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S. K. Hansen · C. S. Rose · C. Glümer · T. Drivsholm · K. Borch-Johnsen · T. Jørgensen · O. Pedersen · T. Hansen

Variation near the hepatocyte nuclear factor (HNF)-4α gene associates with type 2 diabetes in the Danish population

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Abstract *Aims/hypothesis:* The hepatocyte nuclear factor (HNF)-4α is an orphan nuclear receptor, which plays crucial roles in regulating hepatic gluconeogenesis and insulin secretion. The gene encoding HNF-4α (*HNF4A*) is located on chromosome 20q12–q13 in a region that in several studies has shown linkage with type 2 diabetes. Recently, two independent studies identified single nucleotide polymorphisms (SNPs) in a 90-kb region spanning *HNF4A*, which showed strong association with type 2 diabetes in the Finnish and Ashkenazi Jewish populations. In an attempt to replicate and extend these findings, we selected four SNPs in the same *HNF4A* region, which in the Finnish and Ashkenazi Jewish populations were associated with type 2 diabetes, and examined their relationships with type 2 diabetes and prediabetic phenotypes in the Danish Caucasian population. *Methods:* The rs1884614, rs2425637, rs1885088 and rs3818247 were analysed in case-control studies of 1387, 1429, 1417 and 1371 type 2 diabetic patients and 4766, 4727, 4665 and 4748 glucose-tolerant subjects respectively. Genotype–quantitative trait analyses comprised 4430, 4394, 4336 and 4413 middle-aged glucose-tolerant subjects from the population-based Inter99 cohort for the rs1884614, rs2425637, rs1885088 and rs3818247 respectively. *Results:* The risk allele of the rs1884614, which is located 4 kb upstream of the *HNF4A* P2 promoter, was associated with type 2 diabetes (odds ratio [OR]=1.14, p=0.02) and with a subtle increase in post-OGTT plasma glucose levels in glucose-tolerant subjects (additive model, p=0.05). *Conclusions/interpretation:* Consistent with results from studies of Finnish and Ashkenazi Jewish subjects, variation near the P2 region of *HNF4A* is associated with type 2 diabetes in the Danish population.

Keywords Chromosomes, human, pair 20/genetics · Diabetes mellitus, type 2/genetics · Genetic predisposition to disease/genetics · Inter99 · Polymorphism, single nucleotide/genetics · Transcription factors/genetics

Abbreviations FUSION: Finland–United States investigation of NIDDM genetics · HNF-4α: Hepatocyte nuclear factor-4α · OR: Odds ratio · SNP: Single nucleotide polymorphism

S. K. Hansen · C. S. Rose · C. Glümer · K. Borch-Johnsen · O. Pedersen · T. Hansen (✉)
Steno Diabetes Center and Hagedorn Research Institute,
Niels Steensens Vej 2, Gentofte,
2820 Copenhagen, Denmark
e-mail: toha@steno.dk
Tel.: +45-44-439391
Fax: +45-39-681048

C. Glümer · T. Drivsholm · K. Borch-Johnsen · T. Jørgensen
Research Center for Prevention and Health,
Copenhagen County, Glostrup University Hospital,
Glostrup, Denmark

K. Borch-Johnsen · O. Pedersen
Faculty of Health Science, University of Aarhus,
Aarhus, Denmark

Introduction

The hepatocyte nuclear factor (HNF)-4α is an orphan nuclear receptor which regulates expression of genes involved in transport and metabolism of many nutrients including lipids and glucose [1]. It is expressed in various tissues including the liver and the pancreatic beta cells [1]. In the liver, HNF-4α is required for maintaining normal hepatic gluconeogenesis [2], while HNF-4α in the pancreatic beta cells regulates expression of genes involved in glucose metabolism and insulin secretion, including activation of expression of the insulin gene [3–5]. The expression of various isoforms of HNF-4α is driven by two distinct promoters, the P1 and the recently identified P2, the latter being located ~46 kb upstream of P1. The expression of HNF-4α in beta cells is mainly driven by the P2 promoter, whereas P1-driven transcripts are dominant in the liver [6–8].

Rare mutations in the gene encoding HNF-4α (*HNF4A*), including both the P1 and the P2 regions, cause MODY 1 [6, 7, 9]. Results from several linkage studies among Caucasians have, however, indicated that variation in the *HNF4A* region on chromosome 20q12–q13 may also confer

an increased risk of type 2 diabetes [10–17]. Previous mutation analyses of the P1, P2 and the coding exons of *HNF4A* have failed to identify frequent variants which were associated with type 2 diabetes [11, 17–20]. Recently, however, two independent studies have made important progress in explaining the observed linkage on 20q12–q13. These investigators carefully dissected a 90-kb genomic region covering both the P1 and P2 promoters of *HNF4A*, and identified several single nucleotide polymorphisms (SNPs) that were associated with type 2 diabetes among both Ashkenazi Jewish subjects and Finnish subjects [21, 22]. Indeed, the diabetes-associated SNPs were shown to account for most of the previously shown linkage at the 20q12–q13 locus with type 2 diabetes in the two study populations [11, 12, 21, 22].

In the present study, using Danish Caucasian subjects we attempt to replicate the finding that known variation in the region spanning the *HNF4A* is associated with type 2 diabetes or relevant prediabetic traits in glucose-tolerant subjects. We selected four SNPs (rs1884614, rs2425637, rs1885088 and rs3818247), which are evenly distributed throughout the 90-kb region (Table 1) and which have been reported to represent distinct haplotype blocks as measured by linkage disequilibrium in Finnish and Ashkenazi Jewish subjects [21, 22]. In addition, the rs1884614 (located 4 kb upstream of the P2 promoter translation start site) is positioned in the haplotype block that showed significant association with type 2 diabetes in both populations.

Subjects and methods

Subjects From the population-based Inter99 study cohort, which was established at the Research Centre for Prevention and Health during 1999–2001 [23], glucose-tolerant subjects, subjects with IGT, and type 2 diabetic patients were recruited for the present case-control and genotype–quantitative trait analyses (Table 1). Furthermore, middle-aged and elderly glucose-tolerant subjects for the case-control studies were recruited from two additional minor population-based study samples from the Research Centre for Prevention and Health [24] and the Steno Diabetes Center respectively (Table 1). All individuals from the three population-based study samples were randomly recruited through the Danish Central Population Register and from the same geographical area as the type 2 diabetic patients. The remaining type 2 diabetic patients were recruited during 1992–2001 from the outpatient clinics in the greater Copenhagen area (Table 1). Due to varying genotyping success rates of the four different SNPs, differing numbers of type 2 diabetic patients and control subjects are presented for the case-control studies of rs1884614, rs2425637, rs1885088 and rs3818247 respectively (Table 1).

Of the type 2 diabetic patients from the population-based Inter99 cohort and the outpatient clinics in the greater Copenhagen area, 20% were treated with diet alone, 60% with oral hypoglycaemic agents and 15% with insulin, and 5% were receiving a combination of insulin and oral hypoglycaemic agents. Diabetes was diagnosed in accordance with the World Health Organization (WHO) 1999 criteria. All control subjects were subjected to a WHO standard OGTT under fasting conditions and only subjects with normal glucose tolerance were included in the control group.

The 4430, 4394, 4336 and 4413 glucose-tolerant subjects from the Inter99 study with genotypes for rs1884614, rs2425637, rs1885088 and 3818247 respectively (Table 1) were included in the genotype–quantitative trait analyses.

All participants were Danish Caucasians by self-report. Informed consent was obtained from all study participants

Table 1 Characteristics of the glucose-tolerant control subjects, the IGT subjects and the type 2 diabetic patients enrolled in the case-control studies of the rs1884614, rs2425637, rs1885088 and rs3818247 of *HNF4A* respectively

		Middle-aged glucose-tolerant subjects from the population-based Inter99 cohort	Middle-aged and elderly glucose-tolerant subjects from the two minor population-based study samples	IGT subjects from the population-based Inter99 cohort	Type 2 diabetic patients from the population-based Inter99 cohort	Type 2 diabetic patients from outpatient clinics in the greater Copenhagen area
N (M/W)	rs1884614	4430 (2049/2381)	336 (160/176)	680 (333/347)	364 (219/145)	1023 (617/406)
	rs2425637	4394 (2048/2346)	333 (156/177)	673 (333/340)	360 (217/143)	1069 (649/420)
	rs1885088	4336 (2023/2313)	329 (156/173)	672 (331/341)	358 (215/143)	1059 (642/417)
	rs3818247	4413 (2042/2371)	335 (160/175)	676 (331/345)	362 (217/145)	1009 (616/393)
Age (SD) (years)		45.2 (7.8)	62.1 (4.6)	48.5 (7.7)	50.8 (7.1)	58.7 (10.7)
Age at diagnosis (SD) (years)		–	–	–	41.8 (12.4)	52.2 (10.6)
BMI (SD) (kg/m^2)		25.5 (4.1)	26.1 (3.7)	28.2 (5.2)	30.1 (5.7)	29.5 (5.2)

Varying genotyping success rates of the four different SNPs have resulted in differing numbers of type 2 diabetic patients and control subjects for rs1884614, rs2425637, rs1885088 and rs3818247 respectively. The means of age, age at diagnosis and BMI are almost equal for each of the examined SNPs and therefore are only shown for the participants who were genotyped for rs1884614

M men, *W* women

prior to participation. The studies were approved by the ethical committee of Copenhagen and were in accordance with the principles of the Declaration of Helsinki II.

Genotyping of the rs1884614, rs2425637, rs1885088 and rs3818247 The samples were genotyped for the four SNPs (rs1884614, rs2425637, rs1885088 and rs3818247) applying a chip-based matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (DNA MassARRAY) analysis of PCR-generated primer extension products as previously described [25]. The genotyping success rates were 96% for rs1885088 (97% for type 2 diabetic cases, 96% for control subjects), and 97% for rs1884614 (95%/98%), rs2425637 (97%/97%) and rs3818247 (94%/97%). To elucidate the genotyping error rate we examined a minimum of 88 replicate samples for each SNP, and among these there were no mismatches.

The genotype distributions were tested for Hardy–Weinberg equilibrium among both the type 2 diabetic cases and control subjects separately and in combination. The genotype distributions of the rs1884614, rs2425637 and rs3818247 obeyed the Hardy–Weinberg equilibrium (rs1884614, p=0.77 among type 2 diabetic patients/p=0.59 among control subjects/p=0.83 in combination; rs2425637, p=0.36/p=0.13/p=0.20; rs3818247, p=0.90/p=0.54/p=0.83). The genotype distribution of the rs1885088 deviated from the Hardy–Weinberg equilibrium among the type 2 diabetic patients (p=0.03) and when these patients were combined with the control subjects (p=0.03), whereas it was consistent with Hardy–Weinberg equilibrium among the control subjects when analysed separately (p=0.29). However, in the population-based Inter99 cohort, the genotype distribution obeyed the Hardy–Weinberg equilibrium (p=0.33). The deviation among type 2 diabetic patients was due to a higher number of carriers of the *aa* genotype of the rs1885088 than expected. This finding may be due to a causal effect of the rs1885088 (or an SNP in linkage disequilibrium with this). The number of carriers of the *aa* genotype among the glucose-tolerant subjects was, however, not lower than expected, and the rs1885088 showed no association with type 2 diabetes. We therefore considered this minor deviance from the Hardy–Weinberg equilibrium among the type 2 diabetic patients as a chance finding and the rs1885088 was included in the analysis.

Concerning the rs2425637, the minor allele in the Danish Caucasian population was the *g*-allele. Yet, in order to make it comparable with the FUSION and Ashkenazi studies [21, 22], which denoted the *t*-allele as the minor allele, we chose to consider the *t*-allele as the minor allele in our analysis (Table 2).

Biochemical assays and physiological measurements All OGTTs were performed in the morning after a 12-h overnight fast. Plasma glucose and serum insulin levels were analysed using the routine methods of the Steno Diabetes Center.

The insulinogenic index was calculated as (insulin at 30 min−fasting insulin)/glucose at 30 min; the incremental AUC for glucose and insulin during 0–120 min were calculated by applying the trapezoidal method; and HOMA insulin resistance was calculated as (fasting plasma glucose×fasting serum insulin)/22.5.

Statistical analysis Differences in minor allele frequencies and genotype distributions among type 2 diabetic patients and control subjects with corresponding odds ratios (OR) were analysed by likelihood ratio tests with calculation of the p value by chi square approximation to its distribution using the "Web-AssoTest" program (available at http://www.ekstroem.com). All genotype distributions were tested for Hardy–Weinberg equilibrium using likelihood ratio tests at the "Web-AssoTest" program. The p values were not corrected for the number of case-control analyses performed.

Linkage disequilibrium was estimated as R^2 using the linkage disequilibrium estimation v0.1a (available at http://www.ekstroem.com). When R^2=1, this indicates complete linkage disequilibrium, and R^2=0 indicates no linkage disequilibrium.

Differences in continuous variables were tested using a general linear model for analysis of variance with adjustments for age, sex, BMI and potential significant interactions between these covariates. All residuals were tested for normal distribution, and transformation (ln or cube root transformation) of the variables was undertaken if necessary. The analyses of continuous variables were carried out using the Statistical Package for Social Science (SPSS) for Windows version 11.5 (SPSS, Chicago, IL, USA). The p values were not corrected for the number of traits analysed or tests performed.

To avoid assumptions regarding mode of inheritance, the case-control studies and the analyses of differences in continuous variables were performed using additive, recessive and dominant models.

A p value of less than 0.05 was considered significant.

Results

The results of the case-control studies of the four SNPs (rs1884614, rs2425637, rs1885088 and rs3818247) are shown in Table 2. The minor *t*-allele of the rs1884614, which is located 4 kb upstream of the *HNF4A* P2 promoter, associated with type 2 diabetes with an OR of 1.14 (p=0.02). By applying a dominant model, we found an OR for type 2 diabetes of 1.16 for carriers of the *ct* or *tt* genotypes as compared with carriers of the *cc* genotype (p=0.02), whereas an additive model resulted in ORs of 1.15 and 1.31 for the *ct* and *tt* genotypes respectively, as compared with the *cc* genotype (p=0.06). Among 680 IGT subjects from the Inter99 cohort, the frequency of the minor allele of the rs1884614 was 18.5%, which was comparable with the allele frequency among the type 2 diabetic patients. When these 680 IGT subjects were combined with the 1387 type 2 diabetic patients, the differences in both minor allele frequency and genotype distribution between control subjects and cases of type 2 diabetes or IGT were still significant (cases of IGT plus type 2 diabetes: 1371 carriers of the *cc* genotype, 622 carriers of

Table 2 Genotype distributions and corresponding odds ratios for type 2 diabetes from the case-control studies of the rs1884614, rs2425637, rs1885088, and rs3818247 of *HNF4A* analysed by applying an additive, a recessive and a dominant model respectively

Kb position	Name of SNP (major/minor allele)		Type 2 diabetic patients, number (%)	Glucose-tolerant subjects, number (%)	OR (95% CI) for type 2 diabetes, additive model	OR (95% CI) for type 2 diabetes, recessive model	OR (95% CI) for type 2 diabetes, dominant model
−3.926	rs1884614						
	(*c*/*t*)	*cc*	919 (66)	3313 (70)			
		ct	418 (30)	1315 (27)	1.15 (1.00–1.31)[a]		
		tt	50 (4)	138 (3)	1.31 (0.94–1.82)[a]	1.25 (0.90–1.74)	1.16 (1.02–1.32)[b]
	Minor allele frequency[c]		0.187	0.167			
39.604	rs2425637						
	(*g*/*t*)	*gg*	360 (25)	1126 (24)			
		gt	697 (49)	2308 (49)	0.94 (0.82–1.09)		
		tt	372 (26)	1293 (27)	0.90 (0.76–1.06)	0.93 (0.82–1.07)	0.93 (0.81–1.06)
	Minor allele frequency		0.504	0.518			
54.595	rs1885088						
	(*g*/*a*)	*gg*	911 (64)	2982 (64)			
		ga	432 (31)	1481 (32)	0.95 (0.84–1.09)		
		aa	74 (5)	202 (4)	1.20 (0.91–1.58)	1.22 (0.93–1.60)	0.98 (0.87–1.11)
	Minor allele frequency		0.205	0.202			
73.035	rs3818247						
	(*g*/*t*)	*gg*	607 (44)	2008 (42)			
		gt	612 (45)	2174 (46)	0.93 (0.82–1.06)		
		tt	152 (11)	566 (12)	0.89 (0.73–1.09)	0.92 (0.76–1.11)	0.92 (0.82–1.04)
	Minor allele frequency		0.334	0.348			

The kb position indicates kilobase position of the SNP from the *HNF4A* P2 promoter translation start site at chromosome 20, base position 43,669,874 of the human reference sequence (UCSC Genome Browser, July 2003) [22]. In the additive model, the ORs for type 2 diabetes are shown for the carriers of the minor allele in heterozygous and homozygous form respectively, as compared with carriers of the major allele in homozygous form. In the recessive model, the OR for type 2 diabetes is shown for carriers of the minor allele in homozygous form as compared with both carriers of the minor allele in heterozygous form and carriers of the major allele in homozygous form. In the dominant model, the OR for type 2 diabetes is shown for carriers of the minor allele in both heterozygous and homozygous form as compared with carriers of the major allele in homozygous form

OR Odds ratio

[a]Likelihood ratio test for difference in genotype distribution in the additive model p=0.06

[b]Likelihood ratio test for difference in genotype distribution in the dominant model p=0.02

[c]OR for type 2 diabetes for the minor allele, 1.14 (1.03–1.28), likelihood ratio test for difference in minor allele frequency p=0.02

the *ct* genotype, 74 carriers of the *tt* genotype; OR for the minor allele: 1.14 [p=0.006]; additive model: ORs of 1.14 and 1.30 for the *ct* and *tt* genotypes respectively, as compared with the *cc* genotype [p=0.03]; dominant model: OR of 1.16 for carriers of the *ct* or *tt* genotypes as compared with carriers of the *cc* genotype [p=0.009]).

Type 2 diabetic carriers of the rs1884614 risk allele displayed no differences in age of diabetes onset or mode of treatment as compared with type 2 diabetic non-carriers (data not shown).

The three other SNPs (rs2425637, rs1885088 and rs3818247) examined in the present study showed no evidence of association with type 2 diabetes (Table 2).

The degree of linkage disequilibrium between the four SNPs examined (rs1884614, rs2425637, rs1885088 and rs3818247) was estimated by calculation of R^2. There was no evidence of linkage disequilibrium between any of the four SNPs with the R^2 estimates varying between 0.001 and 0.07.

The rs1884614, which associated with type 2 diabetes, was further analysed in genotype–quantitative trait studies of 4430 middle-aged glucose-tolerant subjects. The carriers of the *t*-allele had borderline significantly increased plasma glucose levels both in the fasting (additive model p=0.06, dominant model p=0.11) and post-OGTT states (additive model p=0.05, dominant model p=0.02), while there was no differences in the serum insulin levels (Table 3). Genotype–quantitative trait analyses were also performed for the three other SNPs (rs2425637, rs1885088 and rs3818247); however, we observed no associations with significant alterations in fasting or post-OGTT plasma glucose levels (data not shown). Neither the rs1884614 nor the other three SNPs (rs2425637, rs1885088 and rs3818247)

Table 3 Clinical and biochemical characteristics of 4430 glucose-tolerant Danish Caucasians from the Inter99 cohort stratified according to the rs1884614 (*c*→*t*) and analysed applying an additive (add: *cc* vs *ct* vs *tt*), a recessive (rec: *cc*+*ct* vs *tt*) and a dominant (dom: *cc* vs *ct*+*tt*) model

	cc	*ct*	*tt*	*p* (add)	*p* (rec)	*p* (dom)
N (men/women)	3075 (1424/1651)	1224 (561/663)	131 (64/67)			
Age (years)	45.2 (7.8)	45.1 (8.0)	45.0 (7.8)			
BMI (kg/m^2)	25.6 (4.1)	25.3 (4.0)	25.5 (3.9)			
Fasting plasma glucose (mmol/l)	5.31 (0.40)	5.31 (0.41)	5.38 (0.38)	0.06	0.03	0.11
30-min plasma glucose (mmol/l)	8.15 (1.53)	8.23 (1.54)	8.41 (1.49)	0.03	0.08	0.02
120-min plasma glucose (mmol/l)	5.48 (1.12)	5.54 (1.12)	5.62 (1.16)	0.10	0.20	0.04
AUC plasma glucose	179 (100)	185 (102)	192 (101)	0.05	0.21	0.02
Fasting serum insulin (pmol/l)	37.5 (23.3)	37.7 (23.5)	35.2 (20.1)	0.26	0.17	0.63
30-min serum insulin (pmol/l)	287 (182)	287 (164)	281 (174)	0.41	0.46	0.38
120-min serum insulin (pmol/l)	168 (130)	166 (128)	186 (167)	0.48	0.27	0.43
AUC serum insulin	21046 (13599)	20948 (12427)	21672 (14014)	0.67	0.79	0.37
Insulinogenic index$_{INSULIN}$	30.9 (20.1)	31.0 (19.0)	29.8 (20.0)	0.47	0.34	0.61
HOMA IR	8.9 (5.7)	9.0 (5.7)	8.5 (5.1)	0.35	0.29	0.51

Data are means (SD). AUC denotes incremental area under the curve (0–120 min) during the OGTT. We obtained *p* values using a general linear model with age and BMI as covariates, and sex and genotype as fixed factors on variables or transformed variables
HOMA IR, HOMA insulin resistance

were associated with any of the other prediabetic traits investigated.

Discussion

Four SNPs (rs4810424, rs1884613, rs1884614 and rs2144908) were recently reported to associate with type 2 diabetes in the Finnish and Ashkenazi Jewish populations with ORs varying between 1.31 and 1.46 [21, 22]. These four SNPs are distributed throughout a ~10-kb region upstream of and around the P2 promoter of *HNF4A*, and in both populations these SNPs were in almost complete linkage disequilibrium [21, 22]. Our results show evidence for an association between the rs1884614 in the *HNF4A* P2 region and type 2 diabetes in the Danish population. Additionally, we examined three other SNPs, which were located downstream of the *HNF4A* P2 region: rs2425637 (39.6 kb downstream of the P2), rs1885088 (54.6 kb downstream of the P2) and rs3818247 (73.0 kb downstream of the P2). The rs2425637 and the rs1885088 associated with type 2 diabetes in the Finnish population, whereas the rs3818247 associated with type 2 diabetes in the Ashkenazi Jewish population. In the present study, however, we failed to replicate associations between type 2 diabetes and the rs2425637, the rs1885088 or the rs3818247. Since the four SNPs investigated in the present study (rs1884614, rs2425637, rs1885088 and rs3818247) were selected for analysis based on their reported association with type 2 diabetes in the Ashkenazim and Finnish studies [21, 22], we chose not to adjust the *p* values of the case-control studies for the numbers of case-control analyses performed.

The four SNPs investigated in the present study (rs1884614, rs2425637, rs1885088 and rs3818247) have been reported to represent distinct haplotype blocks in Finnish and Ashkenazi Jewish subjects as measured by linkage disequilibrium [21, 22]. In the Danish population, we found no linkage disequilibrium between any of the four investigated SNPs, substantiating the notion that these SNPs may also be embedded in distinct haplotype blocks in the Danish population.

The rs4810424, rs1884613, rs1884614 or rs2144908, which were in linkage disequilibrium in the Finnish and Ashkenazi Jewish populations, are not located in confirmed transcriptional regulatory regions or in sequences which are conserved between human, mouse and rat [22], indicating that the causal variant in this genomic region may be a yet unknown variant in strong linkage disequilibrium with rs1884614, which has now been shown to associate with type 2 diabetes in three different populations. The discrepancies in association between type 2 diabetes and the three other *HNF4A* SNPs (rs2425637, rs1885088, rs3818247), which were investigated both in this study and in the studies of the Finnish and Ashkenazi Jewish populations, may tentatively be related to population-specific recombinations between the causal variant and the SNPs investigated.

Mutation analyses of both the P1 and P2 promoters and the coding exons of *HNF4A* have not identified any SNPs that were associated with type 2 diabetes [17–20] (own unpublished data). If the observed association with type 2 diabetes is caused by an SNP influencing the expression or function of HNF-4α, it is more likely to be an SNP in a distinct regulatory region of the P2 promoter. Alternatively, the causal SNP(s) is located in a regulatory or a coding region of a yet unknown gene on chromosome 20q12–q13.

In the present investigation, the rs1884614 risk allele associated with type 2 diabetes with an OR of 1.14, which is somewhat lower than the reported ORs of 1.31 and 1.45 for the Finnish and Ashkenazi Jewish populations respec-

tively [21, 22]. Replication studies reporting smaller disease effects than the original association studies are a well-known phenomenon, which has been suggested to be related to an overestimation of the disease effect in the original studies [26, 27]. However, in this context, the finding may also be attributable to differences in the study populations. The studies of the Finnish and Ashkenazi Jewish populations were based on cases from families with type 2 diabetes and unrelated control subjects. The present association study comprised type 2 diabetic cases who were recruited from either outpatient clinics or a population-based screening study and who were compared with a population-based sample of glucose-tolerant control subjects. Aetiologically, random cases may be more heterogeneous than cases with familial diabetes comprising both phenocopies and patients with inherited diabetes. Moreover, the families from both the Finnish and Ashkenazi Jewish studies have shown evidence for linkage to the *HNF4A* locus at 20q12–q13 [11, 12]. Thus, variation at the *HNF4A* locus could potentially have a greater influence on susceptibility to type 2 diabetes in the Finnish and Ashkenazi Jewish study populations as compared with the Danish study population.

The potential influence of the rs1884614 on relevant prediabetic phenotypes was elucidated in a population-based sample of 4430 middle-aged subjects with normal glucose tolerance. Intriguingly, the glucose-tolerant carriers of the rs1884614 risk allele had borderline significantly increased plasma glucose levels, both in the fasting state and during an OGTT. A positive association between the rs2144908 and increased postprandial plasma glucose levels was reported in the Finnish population, and the rs1884614 and rs2144908 were in almost complete linkage disequilibrium in this study population [22]. If the causal variant affects the expression or function of HNF-4α, the observed association between rs1884614 and increased fasting and post-OGTT plasma glucose levels may be explained by a combination of a defective regulation of the hepatic gluconeogenesis and a relative insulin deficiency following stimulation by an oral glucose load, since HNF-4α plays important roles in regulating hepatic gluconeogenesis [2, 28] and glucose metabolism and insulin expression and secretion from the beta cells [3–5].

Even though we analysed several prediabetic traits, potential alterations in plasma glucose levels and/or serum insulin levels were part of our primary working hypothesis. Therefore, we have chosen not to correct the *p* values of the quantitative trait analyses for multiple testing.

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