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# Common variants in MODY genes increase the risk of gestational diabetes mellitus

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**Abbreviations**

| | |
|---|---|
| DBS | dried blood spots |
| ESM | Electronic Supplementary Material |
| GDM | gestational diabetes mellitus |
| *GCK* | glucokinase gene |
| *HNF1A* | hepatocyte nuclear factor-1 α gene |
| *HNF4A* | hepatocyte nuclear factor-4 α gene |
| OR | odds ratio |

## Abstract

***Aims/hypothesis*** Impaired beta cell function is the hallmark of gestational diabetes mellitus (GDM) and MODY. In addition, women with MODY gene mutations often present with GDM, but it is not known whether common variants in MODY genes contribute to GDM.

***Methods*** We genotyped five common variants in the glucokinase (*GCK*, commonly known as *MODY2*), hepatocyte nuclear factor 1-α (*HNF1A*, commonly known as *MODY3*) and 4-α (*HNF4A* commonly known as *MODY1*) genes in 1880 Scandinavian women (648 women with GDM and 1232 pregnant non-diabetic control women).

***Results*** The A allele of the *GCK* −30G→A polymorphism was more common in GDM women than in control subjects (odds ratio [OR] 1.28 [95% CI 1.06−1.53], *p*=0.008, corrected *p*-value, *p*=0.035). Under a recessive model [AA vs GA+GG], the OR increased further to 2.12 (95% CI 1.21−3.72, *p*=0.009). The frequency of the L allele of the *HNF1A* I27L polymorphism was slightly higher in GDM than in controls (1.16 [95% CI 1.001−1.34], *p*=0.048, corrected *p*-value, *p*=0.17). However, the OR increased under a dominant model (LL+IL vs II; 1.31 [95% CI 1.08−1.60], *p*=0.007). The rs2144908, rs2425637 and rs1885088 variants, which are located downstream of the primary beta cell promoter (P2) of *HNF4A*, were not associated with GDM.

***Conclusions/interpretation*** The −30G→A polymorphism of the beta-cell-specific promoter of *GCK* and the I27L polymorphism of *HNF1A* seem to increase the risk of GDM in Scandinavian women.

## Introduction

Gestational diabetes mellitus (GDM) is the most common metabolic disorder during pregnancy, and is defined as glucose intolerance with onset or first recognition during pregnancy [1]. The prevalence of GDM ranges from 0.6 up to 15% [2, 3], and the frequency has increased in several populations during the last decade [4, 5]. Impaired beta cell function and insulin resistance characterise pregnancy complicated by GDM [6]. However, when insulin secretion is adjusted for the degree of insulin resistance, women with GDM have a severe reduction in beta cell function compared with normal pregnant women [7]. This beta cell dysfunction seems to persist in women with a history of GDM post partum [6, 8].

MODY is a clinically and genetically heterogeneous monogenic disease characterised by an autosomal dominant mode of inheritance, early onset (usually before the age of 25 years) and pancreatic beta cell dysfunction [9]. Mutations in the genes encoding the glycolytic enzyme glucokinase (*GCK*, commonly known as *MODY2*) and the transcription factors hepatocyte nuclear factor 4-α (*HNF4A* commonly known as *MODY1*) and 1-α (*HNF1A*, commonly known as *MODY3*), insulin promoter factor 1 (*IPF1*, commonly: *MODY4*), transcription factor 2 (*TCF2*, commonly: *MODY5*) and neurogenic differentiation factor 1 (*NEUROD1*, commonly: *MODY6*) have been shown to cause MODY [9]. The most common forms of the disease are MODY2 and MODY3, which account for 20–65% of all MODY subtypes in Europe [10, 11]. Mutations in the genes involved in MODY1 are less frequent and may account for 5% of subjects with MODY [10, 11], while MODY4–6 are very rare [9, 10].

Women with mutations in *GCK* [12–17] or *HNF1A* [16, 18] often present with GDM. In addition, mutations in *IPF1* have been reported in women with

GDM [16, 19]. Common variants in MODY genes, including *GCK* –30G→A [20, 21] and *HNF1A* I27L [22] variants as well as the rs2144908, rs2425637 and rs1885088 variants in *HNF4A* [23–25], have been associated with beta cell dysfunction, diabetes or related traits.

Since rare mutations in MODY genes are associated with GDM as well as beta cell dysfunction is the hallmark of GDM and MODY, we hypothesised that common variants in MODY genes would also increase the risk of GDM.

Since a comprehensive screening of MODY genes has already been performed in Caucasian patients with type 2 diabetes [26–28] (Winckler W, Weedon M, Graham R et al. unpublished data), we did not perform such screening of these genes and regulatory regions in our study subjects. Instead, we selected five variants in the MODY1–3 genes (i.e. the most common MODY subtypes in Europe) that fulfilled the following criteria: (1) the allele frequency of at least ~15% in order to have sufficient power to detect a relatively modest odds ratio (OR ~1.3); (2) evidence of association with beta cell dysfunction and/or type 2 diabetes or related traits; and (3) for *HNF4A* variants, to represent distinct haplotype blocks as measured by linkage disequilibrium in Caucasians [23, 28]. We genotyped the *GCK* –30G→A, *HNF1A* I27L and *HNF4A* (rs2144908, rs2425637 and rs1885088) variants in a case–control study of 648 unrelated Scandinavian women with GDM and 1232 unrelated Scandinavian pregnant non-diabetic controls.

## Subjects and methods

### Study population

All pregnant women are routinely offered a 75 g OGTT at 27–28 weeks of pregnancy in southern Sweden (Skåne). Women at high risk (previous GDM or a family history of diabetes) are also offered a 75 g OGTT at 12–13 weeks. The tests are performed in the local maternity health-care clinics, using HemoCue® devices (HemoCue, Ängelholm, Sweden) for capillary whole blood analysis. GDM is defined as a 2 h capillary glucose concentration (double test) of at least 9 mmol/l according to the proposal by the Diabetic Pregnancy Study Group of the European Association for the Study of Diabetes [29].

The phenotypic characteristics of the majority of the participants in the present study have been reported earlier [30]. Detailed OGTT data during pregnancy were available only for a small subset of GDM women who were prospectively followed with repeated OGTTs [31]. Briefly, we selected 1880 unrelated Scandinavian women (648 women with GDM and 1232 pregnant non-diabetic controls). Women with GDM were recruited from Malmö or Lund University Hospitals during the period from March 1996 until December 2003 ($n$=226) as well as among women participating in the Diabetes Prediction in Skåne (DiPiS) study, which is a prospective, longitudinal study for the prediction of type 1 diabetes in all newborn infants in southern Sweden during the period from September 2000 to August 2004 ($n$=422) [32]. All pregnant non-diabetic controls ($n$=1232) were ascertained from the DiPiS study. Both GDM groups and the control group are considered to be homogeneous since the GDM women who were recruited from Malmö or Lund hospitals were referred from maternity health-care clinics and underwent the same screening procedure as the DiPiS subjects. In addition, the study groups were

recruited during a similar period, and the population in the southern Sweden is very homogeneous. All women were Scandinavians. Informed voluntary consent was obtained from all study subjects. The study was approved by the ethics committee of Lund University.

## Genetic analyses

### *DNA extraction and template preparation*

Total DNA was isolated from peripheral blood lymphocytes or blood samples were collected as dried blood spots (DBS) on filters (Grade 2992 filters; Schleicher and Schuell, Dassel, Germany).

For DBS samples, initially a template PCR was carried out to amplify the region of interest using the primers listed in Electronic Supplementary Material (ESM), Table 1.

The template PCR was performed with an initial two cycles at 4°C for 30 s followed by 98°C for 3 min, followed by holding at 80°C while the PCR mix was added. Then the PCR was continued with an initial denaturation (94ºC for 5 min), followed by 45 cycles of denaturation (94°C for 30 s), annealing (30 s) and extension (72°C for 30–60 s), followed by final extension (72°C for 10 min). PCR amplification was carried out with a 3 mm DBS in a total volume of 40 μl containing 1 × Pharmacia Amersham buffer (Amersham Pharmacia Biotech, Uppsala, Sweden) (*GCK* –30G→A [rs1799884] and *HNF4A* [rs2425637 and rs1885088]) or 1 × $(NH_4)_2SO_4$ buffer (16 mmol/l $(NH_4)_2SO_4$; 67 mmol/l Tris [pH 8.8]; 0.01% Tween 20) (*HNF1A* I27L [rs1169288] and *HNF4A* [rs2144908]), 4–8 nmol of each dNTP (MBI Fermentas, St Leon-Rot, Germany), 20 pmol of each primer, 60 nmol $MgCl_2$ (*GCK* –30G→A, *HNF1A* I27L and *HNF4A* [rs2144908 and rs2425637]), betaine (Sigma-Aldrich

Sweden, Stockholm, Sweden) (20 μmol: *GCK* –30G→A and *HNF4A* [rs1885088]; 30 μmol: *HNF4A* [rs2425637]) and 1–1.5 U *Taq* polymerase (New England Biolabs, Beverly, MA, USA).

***Genotyping***

SNP genotyping was carried out using the TaqMan allelic discrimination assay or RFLP.

For the TaqMan allelic discrimination assay on an ABI Prism 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), we used 2 μl (5–10 ng) of DNA or 2 μl of template PCR (for DBS samples as described above) according to the manufacturer's instructions. Primers and probes were designed using Assays-by-Design (Applied Biosystems). The primers and probes used are listed in ESM Table 2.

Since TaqMan assay did not work out properly for the *GCK* –30G→A variant on DBS samples, genotyping was carried out using RFLP. The template PCR (see description of template PCR above) product was digested with the enzyme *Alw21*I (MBI Fermentas, St Leon-Rot, Germany) at 37°C for 4 h. PCR products were separated on 2% agarose gel (SeaKem, Rockland, ME, USA) and stained with ethidium bromide to visualise the fragments using UV light.

***Genotyping quality control***

The genotyping success rate was 97.5% for cases (*GCK* –30G→A, 99.1%; *HNF1A* I27L, 94.8%; rs2144908, 96.8%; rs2425637, 99.2%; rs1885088, 97.5%) and 99.0% for controls (*GCK* –30G→A, 99.8%; *HNF1A* I27L, 98.5%; rs2144908, 98.9%; rs2425637, 99.8%; rs1885088, 97.8%). The genotyping error rate was determined to be <0.3% using 943 (10%) duplicate genotypes and 89 double samples (i.e. GDM women who had both peripheral blood DNA and DBS or two DBS spotted at different

deliveries). In the control group, all SNPs conformed to Hardy–Weinberg equilibrium ($\chi^2$ test, $p>0.05$), apart from *HNF4A* rs2144908, which deviated mildly ($p=0.027$). Since the measures described above rule out possible genotyping errors, this deviation might be due to chance variation.

**Statistical analyses**

We used $\chi^2$ analysis to test for differences in allele and genotype frequencies between GDM and control groups. Logistic regression analysis was used to calculate the OR and 95% CI. ANOVA was used to test the significance of differences in continuous variables, such as age, between GDM and control groups using the Number Cruncher Statistical Systems (NCSS, Kaysville, UT, USA). Age was expressed as mean ± SEM. Haplotype analysis was carried out using Haploview software 3.2 [33]. To correct for multiple testing, we permuted the data as implemented in Haploview version 3.2 [33]. We used 10,000 permutations, however, using more permutations gave the same results. This study was not designed to detect differences between genetic models. However, since we did not have predefined genetic models of the potential effect of these variants, we chose to present the data for additive, recessive and dominant models. Two-sided *p*-values less than 0.05 were considered statistically significant.

**Power calculation**

By studying a sample of 648 cases and 1232 controls, the present study had more than 80% power, under a multiplicative model, to detect an effect size of 1.3 (as measured in terms of genotypic relative risk) when the frequency of the predisposing allele equalled 15% (for $\alpha=0.05$). When the predisposing allele frequency was >30%, the study had at least 80% power to detect an OR of 1.22 under a multiplicative model (for $\alpha=0.05$). Power calculations were performed using the Genetic Power Calculator (available at http://ibgwww.colorado.edu/~pshaun/gpc/) [34].

## Results

### Subject characteristics

Women with GDM were slightly older than pregnant non-diabetic controls (32.3±0.2 vs 30.5±0.1, $p<0.0001$). The genotype and allele frequency distributions of all polymorphisms studied are presented in Table 1.

### *GCK* −30G→A

The GG, GA and AA genotype frequencies of the *GCK* −30G→A polymorphism differed significantly between GDM and control women (67.8, 28.2 and 4.0% vs 72.3, 25.7 and 2.0% respectively, $p=0.010$). In addition, the A allele was found to be more common in GDM women than among control subjects (OR 1.28, 95% CI 1.06−1.53, $p=0.008$, corrected $p$-value, $p=0.035$). Under a recessive model (AA vs GA+GG), the OR increased further to 2.12 (95% CI 1.21−3.72, $p=0.009$). Using a dominant model, the OR for GDM in carriers of the GA or AA genotypes compared with carriers of the GG genotype was 1.24 (95% CI 1.01–1.53, $p=0.039$). Of note, the ORs were almost the same, with overlapping 95% CIs, when women who were positive for GAD65Ab, IA–2Ab or both (antibody measurements were not available for all subjects) were removed from the analyses (data not shown).

### *HNF1A* I27L

The II, IL and LL genotype frequencies of the *HNF1A* I27L polymorphism differed significantly between GDM and control women (39.4, 48.5 and 12.1% vs 46.1, 41.8 and 12.1% respectively, $p=0.016$). The L allele was slightly more frequent in GDM women than in controls (OR 1.16, 95% CI 1.001−1.34, $p=0.048$, corrected $p$-value, $p=0.17$). However, the IL genotype was more frequent in GDM women than in

controls, compared with the wild-type II genotype (OR 1.36, 95% CI 1.10−1.67, $p$=0.004). In addition, under a dominant model [IL+LL vs II], the L allele conferred an increased risk of GDM (OR 1.31, 95% CI 1.08−1.60, $p$=0.007). As for the *GCK* −30G→A polymorphism, the ORs and 95% CIs remained almost the same when women who were positive for GAD65Ab, IA–2Ab or both were excluded from the analyses (data not shown).

### *HNF4A* variants

The degree of linkage disequilibrium between *HNF4A* variants (rs2144908, rs2425637 and rs1885088) was estimated using D′ and $r^2$ values. There was no evidence of linkage disequilibrium between these variants; D′ values were between 0.01 and 0.5 and $r^2$ values were between 0.0 and 0.01.

The frequency of the A allele of the rs2144908 variant, which is located 1272 bp downstream of the primary beta cell promoter (P2) of *HNF4A*, did not differ significantly between GDM and controls (OR 1.14, 95% CI 0.96–1.37, $p$=0.14).

Neither was there any difference in the frequency of the T allele of the rs2425637 variant, which is located 39 604 bp downstream of P2, between GDM and control women (OR 1.09, 95% CI 0.95−1.24, $p$=0.23).

The intronic variant (rs1885088) is located 54 595 bp downstream of the P2. Similar frequencies of the A allele were observed in women with GDM and control women (OR 0.96, 95% CI 0.81−1.14, $p$=0.66).

# Discussion

The key finding of the present study was that common variants in two MODY genes, *GCK* and *HNF1A*, increase the risk of GDM.

### *GCK* –30G→A

In the pancreatic islets, glucokinase plays a critical role in the regulation of insulin secretion by acting as a glucose sensor [35]. The −30G→A variant in the beta-cell-specific promoter of the *GCK* was shown to co-segregate with diabetes in a French family in which the proband was a woman with GDM [15]. Subsequently, it has been associated with reduced beta cell function in middle-aged Japanese-American men [36]. In addition, in women in the third trimester of pregnancy, the AA genotype led to a reduction in early-phase insulin secretion [37]. In a recent study of 755 pregnant women, the A allele was associated with increased fasting plasma glucose measured at 28 weeks of gestation in healthy Caucasian women from the UK [38]. In support of this, another recent study reported association of this polymorphism with elevated fasting and post-OGTT glucose levels as well as with impaired glucose regulation (i.e. type 2 diabetes, IGT and IFG) and features of the metabolic syndrome in Caucasians [21]. However, no association of the −30G→A variant with GDM was observed in two small studies that included women of Caucasian, black and oriental origin [39] or in American black women [40]. Interestingly, it has been demonstrated that the A allele increases the risk of coronary artery disease in individuals with and without type 2 diabetes and it was also associated with an increased prevalence of type 2 diabetes in subjects with coronary artery disease [20].

In the present study, the A allele was associated with a modestly increased risk of GDM and this effect was more pronounced under a recessive mode

of inheritance. The previously demonstrated deleterious effect of this polymorphism on beta cell function during pregnancy [37] might be a plausible explanation for the observed association, which is consistent with the key role of impaired beta cell function in the pathogenesis of GDM [6–8].

***HNF1A* I27L**

Defective insulin secretion is the hallmark of patients with *HNF1A* (i.e. *MODY3*) mutations [18]. The I27L polymorphism is located within the dimerisation domain of *HNF1A* [41], and the amino acid isoleucine is conserved among several species, suggesting a potential functional importance of this residue [22]. Chiu et al. have reported association of the I27L polymorphism with lower first- and second-phase insulin secretion in glucose-tolerant subjects [22]. In line with this, we found a nominal association of the L allele of the I27L polymorphism with type 2 diabetes in Scandinavian/Canadian subjects, but this was not the case in the larger sample including also subjects from the US and Poland [27] or in a recent large study in the UK Caucasian population [26]. Moreover, we have recently observed an association of the I27L polymorphism with increased risk of type 2 diabetes in a large new Swedish case–control study [42]. This was supported by in vitro findings that the L allele was associated with decreased transcriptional activity in HeLa and INS-1 cells [42].

In keeping with the findings for the *GCK* −30G→A variant, we observed a modest effect of the L allele of the *HNF1A* I27L polymorphism on the risk of GDM, which might be mediated by its effect on beta cell function [22]. It may be expected that individuals with a slight impairment in their beta cell function are more prone to deteriorated glucose tolerance when becoming insulin-resistant during

pregnancy. It was, however, not possible to address a potential effect on beta cell function in the present study, as this would have required assessment of beta cell function prior to and during pregnancy. Unfortunately, we did not have this information. However, this finding should be interpreted with some caution since the difference in allele frequencies between GDM and controls was not statistically significant after correction for multiple comparisons.

### *HNF4A* variants

Somewhat surprisingly, variants in *HNF4A*, which have repeatedly been associated with a modestly increased risk of type 2 diabetes [23–25], were not associated with GDM in the present study. *HNF4A* is a member of the nuclear receptor family of transcription factors, which is expressed in several tissues, including the liver, gut, kidney and pancreas [43]. Whereas the expression of *HNF4A* in the liver is mediated by a proximal promoter (P1), its expression in beta cells is driven by an alternative beta cell promoter (P2) located 46 kb upstream of P1 [44, 45]. Mutations in the *HNF1A* and *IPF1* binding sites of the P2 promoter have been associated with MODY1 [44, 45]. The rs2144908, rs2425637 and rs1885088 variants, which are located downstream of the P2 promoter, were originally associated with type 2 diabetes in Finns [23]. In addition, the rs2144908 variant has been associated with type 2 diabetes in Ashkenazi Jewish [24] as well as in Caucasians from the UK [25]. Interestingly, the rs2144908 variant was also associated with reduced beta cell function (i.e. decreased acute insulin response to glucose and decreased disposition index) in unaffected Finnish offspring of parents with type 2 diabetes [23]. In the present study, there was no evidence for association of these variants with GDM. This may suggest that the studied variants in the *HNF4A* gene have no major impact on

predisposition to GDM. However, it should be stressed that a smaller effect (OR < 1.22–1.27 depending on the allele frequency) of these variants on the risk of GDM could have been missed. Indeed, the present study had adequate power to detect the ORs (1.23–1.46) reported for type 2 diabetes in the original studies [23, 24], but not the ORs (1.14–1.15) reported in Caucasians from the UK and Denmark in recent large studies [25, 46]. Consistent with the other studies, these three variants were not in linkage disequilibrium in our study and the frequencies of the minor alleles in controls were comparable to that reported in other populations [23–25, 46].

In conclusion, the −30G→A polymorphism of the beta-cell-specific promoter of *GCK* and the I27L polymorphism of *HNF1A* seem to increase the risk of GDM in Scandinavian women, suggesting a role of common variants that are known to affect beta cell function in the aetiology of GDM. However, to demonstrate a direct effect on beta cell function more studies are required, with assessment of beta cell function prior to and during pregnancy in carriers of these polymorphisms.

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**Table 1** Genotype and allele distributions and corresponding odds ratios for GDM

| SNP (rs number) | Genotype or allele | GDM n (%) | Controls n (%) | OR (95% CI) for GDM, additive model/ allelic effect | OR (95% CI) for GDM, recessive model | OR (95% CI) for GDM, dominant model |
|---|---|---|---|---|---|---|
| *GCK* –30G→A (rs1799884) | GG | 435 (67.8) | 889 (72.3) | | | |
| | GA | 181 (28.2) | 316 (25.7) | 1.17 (0.94–1.45) | | |
| | AA | 26 (4.0) | 24 (2.0) [a] | 2.21 (1.26–3.90) [b] | 2.12 (1.21–3.72) [d] | 1.24 (1.01–1.53) [e] |
| | A | 233 (18.1) | 364 (14.8) | 1.28 (1.06–1.53) [c] | | |
| *HNF1A* I27L (rs1169288) | II | 242 (39.4) | 559 (46.1) | | | |
| | IL | 298 (48.5) | 508 (41.8) | 1.36 (1.10–1.67) [f] | | |
| | LL | 74 (12.1) | 147 (12.1) [a] | 1.16 (0.85–1.60) | 0.99 (0.74–1.34) | 1.31 (1.08–1.60) [e] |
| | L | 446 (36.3) | 802 (33.0) | 1.16 (1.001–1.34) [c] | | |
| *HNF4A* (rs2144908) | GG | 425 (67.8) | 854 (70.1) | | | |
| | GA | 167 (26.6) | 316 (25.9) | 1.06 (0.85–1.32) | | |
| | AA | 35 (5.6) | 48 (4.0) | 1.47 (0.93–2.30) | 1.44 (0.92–2.25) | 1.12 (0.91–1.37) |
| | A | 237 (18.9) | 412 (16.9) | 1.14 (0.96–1.37) | | |
| *HNF4A* (rs2425637) | GG | 159 (24.7) | 317 (25.8) | | | |
| | GT | 310 (48.2) | 617 (50.2) | 1.00 (0.79–1.27) | | |
| | TT | 174 (27.1) | 295 (24.0) | 1.18 (0.90–1.54) | 1.17 (0.94–1.46) | 1.06 (0.85–1.32) |
| | T | 658 (51.2) | 1207 (49.1) | 1.09 (0.95–1.24) | | |
| *HNF4A* (rs1885088) | GG | 412 (65.2) | 791 (65.6) | | | |
| | GA | 199 (31.5) | 354 (29.4) | 1.08 (0.87–1.33) | | |
| | AA | 21 (3.3) | 60 (5.0) | 0.67 (0.40–1.12) | 0.66 (0.40–1.09) | 1.02 (0.83–1.25) |
| | A | 241 (19.1) | 474 (19.7) | 0.96 (0.81–1.14) | | |

[a] Differences in genotype frequencies between women with and without GDM (p=0.010 for *GCK* –30G→A and p=0.016 for *HNF1A* I27L)

[b] p=0.006 for comparison of AA genotype vs GG genotype between women with and without GDM

[c] Differences in allele frequencies between women with and without GDM (p=0.008 for *GCK* –30G→A and p=0.048 for *HNF1A* I27L)

[d] p=0.009 for comparison between women with and without GDM using a recessive model

[e] Comparison between women with and without GDM using a dominant model (p=0.039 for *GCK* 30Γ→A and p=0.007 for *HNF1A* I27L)

[f] p=0.004 for comparison of IL genotype vs II genotype between women with and without GDM