Brief Genetics Report

A Novel −192c/g Mutation in the Proximal P2 Promoter of the Hepatocyte Nuclear Factor-4α Gene (*HNF4A*) Associates With Late-Onset Diabetes

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Recently, it has been shown that mutations in the P2 promoter of the hepatocyte nuclear factor (HNF)-4α gene (*HNF4A*) cause maturity-onset diabetes of the young (MODY), while single nucleotide polymorphisms in this locus are associated with type 2 diabetes. In this study, we examined 1,189 bp of the P2 promoter and the associated exon 1D of *HNF4A* for variations associated with diabetes in 114 patients with type 2 diabetes, 72 MODYX probands, and 85 women with previous gestational diabetes mellitus. A −192c/g mutation was found in five patients. We screened 1,587 diabetic subjects and 4,812 glucose-tolerant subjects for the −192c/g mutation and identified 5 diabetic and 1 glucose-tolerant mutation carriers (*P* = 0.004). Examination of the families showed that carriers of the −192c/g mutation had a significantly impaired glucose-stimulated insulin release and lower levels of serum total cholesterol compared with matched control subjects. Furthermore, the mutation disrupted the binding of an unidentified sequence-specific DNA binding complex present in human islet extracts. Also, two novel linked polymorphisms in the P2 promoter at positions −1107g/t and −858c/t were identified. These variants were not significantly associated with type 2 diabetes or any pre-diabetic traits. In conclusion, a rare, novel mutation that disrupts a protein binding site in the pancreatic *HNF4A* promoter associates with late-onset diabetes. *Diabetes* 55: 1869–1873, 2006

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GDM, gestational diabetes mellitus; HNF, hepatocyte nuclear factor; IGT, impaired glucose tolerance; MODY, maturity-onset diabetes of the young; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

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Mutations in the coding region and the P2 promoter of the hepatocyte nuclear factor (HNF)-4α gene (*HNF4A*) cause maturity-onset diabetes of the young (MODY) (1–3). Interestingly, studies in Caucasian populations have shown linkage between late-onset type 2 diabetes and the 20q region (4–10), in which the *HNF4A* gene is located. Recently, a 90-kb genomic region covering the two *HNF4A* promoters, P1 and P2, which are located 46 kb apart, has been examined. In this region and close to the P2 promoter, several single nucleotide polymorphisms have been identified that associate with type 2 diabetes among both Ashkenazi Jewish subjects and Finns (11,12). These findings have been confirmed in the U.K. population, the Amish population, and the Danish population (13–15). However, only a few studies have examined the *HNF4A* P2 promoter for variation associated with diabetes. We and others have previously identified mutations in the P2 promoter causing MODY or forms of early-onset diabetes (2,3,16). Furthermore, Mitchell et al. (17) have examined 25 MODYX probands and 48 early-onset type 2 diabetic patients for variants in 534 bp covering the minimal *HNF4A* P2 promoter and exon 1D. However, these authors failed to detect any diabetes-associated mutations.

In the present study, we have examined 1,189 bp of the P2 region and exon 1D of *HNF4A* for variation among different groups of non–type 1 diabetic patients. Furthermore, we tested for an association between identified gene variants and type 2 diabetes and/or intermediary pre-diabetic phenotypes.

RESEARCH DESIGN AND METHODS

The mutation screening was carried out on genomic DNA from 114 unrelated Danish type 2 diabetic patients of Caucasian origin (47 with diabetes diagnosed at ≤40 years of age), 85 women with previously diagnosed gestational diabetes mellitus (GDM) and with a family history of diabetes (at least one parent with diabetes), and 72 unrelated Caucasian MODYX patients from the Czech Republic and Denmark with clinical MODY (diabetes in at least two consecutive generations and at least one affected member of the family with onset before age 25 years) but without any known mutations in the genes encoding glucokinase (MODY2), HNF-1α (MODY3), and the P1 promoter and previous known exons of *HNF4A* (MODY1). All patients were recruited from the outpatient clinic at Steno Diabetes Center (Copenhagen), the Department of Obstetrics, National University Hospital (Copenhagen), the Department of Medical Endocrinology, Odense University Hospital (Odense), or the outpatient clinic at the Department of Pediatrics, Charles University (Prague).

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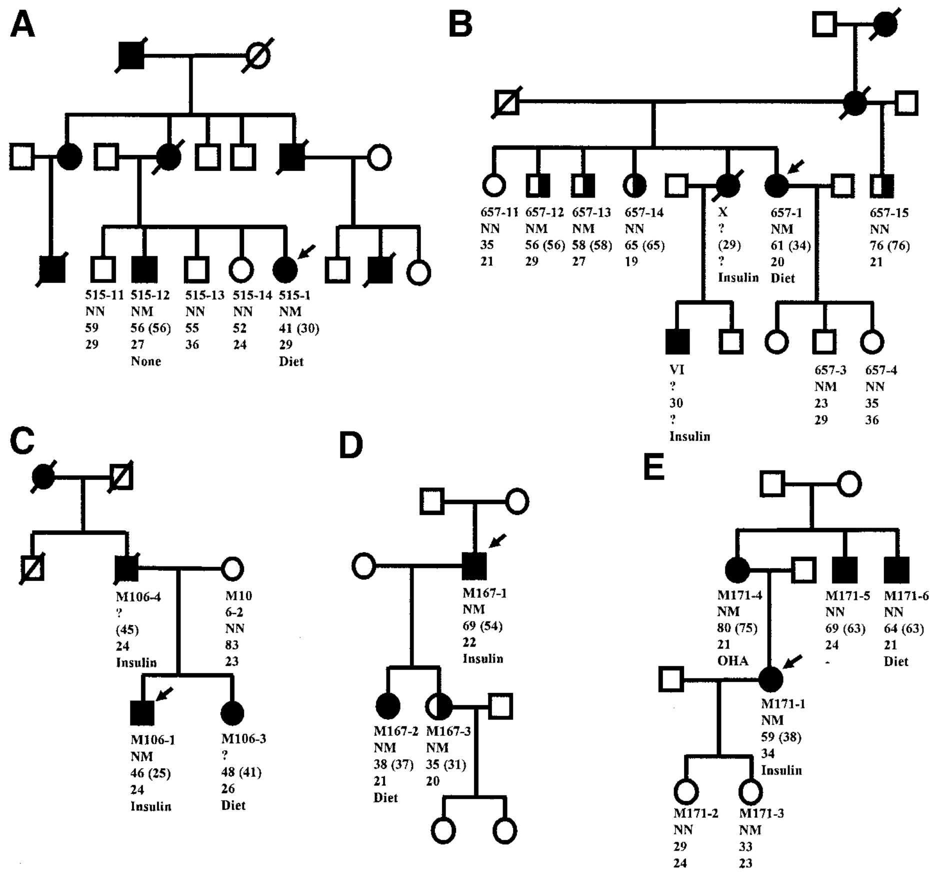

FIG. 1. Pedigrees of the families with the −192c/g mutation in the P2 promoter of *HNF4A*. The probands are patients with GDM (*A* and *B*), MODY (*C*), and type 2 diabetes (*D* and *E*). Square, man; circle, woman; empty symbol, subject with NGT; half-filled symbol, subject with IGT; filled symbol, diabetic patient; symbol with arrowhead, proband. The text below each individual represents the following: identity number, genotype (N denotes the −192c allele; M denotes the −192g mutant allele), age (age of diagnosis) in years, BMI in kg/m^2, and treatment.

Diabetes was diagnosed in accordance with 1999 World Health Organization criteria. For the case-control study, 1,430 type 2 diabetic patients were recruited from the outpatient clinic at Steno Diabetes Center (n = 1,064 patients, including the 114 type 2 diabetic subjects included in the mutation screening) and the population-based Inter99 study, which was performed at the Research Centre for Prevention and Health (Glostrup, Denmark) (n = 366 patients). Control subjects comprised 4,812 individuals with normal glucose tolerance (NGT) and normoglycemia who were recruited from the population-based Inter99 study (n = 4,456) and randomly selected subjects from the Danish Central Population Register (n = 356) (18,19). The studies were approved by the local ethical committees of Copenhagen and Charles University (Prague) and were carried out in accordance with the principles of the Declaration of Helsinki II.

Mutation screening and genotyping. The mutation screening was performed by either denaturing high-performance liquid chromatography (20) or single-stranded conformation polymorphism–heteroduplex analysis (21). In our laboratory, these methodologies have an estimated sensitivity of >95% for detecting a variety of known mutations. The −858 polymorphism was genotyped applying a mass spectrometry–based method as described (15), and the −192 variant was genotyped by Taqman allelic discrimination (BioScience, Herts, U.K.).

Haplotypes. The haplotypes were inferred with the expectation-maximization algorithm, using multiple starting points. Associations were tested using a general linear model implemented in the *haplo.stats* R-package (22).

Promoter functional analyses. The −192 variant was introduced in a luciferase reporter plasmid containing the P2 promoter sequence from positions −371 to −37 relative to the initiator codons (P2.371) and assessed by transient transfection analysis in MIN6 and CaCo cells as described (3).

Electrophoretic mobility shift assays. Protein binding to the −192 region of the P2 promoter was analyzed in nuclear extracts as previously described (23), except that the binding reaction contained 1 μg poly(dI/dC), 10 mmol/l Tris HCl (pH 8.0), 0.5 mmol/l dithiothreitol, 1 μg/μl BSA, 7% glycerol, and 2.5 mmol/l $MgCl_2$.

Statistical analysis. Fisher's exact test was applied to test for significant differences in distribution of allelic frequencies or genotypes among type 2 diabetic patients and control subjects in the case-control study. All genotype distributions were tested for Hardy-Weinberg equilibrium using a likelihood ratio test. Differences in continuous variables between carriers of the polymorphisms were tested using a general linear model with age and BMI as covariates, sex and genotype as fixed factors, and familial structure as the random factor. SPSS for Windows (version 12.0) and SAS software were used for statistical analysis. A P value <0.05 was considered significant.

RESULTS

The mutation analysis revealed four nucleotide substitutions: a IVS1 +89g/a mutation, a −192c/g mutation, and two polymorphisms (a −1107g/t substitution and a −858c/t substitution). The IVS1 +89g/a substitution was identified in one Czech MODYX patient. No cosegregation with diabetes was demonstrated (data not shown). The −192c/g mutation was identified in a Danish MODYX proband, two type 2 diabetic patients, and two women with previously diagnosed GDM. Available family members (n = 19) were genotyped for the variant, and 8 additional mutation carriers were identified (Fig. 1). Furthermore, we screened for the variant among 4,812 glucose-tolerant Caucasian subjects and an additional 1,316 type 2 diabetic subjects and identified 1 additional mutation carrier, a 49-year-old glucose-tolerant woman. No family members of this mutation carrier were available for examination. Thus, we identified 1 glucose-tolerant mutation carrier among 4,812 control subjects and 5 diabetic mutation carriers among 1,587 unrelated diabetic subjects (1,430 type 2 diabetic subjects, 85 GDM women, and 72 MODYX subjects) (P = 0.004). Diabetic mutation carriers were on average diagnosed with diabetes at an age of 45.0 ± 15.5 years, slightly earlier than wild-type type 2 diabetic patients, who were diagnosed at an age of 52.2 ± 10.1 years.

The clinical and biochemical characteristics of the probands and their family members carrying the −192c/g mutation, as compared with nonrelated subjects, are

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TABLE 1
Clinical and biochemical characteristics of 14 *HNF4A* −192c/g mutation carriers and 140 *HNF4A* −192c/c wild-type subjects matched on glucose tolerance status, sex, age, BMI, and fasting plasma glucose

	HNF4A −192c/g mutation carriers	*HNF4A* −192c/c wild-type subjects	*P*
n (M/F)	14 (7/7)	140 (70/70)	
Glycemic status (NGT/IGT/type 2 diabetes) (*n*)	3/3/8	30/30/80	
Age (years)	50.3 ± 15.4	47.2 ± 10.3	0.2
Age at diagnosis (years)	45.0 ± 15.5	42.4 ± 14.2	0.6
BMI (kg/m^2)	25.1 ± 4.2	26.2 ± 3.5	0.2
Waist-to-hip ratio	0.91 ± 0.09	0.87 ± 0.08	0.3
Plasma glucose (mmol/l)			
Fasting	7.6 ± 3.1	7.4 ± 3.3	0.8
30 min	11.5 ± 3.7	9.8 ± 2.7	0.07
120 min	13.1 ± 7.1	8.7 ± 4.1	**0.02**
Serum insulin (pmol/l)*			
Fasting	30.5 ± 12.3	54.9 ± 43.3	0.1
30 min	107 ± 76	279 ± 192	**0.005**
120 min	103 ± 66	306 ± 312	**0.02**
Insulinogenic index*	9.1 ± 12.0	26.3 ± 19.0	0.2
HOMA-IR*	12 ± 10	19 ± 19	0.2
Fasting serum total cholesterol (mmol/l)	4.6 ± 0.9	5.6 ± 1.1	**0.01**
Fasting serum HDL cholesterol (mmol/l)	1.7 ± 0.5	1.4 ± 0.4	0.4
Fasting serum triglycerides (mmol/l)	1.1 ± 0.4	1.6 ± 1.2	0.3

Data are means ± SD. Differences in continuous variables between carriers of the mutation were tested using a general linear model with age and BMI as covariates, sex and genotype as fixed factors, and familial structure as random factor. *Examined variable was ln transformed in the analysis. *P* values in bold indicate statistical significance. Subjects with NGT, IGT, or type 2 diabetes were recruited from the population-based Inter99 study, which was performed at the Research Centre for Prevention and Health. For each −192c/g mutation carrier, we selected 10 subjects matched for glycemic status (NGT, IGT, or type 2 diabetes), sex, age, BMI, and fasting plasma glucose. The insulinogenic index was calculated as fasting serum insulin (in pmol/l) subtracted from 30-min post-OGTT serum insulin (in pmol/l) and divided by 30-min post-OGTT plasma glucose (in mmol/l). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting plasma glucose (in mmol/l) multiplied by fasting serum insulin (in mmol/l) and divided by 22.5.

shown in Table 1. Subjects with NGT, impaired glucose tolerance (IGT), or type 2 diabetes were recruited from the population-based Inter99 study, which was performed at the Research Centre for Prevention and Health. For each −192c/g mutation carrier, we selected 10 subjects matched on glucose tolerance status (NGT, IGT, or type 2 diabetes), sex, age, BMI, and fasting plasma glucose. The −192c/g carriers had a low 30- and 120-min post–oral glucose tolerance test (OGTT) serum insulin response ($P = 0.005$ and 0.02, respectively) and higher levels of plasma glucose at 120 min during an OGTT ($P = 0.02$) compared with matched subjects. Additionally, the −192c/g carriers had significantly lower fasting serum total cholesterol ($P = 0.01$) (Table 1). When stratifying mutation carriers and matched control subjects into groups with NGT, IGT, and type 2 diabetes, we found that mutation carriers had a low 30- and 120-min post-OGTT serum insulin response in all subgroups (supplementary Tables 1–3 [online appendix available at http://diabetes.diabetesjournals.org]).

Electrophoretic mobility shift assays showed that double-stranded oligonucleotides encompassing position −192 of the P2 promoter exhibit sequence-specific, high-affinity binding to a single major nuclear complex that is not only enriched in human pancreatic islet nuclear extracts and INS1 β-cells but also present in numerous other cell types (Fig. 2*B* and data not shown). Remarkably, the mutated sequence was unable to form a retardation complex and failed to compete with the complex formed with the wild-type sequence (Fig. 2*B*). However, transient transfection assays in cultured MIN6 and CaCo cells using a 334-bp P2 promoter luciferase reporter gene showed no significant impact of the mutation on basal or HNF-1α–dependent activity (data not shown).

The two upstream polymorphisms, at positions −1107 and −858, were in perfect linkage disequilibrium ($R^2 = 1$); therefore we only made further analyses on the −858 polymorphism. The allelic frequency of the −858 polymorphism was 12.0% (95% CI 11.4–12.7) among 4,812 control subjects and 11.8% (10.6–13.0) among 1,430 type 2 diabetic patients (NS). Among 4,456 subjects with NGT and normoglycemia, there was a significant association between the polymorphism and alterations in fasting levels of serum total cholesterol and HDL cholesterol ($P = 0.02$ and 0.02, respectively) (Table 2). Association studies were also carried out using haplotypes based on single nucleotide polymorphisms identified and investigated in previous studies (15,18). However, there were no extended significant associations with diabetes or any relevant pre-diabetic trait for any combinations, including combinations of the −858c/t polymorphism and the two previously reported diabetes-associated polymorphisms (rs1884614 and Thr130Ile) (data not shown) (15,18).

DISCUSSION

We report the identification of a −192c/g mutation in the *HNF4A* P2 promoter that associates with diabetes. The mutation was identified among one MODYX proband, two type 2 diabetic patients, two women with previously diagnosed GDM, and one glucose-tolerant 49-year-old woman. The probands and the family members carrying the variant ($n = 14$) had a low serum insulin response during an OGTT compared with matched control subjects, indicating that they may have a pancreatic β-cell defect. Their β-cell function seems similar to what has been reported among two previously identified MODY mutations in the *HNF4A* P2 promoter (2,3) and is consistent with a decreased expression of *HNF4A* in pancreatic islets. Moreover, the fasting serum total cholesterol concentration of the −192c/g mutation carriers was on average reduced by 18%. This finding may indicate that the expression of *HNF4A* in the liver is also decreased. Interestingly, a missense Thr130Ile *HNF4A* polymorphism has been described to be associated with decreased circulating total cholesterol levels (18). In contrast to what is typically seen with the majority of MODY1 mutations, the −192c/g mutation does not seem to be highly penetrant. The ages for the diagnosis of diabetes varies between 25 and 75 years, and six mutation carriers have NGT or IGT at 35, 56, and 58 and 23, 33, and 49 years of age, respectively. Thus, our data indicate that the −192c/g mutation is associated with late-onset familial diabetes. That the

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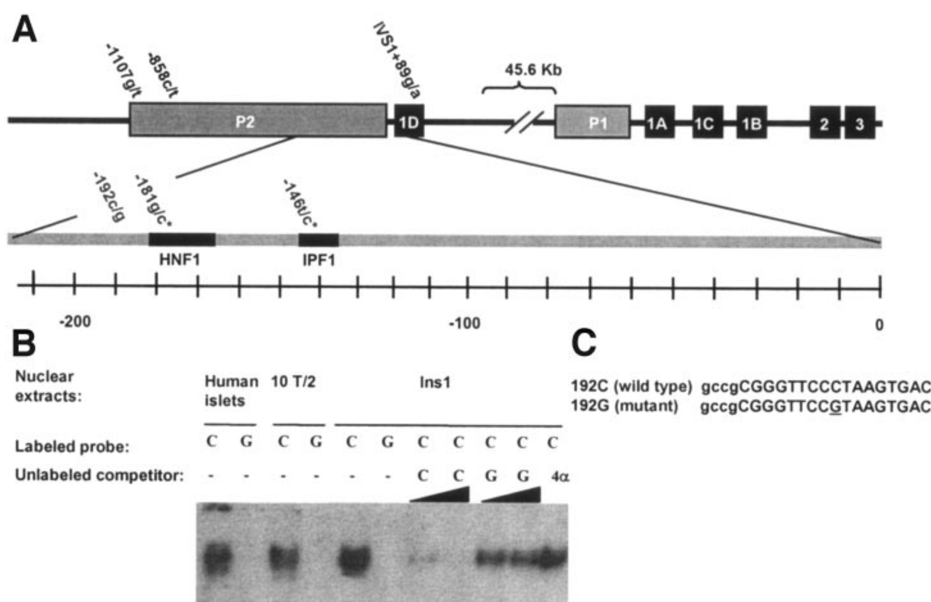

FIG. 2. ***A*****: Summary of genetic variation identified in 1,189 bp of the *HNF4A* P2 promoter region. Positions are numbered according to the translation start site of exon 1D. Mutations shown with an asterisk (*) have previously been described to cause MODY by destroying a HNF-1α binding site or an insulin promoter factor (IPF)1 binding site (2,3).** ***B*****: The −192 c/g mutation eliminates high-affinity, sequence-specific binding to a human islet nuclear complex. Nuclear extracts from human islets, C3H 10 T1/2 fibroblasts, or INS1 β-cells were incubated with labeled oligonucleotide probes containing the wild-type −192C sequence (*C*) or the mutated sequence (*G*). Binding to the 192C probe was competed with 10- or 100-fold molar excess of indicated unlabeled competitor probes. 4α indicates an oligonucleotide containing the HNF-4α binding element of the apoCIII gene. Sequences of oligonucleotide probes are shown in *C*.**

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−192c/g mutation can be associated with late-onset monogenic diabetes is further strengthened by a recent Norwegian study in which the −192 mutation of *HNF4A* is shown to cosegregate with diabetes in two extended families (24).

The mutation did not affect basal and HNF-1α–induced promoter activity in transient transfection assays in cell lines using a 334-bp reporter minigene. However, such an in vitro assay with a restricted DNA segment cannot by any means negate that this mutation may be functionally important in vivo in human pancreatic cells in the physiological chromatin and full genomic context. In favor of the idea that this mutation may indeed be functionally relevant for *HNF4A* gene activity is provided by the observation that it completely disrupts a high-affinity interaction site of the wild-type sequence with a human islet nuclear complex. This finding suggests that the −192c/g mutation may disrupt a novel uncharacterized regulatory interaction in the *HNF4A* P2 promoter, which is analagous to two recently identified *cis* element mutations in this same segment of the P2 promoter causing MODY1 (Fig. 2*A*) (2,3). We therefore propose that the −192c/g mutation may cause non–type 1 subtypes of diabetes through an abolished binding of a protein important in *HNF4A* gene regulation. However, in vivo functional assays will be

TABLE 2

Clinical and biochemical characteristics of 4,456 subjects recruited from the population-based Inter99 study with NGT stratified according to the −858c/t genotype of *HNF4A*

	−858c/c	−858c/t	−858t/t	P_a	P_b	P_c
n (M/F)	3,449 (1,619/1,830)	933 (415/518)	74 74 (34/40)			
Age (years)	45.2 ± 7.9	45.0 ± 7.8	46.4 ± 8.4	0.3	0.8	0.2
BMI (kg/m^2)	25.5 ± 4.1	25.5 ± 4.0	25.5 ± 4.3	0.9	0.7	0.9
Waist-to-hip ratio	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7	0.6	0.5
Plasma glucose (mmol/l)						
Fasting	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.4	0.5	0.3	0.9
30 min	8.2 ± 1.5	8.2 ± 1.5	8.0 ± 1.4	0.4	0.6	0.2
120 min	5.5 ± 1.1	5.5 ± 1.1	5.5 ± 1.0	0.4	0.2	0.9
Serum insulin (pmol/l)*						
Fasting	37 ± 23	39 ± 25	37 ± 22	0.06	**0.02**	0.8
30 min	288 ± 177	285 ± 173	271 ± 169	0.4	0.5	0.2
120 min	167 ± 131	171 ± 130	184 ± 151	0.5	0.4	0.3
Insulinogenic index*	31 ± 20	31 ± 20	30 ± 21	0.7	0.5	0.6
HOMA-IR*	8.8 ± 5.5	9.2 ± 6.0	8.9 ± 5.4	0.1	0.05	0.8
Fasting serum total cholesterol (mmol/l)	5.4 ± 1.0	5.5 ± 1.0	5.3 ± 1.0	**0.02**	**0.03**	0.3
Fasting serum HDL cholesterol (mmol/l)	1.5 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	**0.02**	0.1	0.05
Fasting serum triglycerides (mmol/l)	1.2 ± 1.0	1.2 ± 0.8	1.2 ± 0.7	0.9	0.9	0.7

Data are means ± SD. Differences in continuous variables were tested using an additive model (P_a, comparing c/c vs. c/t vs. t/t), a dominant model (P_b, comparing c/c vs. c/t + t/t), or a recessive model (P_c, comparing c/c + c/t vs. t/t). *P* values were obtained using a general linear model with age and BMI as covariates and sex and genotype as fixed factors on variables or *ln-transformed variables. *P* values in bold indicate statistical significance. The insulinogenic index was calculated as fasting serum insulin (in pmol/l) subtracted from 30-min post-OGTT serum insulin (in pmol/l) and divided by 30-min post-OGTT plasma glucose (in mmol/l). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the fasting plasma glucose (in mmol/l) multiplied by fasting serum insulin (in pmol/l) and divided by 22.5.

required to conclusively test if this mutation can indeed alter the *HNF4A* gene transcription.

We identified two linked polymorphisms upstream of the transcription start site in the P2 promoter, −1107g/t and −858c/t, respectively. In a case-control study with a power of >80% to detect a relative risk of <1.2 with the observed allele frequency of 12%, we did not find an association with type 2 diabetes. Also, haplotype studies, including previously examined variants in the genomic region (15,18), did not reveal an association with type 2 diabetes. Genotype–quantitative trait studies in 4,456 subjects with NGT and normoglycemia suggested an association between the polymorphism and a reduced fasting level of serum total cholesterol and serum HDL cholesterol, indicating that these linked polymorphisms may affect the expression of *HNF4A* in the liver.

In conclusion, identified variations in the proximal P2 promoter of *HNF4A* do not contribute to the association to type 2 diabetes observed in this genomic region (11,12). However, a rare, novel −192c/g mutation in the proximal *HNF4A* P2 promoter shown to affect binding of a human islet nuclear complex is associated with late-onset familial forms of diabetes.

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REFERENCES

1. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458–460, 1996
2. Thomas H, Jaschkowitz K, Bulman M, Frayling TM, Mitchell SMS, Roosen S, Lingott-Frieg A, Tack CJ, Ellard S, Ryffel GU, Hattersley AT: A distant upstream promoter of the HNF-4alpha gene connects the transcription factors involved in maturity-onset diabetes of the young. *Hum Mol Genet* 10:2089–2097, 2001
3. Hansen SK, Parrizas M, Jensen ML, Pruhova S, Ek J, Boj SF, Johansen A, Maestro MA, Rivera F, Eiberg H, Andel M, Lebl J, Pedersen O, Ferrer J, Hansen T: Genetic evidence that HNF-1a-dependent transcriptional control of HNF-4alpha is essential for human pancreatic beta-cell function. *J Clin Invest* 110:827–833, 2002
4. Hani E, Zouali H, Philippi A, Beaudoin JC, Vionnet N, Passa P, Demenais F, Froguel P: Indication for genetic linkage of the phosphoenolpyruvate carboxykinase (PCK1) gene region on chromosome 20q to non-insulin-dependent diabetes mellitus. *Diabetes Metab* 22:451–454, 1996
5. Ji L, Malecki M, Warram JH, Yang Y, Rich SS, Krolewski AS: New susceptibility locus for NIDDM is localized to human chromosome 20q. *Diabetes* 46:876–881, 1997
6. Bowden DW, Sale M, Howard TD, Qadri A, Spray BJ, Rothschild CB, Akots G, Rich SS, Freedman BI: Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. *Diabetes* 46:882–886, 1997
7. Ghosh S, Watanabe RM, Hauser ER, Valle T, Magnuson VL, Erdos MR, Langefeld CD, Balow J, Ally DS, Kohtamaki K, Chines P, Birznieks G, Kaleta HS, Musick A, Te C, Tannenbaum J, Eldridge W, Shapiro S, Martin C, Witt A, So A, Chang J, Shurtleff B, Porter R, Boehnke M: Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. *Proc Natl Acad Sci U S A* 96:2198–2203, 1999
8. Klupa T, Malecki MT, Pezzolesi M, Ji L, Curtis S, Langefeld CD, Rich SS, Warram JH, Krolewski AS: Further evidence for a susceptibility locus for type 2 diabetes on chromosome 20q13.1-q13.2. *Diabetes* 49:2212–2216, 2000
9. Malecki MT, Antonellis A, Casey P, Ji L, Wantman M, Warram JH, Krolewski AS: Exclusion of the hepatocyte nuclear factor 4α as a candidate gene for late-onset NIDDM linked with chromosome 20q. *Diabetes* 47:970–972, 1998
10. Permutt MA, Wasson JC, Suarez BK, Lin J, Thomas J, Meyer J, Lewitzky S, Rennich JS, Parker A, DuPrat L, Maruti S, Chayen S, Glaser B: A genome scan for type 2 diabetes susceptibility loci in a genetically isolated population. *Diabetes* 50:681–685, 2001
11. Love-Gregory LD, Wasson J, Ma J, Jin CH, Glaser B, Suarez BK, Permutt MA: A Common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4α gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an Ashkenazi Jewish population. *Diabetes* 53:1134–1140, 2004
12. Silander K, Mohlke KL, Scott LJ, Peck EC, Hollstein P, Skol AD, Jackson AU, Deloukas P, Hunt S, Stavrides G, Chines PS, Erdos MR, Narisu N, Conneely KN, Li C, Fingerlin TE, Dhanjal SK, Valle TT, Bergman RN, Tuomilehto J, Watanabe RM, Boehnke M, Collins FS: Genetic variation near the hepatocyte nuclear factor-4α gene predicts susceptibility to type 2 diabetes. *Diabetes* 53:1141–1149, 2004
13. Weedon MN, Owen KR, Shields B, Hitman G, Walker M, McCarthy MI, Love-Gregory LD, Permutt MA, Hattersley AT, Frayling TM: Common variants of the hepatocyte nuclear factor-4α P2 promoter are associated with type 2 diabetes in the U.K. population. *Diabetes* 53:3002–3006, 2004
14. Damcott CM, Hoppman N, Ott SH, Reinhart LJ, Wang J, Pollin TI, O'Connell JR, Mitchell BD, Shuldiner AR: Polymorphisms in both promoters of hepatocyte nuclear factor 4-α are associated with type 2 diabetes in the Amish. *Diabetes* 53:3337–3341, 2004
15. Hansen SK, Rose CS, Glumer C, Drivsholm T, Borch-Johnsen K, Jorgensen T, Pedersen O, Hansen T: Variation near the hepatocyte nuclear factor (HNF)-4 alpha gene associates with type 2 diabetes in the Danish population. *Diabetologia* 48:452–458, 2005
16. Gloyn AL, Ellard S, Shepherd M, Howell RT, Parry EM, Jefferson A, Levy ER, Hattersley AT: Maturity-onset diabetes of the young caused by a balanced translocation where the 20q12 break point results in disruption upstream of the coding region of hepatocyte nuclear factor-4α (*HNF4A*) gene. *Diabetes* 51:2329–2333, 2002
17. Mitchell SM, Vaxillaire M, Thomas H, Parrizas M, Benmezroua Y, Costa A, Hansen T, Owen KR, Tuomi T, Pirie F, Ryffel GU, Ferrer J, Froguel P, Hattersley AT, Frayling TM: Rare variants identified in the HNF-4 alpha beta-cell-specific promoter and alternative exon 1 lack biological significance in maturity onset diabetes of the young and young onset type II diabetes. *Diabetologia* 45:1344–1348, 2002
18. Ek J, Rose CS, Jensen DP, Glumer C, Borch-Johnsen K, Jorgensen T, Pedersen O, Hansen T: The functional Thr130Ile and Val255Met polymorphisms of the hepatocyte nuclear factor-4alpha (HNF4A): gene associations with type 2 diabetes or altered beta-cell function among Danes. *J Clin Endocrinol Metab* 90:3054–3059, 2005
19. Jørgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glümer C, Pisinger C: A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. *Eur J Cardiovasc Prev Rehabil* 10:377–386, 2003
20. Pruhova S, Ek J, Lebl J, Sumnik Z, Saudek F, Andel M, Pedersen O, Hansen T: Genetic epidemiology of MODY in the Czech republic: new mutations in the MODY genes HNF-4alpha, GCK and HNF-1alpha. *Diabetologia* 46:291–295, 2003
21. Urhammer SA, Rasmussen SK, Kaisaki PJ, Oda N, Yamagata K, Moller AM, Fridberg M, Hansen L, Hansen T, Bell GI, Pedersen O: Genetic variation in the hepatocyte nuclear factor-1alpha gene in Danish Caucasians with late-onset NIDDM. *Diabetologia* 40:473–475, 1997
22. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA: Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 70:425–434, 2002
23. Boj SF, Parrizas M, Maestro MA, Ferrer J: A transcription factor regulatory circuit in differentiated pancreatic cells. *Proc Natl Acad Sci U S A* 98:14481–14486, 2001
24. Ræder H, Bjørkhaug L, Johansson S, Mangseth K, Sagen JV, Hunting A, Følling I, Johansen O, Bjørgaas M, Paus PN, Søvik O, Molven A, Njølstad PR: A hepatocyte nuclear factor-4α gene (*HNF4A*) P2 promoter haplotype linked with late-onset diabetes: studies of *HNF4A* variants in the Norweigian MODY Registry. *Diabetes* 55:1899–1903, 2006

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