Lack of association between PKLR rs3020781 and NOS1AP rs7538490 and type 2 diabetes, overweight, obesity and related metabolic phenotypes in a Danish large-scale study: case-control studies and analyses of quantitative traits

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Abstract

Background: Several studies in multiple ethnicities have reported linkage to type 2 diabetes on chromosome 1q21-25. Both PKLR encoding the liver pyruvate kinase and NOS1AP encoding the nitric oxide synthase 1 (neuronal) adaptor protein (CAPON) are positioned within this chromosomal region and are thus positional candidates for the observed linkage peak. The C-allele of PKLR rs3020781 and the T-allele of NOS1AP rs7538490 are reported to strongly associate with type 2 diabetes in various European-descent populations comprising a total of 2,198 individuals with a combined odds ratio (OR) of 1.33 [1.16–1.54] and 1.53 [1.28–1.81], respectively. Our aim was to validate these findings by investigating the impact of the two variants on type 2 diabetes and related quantitative metabolic phenotypes in a large study sample of Danes. Further, we intended to expand the analyses by examining the impact of the variants in relation to overweight and obesity.

Methods: PKLR rs3020781 and NOS1AP rs7538490 were genotyped, using TaqMan allelic discrimination, in a combined study sample comprising a total of 16,801 and 16,913 individuals, respectively. The participants were ascertained from four different study groups; the population-based Inter99 cohort (nPKLR = 5,962, nNOS1AP = 6,008), a type 2 diabetic patient group (nPKLR = 1,873, nNOS1AP = 1,874) from Steno Diabetes Center, a population-based study sample (nPKLR = 599, nNOS1AP = 596) from Steno Diabetes Center and the ADDITION Denmark screening study cohort (nPKLR = 8,367, nNOS1AP = 8,435).
Results: In case-control studies we evaluated the potential association between rs3020781 and rs7538490 and type 2 diabetes and obesity. No significant associations were observed for type 2 diabetes (rs3020781: $p_{\text{AF}} = 0.49$, OR = 1.02 [0.96–1.10]; rs7538490: $p_{\text{AF}} = 0.84$, OR = 0.99 [0.93–1.06]). Neither did we show association with overweight or obesity. Additionally, the PKLR and the NOS1AP genotypes were demonstrated not to have a major influence on diabetes-related quantitative metabolic phenotypes.

Conclusion: We failed to provide evidence of an association between PKLR rs3020781 and NOS1AP rs7538490 and type 2 diabetes, overweight, obesity or related quantitative metabolic phenotypes in large-scale studies of Danes.

Background
Type 2 diabetes (T2D) is a complex metabolic disease, where several tissues and organs, including pancreatic β-cells, skeletal muscle, adipose tissue, liver and the central nervous system have been suggested to be directly or indirectly involved in the pathogenesis [1].

Several independent studies have shown evidence for linkage between chromosome 1q21-25 and T2D in multiple ethnicities [2-14]. Both PKLR encoding the liver pyruvate kinase and NOS1AP encoding the nitric oxide synthase 1 (neuronal) adaptor protein (CAPON), are located in the 1q21-25 region and are therefore positional candidate genes for T2D susceptibility. The pyruvate kinase enzyme catalyses the last step in glycolysis converting phosphoenolpyruvate to pyruvate under the generation of ATP. PKLR is, in addition to the liver, expressed in pancreatic β-cells, the kidneys and the small intestine [15], and its expression is upregulated by glucose through a carbohydrate response element in the promoter [16]. Moreover, a binding site for hepatocyte nuclear factor 1-α is located in the PKLR promoter and patients with maturity-onset diabetes of the young type 1 and 3 show decreased expression of the gene [17,18]. Hence, PKLR is a strong biological candidate gene for impaired blood glucose regulation and thus T2D. The CAPON protein binds nitric oxide synthase, which results in downregulation of N-methyl-D-aspartate receptor-mediated glutamate signalling [19], however, the link between dysfunctional CAPON protein and T2D is as yet unexplained.

A substantial number of genes, in this very gene-dense 1q21-25 region, have already been investigated for susceptibility to T2D, however, none have so far explained the observed linkage [1]. As a part of The International Type 2 Diabetes 1q Consortium 5,285 single-nucleotide polymorphisms (SNPs), covering 22.7 Mb of the 1q linkage region were genotyped in 1,000 cases and 1,198 matched controls from four different European-descent populations.

Two SNPs, rs3020781 in PKLR and rs7538490 in NOS1AP were reported to associate with T2D. Applying an additive model the C-allele of PKLR rs3020781 associated with T2D with an odds ratio (OR) of 1.33 [1.16–1.54] ($p = 1 \cdot 10^{-6}$), and under a dominant model the T-allele of NOS1AP rs7538490 associated with T2D with an OR of 1.53 [1.28–1.81] ($p = 2 \cdot 10^{-6}$) [38]. PKLR has previously been examined in two independent studies, where four SNPs, (rs3020781, rs2071053, rs1052176, rs1052177), showed association with T2D when analysing a total 909 individuals of European descent [20,21]. No further association studies regarding the role of NOS1AP in T2D pathogenesis have been performed.

The aim of the present study was to validate the association of PKLR rs3020781 and NOS1AP rs7538490 with T2D. In addition we intend to expand with analyses of overweight and obesity and the relationship with diabetes-related metabolic quantitative phenotypes.

Methods
Subjects
PKLR rs3020781 and NOS1AP rs7538490 were successfully genotyped in 16,801 and 16,913 Danes, respectively, involving four study groups 1) the population-based Inter99 cohort (ClinicalTrials.gov ID no: NCT00289237) ($n_{\text{PKLR}} = 5,962$, $n_{\text{NOS1AP}} = 6,008$), with an average age of 46 ± 8 years and a mean BMI of 26.2 ± 4.6 kg/m², sampled at the Research Centre for Prevention and Health [22] 2) unrelated T2D patients ($n_{\text{PKLR}} = 1,873$, $n_{\text{NOS1AP}} = 1,874$), with an average age of 62 ± 11 years and a mean BMI of 30.0 ± 5.6 kg/m², sampled through the out-patient clinic at Steno Diabetes Center 3) a population-based group of unrelated middle-aged individuals ($n_{\text{PKLR}} = 599$, $n_{\text{NOS1AP}} = 596$), with an average age of 59 ± 8 years and a mean BMI of 26.5 ± 4.2 kg/m², examined at Steno Diabetes Center 4) the ADDITION Denmark screening study cohort (ClinicalTrials.gov ID no: NCT00237548) ($n_{\text{PKLR}} = 8,367$, $n_{\text{NOS1AP}} = 8,435$), with an average age of 60 ± 7 years and a mean BMI of 28.6 ± 4.9 kg/m², sampled by Department of General Practice at University of Aarhus [23]. The different study groups are further described in Additional file 1. In study group 1 and 3 all non-diabetic individuals underwent a standard 75 g oral glucose tolerance test (OGTT) and only glucose-tolerant and normoglycaemic
individuals were included as control subjects in the case-control study of T2D. Analyses of quantitative metabolic phenotypes were performed in the population-based Inter99 cohort exclusively, excluding T2D patients receiving pharmacological treatment. Informed written consent was obtained from all individuals before participation. The studies were approved by the regional Ethical Committee (Ethics Committee, Copenhagen County for study group 1, 2 and 3 and Ethics Committee, Aarhus County for study group 4) and were in accordance with the principles of the Helsinki Declaration. T2D and normal glucose tolerance (NGT) were defined according to the World Health Organization [24].

Biochemical and anthropometrical measurements

In all study groups body weight and height were measured in light indoor clothes and without shoes [22,23]. In study groups 1 and 3 serum insulin and plasma glucose were measured at fasting and 30 and 120 minutes after an OGTT. Serum insulin levels excluding des(31,32)- and intact proinsulin were measured using the AutoDELFA insulin kit (Perkin-Elmer, Wallac, Turku, Finland). Plasma glucose was analysed using a glucose oxidase method (Granulene; Merck, Darmstadt, Germany) [25]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) divided by 22.5. The BIGTT insulin sensitivity index (BIGTT-S) and BIGTT acute insulin response (BIGTT-AIR) were calculated as described [26,27].

Genotyping

PKLR rs3020781 and NOS1AP rs7538490 were genotyped using Taqman allelic discrimination (K Bioscience, Herts, UK). Discordances between 1,202 random duplicate samples were 0.1% and 0.2%, respectively, and the genotyping success rates were 96.3% and 96.8%, respectively. Both genotype groups obeyed Hardy-Weinberg equilibrium (p > 0.05).

Statistical analysis

Fisher’s exact test was applied to examine differences in allele frequencies (AF) between affected and unaffected individuals. A general linear model was used to test quantitative metabolic variables for differences between genotype groups assuming an additive (Add) model for PKLR rs3020781, and a dominant (Dom) model for NOS1AP rs7538490. Values of plasma glucose, serum insulin, HOMA-IR and BIGTT-AIR were logarithmically transformed before statistical analysis to obtain normal distribution. Adjustment for sex, age and BMI was applied when appropriate. All analyses were performed in RGui version 2.5.0 [28], and p-values < 0.05 were considered significant. Statistical power was determined using the CaTS power calculator version 0.0.2. A test for homogeneity between the population-based Inter99 cohort, the T2D patients and the population-based sample from Steno Diabetes Center and the ADDITION Denmark screening study cohort, was performed by means of the Mantel-Haenszel method (fixed effects model) for both genotypes, revealing no significant heterogeneity between study groups (rs3020781: p = 0.8, rs7538490: p = 0.4).

Results and discussion

The minor allele frequencies (MAF) of the PKLR rs3020781 C-allele and the NOS1AP rs7538490 T-allele were 26.2% and 28.0%, respectively, and comparable to the 32.5% and 29.7%, reported for the HapMap CEU population. Using the population-based Inter99 cohort as reference the prevalence of T2D is estimated to 6% in the Danish population of middle-aged people. Combining the four study samples, gives us a statistical power of 100% observing an association with T2D with a relative risk above 1.3, and a MAF as reported for the two variants assuming either an additive or a dominant model. The potential associations between PKLR rs3020781 and NOS1AP rs7538490 and T2D were evaluated in case-control studies including 8,410 and 8,447 individuals, respectively. No difference in allele frequencies between T2D patients and glucose-tolerant subjects were found for either SNP (rs3020781: pAF = 0.49, OR [95% CI] = 1.02 [0.96–1.10]; rs7538490: pAF = 0.84, OR [95% CI] = 0.99 [0.93–1.06]), Table 1.

Case-control studies comparing allele frequencies between body mass index (BMI) defined normal weight (BMI < 25 kg/m2) and overweight (25 kg/m2 ≤ BMI < 30 kg/m2) or obese (BMI ≥ 30 kg/m2) individuals, respectively, were performed in the combined study sample including T2D patients. No statistically significant association with overweight or obesity were demonstrated for PKLR rs3020781 (overweight: pAF = 0.49, OR [95% CI] = 0.98 [0.92–1.04]; obesity: pAF = 0.81, OR [95% CI] = 0.99 [0.93–1.06]) nor for NOS1AP rs7538490 (overweight: pAF = 0.48, OR [95% CI] = 0.95 [0.83–1.09]; obesity: pAF = 0.68, OR [95% CI] = 0.99 [0.93–1.05]), Table 1. As pharmacological treatment can influence on BMI, we additionally performed case-control studies of overweight and obesity considering T2D patients and treatment-naive individuals separately, however, neither variant showed association with overweight or obesity when stratifying according to glucose tolerance status (data not shown).

Furthermore, we investigated PKLR rs3020781 and NOS1AP rs7538490 for influence on diabetes-related quantitative metabolic phenotypes in 5,590 and 5,630 treatment-naive Danish people from the population-based Inter99 cohort, respectively. No association with plasma glucose or serum insulin levels at fasting, 30 or 120 min during an OGTT or with OGTT-derived surrogate
indices of insulin sensitivity or beta-cell function was demonstrated, Table 2. To evaluate the effect of the variants in individuals without impaired blood glucose regulation, analyses of quantitative metabolic phenotypes were conducted in the population-based Inter99 cohort including only glucose-tolerant individuals (\(n_{PKLR} = 4,248, n_{NOS1AP} = 4,269\)). However, no significant differences in genotype distribution of the two SNPs were demonstrated (data not shown).

Despite successful identification of several T2D susceptible genes only a small percentage of T2D heritability is explained, thus, more T2D genes are to be found.

Originally two clusters of SNPs located within the T2D linkage peak were identified by The International Type 2 Diabetes 1q Consortium to associate with T2D among a total of 5,285 SNPs tagging the linkage peak. The first cluster of 9 SNPs were located in a linkage disequilibrium (LD) region including \(PKLR\) while the second cluster of 4 SNPs resided within \(NOS1AP\). Replication of such potential associations, in statistically well-powered studies, is essential to substantiate the initial findings. Therefore, we aimed specifically at replicating the strongest associations in the two clusters of SNPs within \(PKLR\) and \(NOS1AP\), which are rs3020781 and rs7538490, respectively. However, we did not show any association with T2D for either

Table 1: Case-control studies of \(PKLR\) rs3020781 and \(NOS1AP\) rs7538490 in relation to type 2 diabetes, overweight and obesity

|     | PKLR rs3020781 |     | NOS1AP rs7538490 |
|-----|---------------|-----|-----------------|
|     | \(n\) (men/women) | TT (%) | TC (%) | CC (%) | MAF (95% CI) | \(p_{AF}\) | OR (95% CI) | \(n\) (men/women) | CC (%) | CT (%) | TT (%) | MAF (95% CI) | \(p_{AF}\) | OR (95% CI) |
| NGT | 4,736 (2,209/2,527) | 2,602 (55) | 1,812 (38) | 322 (7) | 25.9 (25.0–26.8) | 0.49 | 1.02 (0.96–1.10) | 4,755 (2,218/2,537) | 2,479 (52) | 1,914 (40) | 362 (8) | 27.7 (26.8–28.7) | 0.84 | 0.99 (0.93–1.06) |
| T2D | 3,674 (2,187/1,487) | 1,998 (54) | 1,412 (39) | 264 (7) | 26.4 (25.4–27.4) |     |          | 5,036 (2,111/2,925) | 2,732 (54) | 1,952 (39) | 352 (7) | 26.4 (25.5–27.2) | 0.49 | 0.98 (0.92–1.04) |
| BMI < 25 (kg/m²) | 6,985 (4,359/2,626) | 3,821 (55) | 2,700 (39) | 464 (6) | 26.0 (25.2–26.7) | 0.48 | 0.95 (0.83–1.09) | 4,780 (2,467/2,313) | 2,612 (55) | 1,845 (38) | 323 (7) | 26.2 (25.3–27.1) | 0.81 | 0.99 (0.93–1.06) |
| BMI ≤ 30 (kg/m²) | 25 ≤ BMI < 30 (kg/m²) | 7,030 (4,384/2,646) | 3,628 (51) | 2,857 (41) | 545 (8) | 28.1 (27.2–29.0) | 0.48 | 0.95 (0.83–1.09) | 5,064 (2,128/2,936) | 2,635 (52) | 2,013 (40) | 416 (8) | 28.1 (27.3–28.8) | 0.48 | 0.95 (0.83–1.09) |
| BMI ≥ 30 (kg/m²) | 4,819 (2,490/2,329) | 2,517 (52) | 1,923 (40) | 379 (8) | 27.8 (26.9–28.7) | 0.68 | 0.99 (0.93–1.05) |     |          |     |          |     |          |

Data are number of individuals, divided into genotype groups (% in each group), and frequencies of the minor allele (MAF) in percentages. Fisher’s exact test was used to compare allele frequencies (\(p_{AF}\)). The odds ratios (OR) and 95% confidence interval (CI) are given for comparison of allele frequency. NGT: individuals with normal glucose tolerance, T2D: type 2 diabetic patients.
of the two variants, despite having the statistical power to
detect the reported effect sizes. Neither did we find an
association with pertinent metabolic phenotypes, which
could indicate an impaired blood glucose regulation ulti-
mately leading to T2D.

From our studies we can exclude rs3020781 and
rs7538490 as T2D susceptibility variants in the Danish
population, but PKLR and NOS1AP may still represent
true T2D susceptibility loci. That PKLR represents a true
T2D candidate gene, is supported by a study analysing two
SNPs (rs1052176 and rs1052177) within PKLR, both
showing association with T2D and both being in perfect
LD with rs3020781 according to HapMap [21]. HapMap
further outlined that rs3020781 is located at the border of
a LD block near a recombination hotspot. Therefore, if the
LD pattern is slightly shifted in our population, compared
to the populations in which association is observed,

| PKLR rs3020781 | NOSIAP rs7538490 |
|----------------|-----------------|
| TT             | TT              |
| (men/women)    | (men/women)     |
| 3,065          | 2,954           |
| (1,544/1,521)  | (1,474/1,480)   |
| Age (years)    | Age (years)     |
| 46 ± 8         | 46 ± 8          |
| BMI (kg/m²)    | BMI (kg/m²)     |
| 26.2 ± 4.6     | 26.2 ± 4.6      |
| Plasma glucose (mmol/l) |
| Fasting       | Fasting        |
| 5.5 ± 0.8      | 5.5 ± 0.8       |
| 30-min         | 30-min          |
| 8.7 ± 1.9      | 8.7 ± 1.9       |
| 120-min        | 120-min         |
| 6.2 ± 2.2      | 6.2 ± 2.2       |
| Serum insulin (pmol/l) |
| Fasting       | Fasting        |
| 34 (23–50)     | 34 (23–51)      |
| 30-min         | 30-min          |
| 243 (173–350)  | 247 (176–354)   |
| 120-min        | 120-min         |
| 154 (93–253)   | 157 (97–257)    |
| Derived indices |
| BIGTT-Si       | BIGTT-Si       |
| 9.2 (6.4–12.1) | 9.2 (6.5–12.1)  |
| BIGTT-AIR      | BIGTT-AIR      |
| 1,622 (1,282–2,083) | 1,643 (1,301–2,118) |
| HOMA-IR        | HOMA-IR        |
| 8.2 (5.6–12.6) | 8.4 (5.5–13.1)  |

Data are means ± standard deviation or median (interquartile range). Values of plasma glucose, serum insulin, HOMA-IR and BIGTT-AIR were
logarithmically transformed before statistical analysis to obtain normal distribution. All analyses of PKLR rs3020781 were made using an additive
model (Add), while analyses of NOSIAP rs7539480 were made using a dominant model. Calculated p-values were adjusted for age and sex for BMI
measures, for sex, age and BMI for serum insulin, plasma glucose and HOMA-IR, and for age for the BIGTT-Si and BIGTT-AIR index. HOMA-IR was
calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) divided by 22.5. BIGTT-Si and BIGTT-AIR were calculated as
described [26].
rs3020781 may fail as a marker for the functional variant. Similar may be true for rs7538490, as LD is sparse in the region where NOS1AP is located. Thus, different LD patterns could explain the lack of association between rs3020781 and rs7538490 and T2D in our population.

In regards to the identification of T2D susceptibility genes, the linkage analysis, used for the identification of PKLR and NOS1AP, has been less successful due to inconsistent replication.

However, genome-wide association (GWA) studies have added to progress in finding common T2D susceptible gene variants with modest impact on diabetes risk [29-34], with the identification of non-obvious biological candidate genes and where replication have been predominantly successful [35-37]. We have investigated results of available data from GWA studies in web-based databases, but neither PKLR nor NOS1AP were among the high priority candidate genes as estimated from genome-wide significance levels [29,30]. Two markers in LD with PKLR rs3020781 were available in the public GWA data, however, none of these associated with T2D. No markers were available for NOS1AP rs7538490 [29]. The lack of association could either be due to small effect sizes, or the possibility that the variants represent false positive findings, thus explaining our failure to demonstrate an association.

**Conclusion**

In statistically well-powered case-control studies and in studies of pertinent quantitative phenotypes we failed to validate the proposed association of the C-allele of rs3020781 and the T-allele of rs7538490 with T2D or intermediate phenotypes.

**Abbreviations**

Add: additive model; AF: allele frequency; BIGTT-AIR: BIGTT acute insulin response; BIGTT-Sr: BIGTT insulin sensitivity index; BMI: body mass index; CAPON: nitric oxide synthase 1 (neuronal) adaptor protein; CI: confidence interval; Dom: dominant model; GWA: genome-wide association; HOMA-IR: homeostasis model assessment of insulin resistance; LD: linkage disequilibrium; MAF: minor allele frequency; NGT: normal glucose tolerance; OR: odds ratio; SNP: single-nucleotide polymorphism; T2D: type 2 diabetes

**Competing interests**

KBJ and OP hold stock in Novo Nordisk and have received lecture fees from pharmaceutical companies. All other authors declare that there is no competing interest associated with this manuscript.

**Authors’ contributions**

The original hypothesis was conceived by CHA and MSM and approved by OP and TH. Detail planning of analyses and study design was performed by CHA, MSM and approved by OP and TH. TJ, KBJ, TL, AS, OP and TH contributed to the epidemiological part of the recruitment of study populations. CHA, MSM, KA, LH, OP and TH contributed to the preparation of study populations for statistical analyses. Statistical analyses were performed by CHA and MSM. All authors contributed the interpretation of data. The first manuscript was written by CHA and MSM and the final draft was finalised by CHA, MSM, OP and TH. All authors revised the manuscript and contributed to the discussion of the results.

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**Additional material**

**Additional file 1**

Supplementary table 1.

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**Footnotes**

1 EASD 2007 Abstract no. 0169 (http://www.easd.org/easdwebfiles/annualmeeting/43rdmeeting/abstracts/documents/0169.doc); Prokopenko I, Zeggini E, Rayner NW, Groves CJ, Hanson RL, Mitchell BD et al.
High-density association mapping and comprehensive tagging of the type 2 diabetes linkage region on chromosome 1q in 4 European populations.

References

1. Das SK, Elbein SC: The search for type 2 diabetes susceptibility loci: the chromosome 1q story. Curr Diab Rep 2007, 7:154-164.
2. Das SK, Hasstedt SJ, Zhang Z, Elbein SC: Linkage and association mapping of a chromosome 1q21-q24 type 2 diabetes susceptibility locus in northern European Caucasians. Diabetes 2004, 53:492-499.
3. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ: A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. Diabetes 1999, 48:1175-1182.
4. Hanson RL, Ehm MG, Pettitt DJ, Timi DB, Tomberg DB, Timi DJ, et al: An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. Am J Hum Genet 1998, 63:1130-1138.
5. Hansen RL, Imperatore G, Narayan KM, Rouman J, Fagot-Campagna A, Pettit DJ, et al: Family and genetic studies of indices of insulin sensitivity and insulin secretion in Pima Indians. Diabetes Metab Res Rev 2001, 17:296-303.
6. Huseh WC, Stjepan PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, et al: Genome-wide fine and mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish: evidence for a new diabetes locus on chromosome 1q41q11 and confirmation of a locus on chromosome 1q21-q24. Diabetes 2002, 51:251-257.
7. Langefeld CW, Wagenknecht LE, Rotter JI, Williams AH, Hokanson JE, Saad MF, et al: Linkage of the metabolic syndrome to 1q32-3q13 in Hispanic families: the Insulin Resistance Atherosclerosis Study Family Study. Diabetes 2004, 53:1170-1174.
8. Nejts J, Bandin AS, Myers RH, Wilson PW, Cupples LA: A genome-wide scan for loci linked to plasma levels of glucose and HbA1c in a community-based sample of Caucasian pedigrees: The Framingham Oftspring Study. Diabetes 2002, 51:833-840.
9. Ng MC, So WY, Lam VK, Cockram CS, Critchley JA, et al: Genomewide scan for type 2 diabetes loci in Hong Kong Chinese and confirmation of a susceptibility locus on chromosome 1q21-25. Diabetes 2004, 53:1609-1613.
10. Ng MC, So WY, Lam VK, Cockram CS, Bell GI, Cox NJ, et al: Genomewide scan for metabolic syndrome and related quantitative traits in Hong Kong Chinese and confirmation of a susceptibility locus on chromosome 1q21-24. Diabetes 2004, 53:2676-2683.
11. Vernet N, Huguet EH, Doupont S, Gallina S, Francke S, Dotte S, et al: Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 1q27-qter and independent replication of a type 2 diabetes locus on chromosome 1q21-q24. Am J Hum Genet 2000, 67:140-148.
12. Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M, et al: A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. Am J Hum Genet 2001, 69:553-569.
13. Xiang K, Wang Y, Zheng T, Jia W, Li J, Chen L, et al: Genomewide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese: significant linkage to chromosome 1q21-q23 and chromosome 1q21-q24. Diabetes 2004, 53:228-234.
14. Zhao JY, Xiong MM, Huang W, Wang H, Zuo J, Wu GD, et al: An autosomal genomic scan for loci linked to type 2 diabetes in northern Han Chinese. J Mol Med 2005, 83:209-215.
15. Noguchi T, Yamada K, Tanaka T, Noguchi T, Hasstedt SJ: Characterization and purification of carbohydrate response element-binding protein of the rat liver pyruvate kinase gene promoter. Biochem Biophys Res Commun 1999, 257:44-49.
16. Wang H, Antinoozi PA, Hagenfeldt KA, Maechler P, Wollheim CB: Molecular targets of a human HNF1 alpha mutation responsible for pancreatic beta-cell dysfunction. EMBO J 2000, 19:4257-4264.
17. Shih DQ, Screen S, Munoz KN, Phillipson L, Pontoglio M, Yaniv M, et al: Loss of HNF1-alpha function in mice leads to abnormal expression of genes involved in pancreatic islet development and metabolism. Diabetes 2001, 50:2472-2480.
18. Jaffrey SR, Snowman AM, Eliaisson MJ, Cohen NA, Snyder SH: C4q: a gene product associated with nonenzymatic oxidative stress that regulates its interactions with PSD95. Neuron 1998, 20:115-124.
19. Wang H, Chu W, Das SK, Ren Q, Hasstedt SJ, Elbein SC: Liver pyruvate kinase polymorphisms are associated with type 2 diabetes in northern European Caucasians. Diabetes 2002, 51:2861-2865.
20. Hasstedt SJ, Chu WS, Das SK, Wang H, Elbein SC: Type 2 diabetes susceptibility genes on chromosome 1q21-24. Ann Hum Genet 2008, 72:163-169.
21. Jorgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glumner C, Pisinger C: A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. Eur J Cardiovasc Prev Rehabil 2003, 10:377-386.
22. Lauritzen T, Griffin S, Borch-Johnsen K, Wareham NJ, Woffendenbustel BH, Buu Tran G: The ADDITION study: proposed trial of the cost-effectiveness of an intensive multifactorial intervention on morbidity and mortality among people with Type 2 diabetes detected by screening. Int J Obes Relat Metab Disord 2000, 24(Suppl 3):S6-11.
23. World Health Organization Study Group: Part I: Diagnosis and Classification of Diabetes Mellitus. In Tech. Rep. Ser., no. WHO/ NCD/INS/99.2 World Health Organization, Geneva: 1999.
24. Glumner C, Jorgensen T, Borch-Johnsen K: Prevalence of diabetes and impaired glucose regulation in a Danish population: the Inter99 study. Diabetes Care 2003, 26:2335-2340.
25. Hansen T, Drivsholm T, Urumhammer SA, Palacios RT, Volund A, Borch-Johnsen K, et al: The BIGTT: a novel test for simultaneous measurement of pancreatic beta-cell function, insulin sensitivity, and glucose tolerance. Diabetes Care 2007, 30:257-262.
26. Benyamin B, Sorensen TI, Schousboe K, Fenger M, Visscher PM, Kyvik KO: Are there common genetic and environmental factors behind the endophenotypes associated with the metabolic syndrome? Diabetes Care 2007, 30:2135-2140.
27. R Development Core Team: R: A language and environment for statistical computing, R. 2008 [http://www.R-project.org], Foundation for Statistical Computing, Vienna, Austria.
28. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, et al: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007, 316:1331-1336.
29. The Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007, 447:661-678.
30. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al: A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 2007, 445:811-815.
31. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, et al: A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet 2007, 39:770-775.
32. Scott LJ, Muhlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007, 316:1341-1345.
33. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al: Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007, 316:1346-1351.
34. Grarup N, Rose CS, Andersen EA, Andersen G, Nielsen AL, Albrechtsen A, et al: Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. Diabetes 2007, 56:3105-3111.
35. Sparso T, Andersen G, Nielsen T, Burgdorf KS, Qvist AI, Nielsen AL, et al: The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting
and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. *Diabetologia* 2008, 51:70-75.

37. Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wagenandt L, Andersen G, et al.: Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* 2008, 57:95-101.

38. Prokopenko I, Zeggini E, Raymer NW, Groves CJ, Hansson RL, Mitchell BD, et al.: High-density association mapping and comprehensive tagging of the type 2 diabetes linkage region on chromosome 1q in 4 European populations. *EASD 2007* Abstract no. 0169 [http://www.easd.org/easdwebfiles/annualmeeting/43rdmeeting abstracts/documents/0169.doc].

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