± 2 years and BMI 26.0 ± 4.6 kg/m^2. The genotype-phenotype correlation study comprised the first group of unrelated glucose-tolerant control subjects, as described above.

Diabetes was diagnosed according to 1999 World Health Organization criteria (31). All participants were Danish whites by self-report. Informed written consent was obtained from all subjects before participation. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki II.

Biochemical assays. Blood samples for measurement of serum levels of insulin and plasma glucose were drawn after a 12-h overnight fast. All glucose-tolerant subjects underwent a 75-g OGTT with measurement in duplicate of plasma glucose and serum insulin at 0, 30, 60, and 120 min after glucose intake. The plasma glucose concentration was analyzed by a glucose oxidase method (Granutest; Merck, Darmstadt, Germany), and serum-specific insulin [excluding des(31,32) and intact proinsulin] was measured by enzyme-linked immunosorbent assay (Dako insulin kit K6219; Dako Diagnostics, Ely, U.K.). HbA_{1c} was measured by ion-exchange high-performance liquid chromatography (normal reference range 4.1–6.4%). The insulinogenic index was calculated as [(serum insulin t = 30 min − serum insulin t = 0 min)/plasma glucose t = 30 min] (32). AUC 0–120 min for serum insulin was calculated as [(30 × serum insulin t = 30 min) + (45 × serum insulin t = 60 min) + (30 × serum insulin t = 120 min) − (105 × serum insulin t = 0 min)], according to the trapezoidal rule, as the incremental values (AUC when expressed above basal values).

Anthropometric measures. All subjects had measured body height, weight, waist circumference, and hip circumference.

Genotyping. The E23K polymorphism was genotyped by PCR on genomic DNA isolated from human leukocytes with the forward primer: 5′-GACTCTGCAGTGAGGC CCTA-3′ and reverse primer: 5′-ACGTTGCAGTTGCCTT TCTT-3′. PCR amplification was carried out in a volume of 25 μl containing 100 ng genomic DNA, 1× PCR buffer, 0.2 μmol/l of each primer, 0.2 mmol/l dNTP, 1.5 mmol/l $MgCl_2$, and 0.35 units AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). The cycling program was a denaturing step at 95°C for 3 min followed by 35 cycles of 95°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s with a final elongation step at 72°C for 9 min using a GeneAmp 9,600 thermal cycler (Perkin Elmer, Norwalk, CT). The PCR product was digested with *Ban*II (New England Biolabs, Beverly, MA) and separated on 3% agarose gels.

Subjects for the meta-analysis. The meta-analysis was carried out using published data on the frequency of the E23K variant. Studies included in the meta-analysis were identified through PubMed by applying a combination of the following keywords: Kir6.2, scanning, variation, mutation, polymorphism, Glu23Lys, E23K, and Bir. In the present meta-analysis 1,473 type 2 diabetic patients and 1,351 control subjects were included (11,15,16).

Statistical methods. Before the present case-control study, a statistical power analysis was undertaken. An OR of 2.0 associated with a genotype frequency of 15%, as reported by Hani et al. (15), required a sample size of 180 type 2 diabetic patients and 180 control subjects to attain 80% power, and 300 type 2 diabetic patients and 300 control subjects to attain 95% power (33).

The case-control study was analyzed by logistic regression, adjusted for age and sex. Models for the effect of the E23K variant were tested against the general model with separate effects of genotypes. A recessive, a codominant, and a dominant model for the effect of the E23K variant were examined. The meta-analysis was performed by logistic regression on the basis of genotypes, allowing for different effects between studies, disregarding age and sex. In the meta-analysis we tested the dominant model (P = 0.001), the codominant model (P = 0.024), and the recessive model (P = 0.167). Thus, only the recessive model gave an adequate description of data. The recessive effect could be described with a common OR for the studies (test for homogeneity, P = 0.071). The combined effect was estimated using logistic regression, adjusting for study (equivalent to Mantel-Haenszel analysis).

A multiple linear regression model was used to test

variables or transformed variables for differences between genotypes. Genotype and sex were considered fixed factors and age and BMI as covariates. All analyses allowed for interaction between age times BMI, age times sex, and sex times BMI and were included in the analysis if the *P* value was significant at a 5% level. The residuals for the (transformed) variables were all normally distributed. Estimates are reported with 95% CIs. All tests were two-sided, and a P value <0.05 was considered significant.

ACKNOWLEDGMENTS

The study was supported by the Danish Medical Research Council, the Danish Diabetes Association, the Danish Heart Foundation, and EEC grants (QLRT-1999-00546, BMH4-CT98-3084, and QLK-CT-2000-01038).

The authors thank Annemette Forman, Inge Lise Wantzin, Lene Aabo Jensen, and Christina B.P. Søholm for dedicated and careful technical assistance and Grete Lademann for secretarial support.

REFERENCES

1. Ashcroft FM, Rorsman P: Electrophysiology of the pancreatic beta-cell. *Prog Biophys Mol Biol* 54:87–144, 1989
2. Misler S, Barnett DW, Gillis KD, Pressel DM: Electrophysiology of stimulus-secretion coupling in human β-cells. *Diabetes* 41:1221–1228, 1992
3. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP, Boyd AE, Gonzblez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA: Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 268:423–426, 1995
4. Seino S, Inagaki N, Namba N, Gonoi T: Molecular biology of the beta-cell ATP-sensitive K+ channel. *Diabetes Rev* 4:177–190, 1996
5. Aguilar-Bryan L, Bryan J: Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocr Rev* 20:101–135, 1999
6. Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J: Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 270:1166–1170, 1995
7. Nestorowicz A, Inagaki N, Gonoi T, Schoor KP, Wilson BA, Glaser B, Landau H, Stanley CA, Thornton PS, Seino S, Permutt MA: A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. *Diabetes* 46:1743–1748, 1997
8. Thomas P, Ye Y, Lightner E: Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 5:1809–1812, 1996
9. Koster JC, Marshall BA, Ensor N, Corbett JA, Nichols CG: Targeted overactivity of beta cell K-ATP channels induces profound neonatal diabetes. *Cell* 100:645–654, 2000
10. Sakura H, Wat N, Horton V, Millns H, Turner RC, Ashcroft FM: Sequence variations in the human Kir6.2 gene, a subunit of the beta-cell ATP-sensitive K-channel: no association with NIDDM in white Caucasian subjects or evidence of abnormal function when expressed in vitro. *Diabetologia* 39:1233–1236, 1996
11. 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
12. 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
13. Yamada Y, Kuroe A, Li Q, Someya Y, Kubota A, Ihara Y, Tsuura Y, Seino Y: Genomic variation in pancreatic ion channel genes in Japanese type 2 diabetic patients. *Diabetes-Metab Res Rev* 17:213–216, 2001
14. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPAR gamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nature Gene* 26:76–80, 2000
15. Hani EH, Boutin P, Durand E, Inoue H, Permutt MA, Velho G, Froguel P: Missense mutations in the pancreatic islet beta cell inwardly rectifying K+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of type II diabetes mellitus in Caucasians. *Diabetologia* 41:1511–1515, 1998
16. Gloyn AL, Hashim Y, Ashcroft SJH, Ashfield R, Wiltshire S, Turner RC: Association studies of variants in promoter and coding regions of beta-cell ATP-sensitive K-channel genes SUR1 and Kir6.2 with type 2 diabetes mellitus (UKPDS 53). *Diabet Med* 18:206–212, 2001
17. 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
18. Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M, O'Rahilly S, Frayling TM, Bell JI, Lathrop GM, Bennett A, Dhillon R, Fletcher C, Groves CJ, Jones E, Prestwich P, Simecek N, Subba-Rao PV, Wishart M, Foxon R, Howell S, Smedley D, Cardon LR, Menzel S, McCarthy MI: A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (The diabetes U.K. Warren 2 repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. *Am J Hum Genet* 69:553–569, 2001
19. Vionnet N, Hani E, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P: Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–q24. *Am J Hum Genet* 67:1470–1480, 2000
20. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ: A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes* 48:1175–1182, 1999
21. Ek J, Andersen G, Urhammer SA, Hansen L, Carstensen B, Borch-Johnsen K, Drivsholm T, Berglund L, Hansen T, Lithell H, Pedersen O: Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) gene in relation to insulin sensitivity among glucose tolerant Caucasians. *Diabetologia* 44:1170–1176, 2001
22. Tschritter O, Stumvoll M, Machicao F, Holzwarth M, Weisser M, Maerker E, Teigeler A, Sring H, Fritsche A: The prevalent Glu23Lys polymorphism in the potassium inward rectifier 6.2 (KIR6.2) gene is associated with impaired glucagon suppression in response to hyperglycemia. *Diabetes* 51:2854–2860, 2002
23. 't Hart LM, van Haeften TW, Dekker JM, Bot M, Heine RJ, Maassen JA: Variations in insulin secretion in carriers of the E23K variant in the KIR6.2 subunit of the ATP-sensitive K^+ channel in the β-cell. *Diabetes* 51:3135–3138, 2002
24. Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH: Insulin within islets is a physiologic glucagon release inhibitor. *J Clin Invest* 74:2296–2299, 1984
25. Samols E, Stagner JI: Intra-islet cell-cell interactions and insulin secretion. *Diabetes Rev* 4:207–223, 1996
26. Reimann F, Gribble FM: Glucose-sensing in glucagon-like peptide-1–secreting cells. *Diabetes* 51:2757–2763, 2002
27. Ahren B, Larsson H: Impaired glucose tolerance (IGT) is associated with reduced insulin-induced suppression of glucagon concentrations. *Diabetologia* 44:1998–2003, 2001
28. Song Z, Levin BE, McArdle JJ, Bakhos, N, Routh VH: Convergence of pre- and postsynaptic influences on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes* 50:2673–2681, 2001
29. Drivsholm T, Ibsen H, Schroll M, Davidsen M, Borch-Johnsen K: Increasing prevalence of diabetes mellitus and impaired glucose tolerance among 60-year-old Danes. *Diabet Med* 18:126–132, 2001
30. Glumer C, Jorgensen T, Borch-Johnsen K: DiaRisk, a population based study of previously undiagnosed diabetes mellitus, impaired glucose tolerance and the metabolic syndrome (Abstract). *Diabetologia* 43(Suppl.1) A112, 2000
31. World Health Organization: *Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications: Report of a WHO consultation*. Part 1. *Diagnosis and classification of diabetes mellitus*. Geneva, Department of Noncommunicable Disease Surveillance, World Health Org., 1999
32. Byrne CD, Wareham NJ, Brown DC, Clark PM, Cox LJ, Day NE, Palmer CR, Wang TW, Williams DR, Hales CN: Hypertriglyceridaemia in subjects with normal and abnormal glucose tolerance: relative contributions of insulin secretion, insulin resistance and suppression of plasma non-esterified fatty acids. *Diabetologia* 37:889–896, 1994
33. Breslow NE, Day NE: The Design and Analysis of Cohort Studies. In *Statistical Methods in Cancer Research*. Vol. 2. International Agency for Research on Cancer, 1987, p. 1–406