

Screening for MODY Mutations, GAD Antibodies, and Type 1 Diabetes-Associated HLA Genotypes in Women With Gestational Diabetes Mellitus

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OBJECTIVE — To investigate whether genetic susceptibility to type 1 diabetes or maturity-onset diabetes of the young (MODY) increases susceptibility to gestational diabetes mellitus (GDM).

RESEARCH DESIGN AND METHODS — We studied mutations in MODY1–4 genes, the presence of GAD antibodies, and HLA DQB1 risk genotypes in 66 Swedish women with GDM and a family history of diabetes. An oral glucose tolerance test was repeated in 46 women at 1 year postpartum.

RESULTS — There was no increase in type 1 diabetes-associated HLA-DQB1 alleles or GAD antibodies when compared with a group of type 2 diabetic patients ($n = 82$) or healthy control subjects ($n = 86$). Mutations in known MODY genes were identified in 3 of the 66 subjects (1 MODY2, 1 MODY3, and 1 MODY4). Of the 46 GDM subjects, 2 had diabetes (4%) and 17 had impaired glucose tolerance (IGT) (37%) at 1 year postpartum. Of the two subjects who developed manifest diabetes, one carried a MODY3 mutation (A203H in the hepatocyte nuclear factor-1 α gene). There was no increase in high-risk HLA alleles or GAD antibodies in the women who had manifest diabetes or IGT at 1 year postpartum.

CONCLUSIONS — MODY mutations but not autoimmunity contribute to GDM in Swedish women with a family history of diabetes and increase the risk of subsequent diabetes.

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Abbreviations: GCK, glucokinase; GDM, gestational diabetes mellitus; HNF, hepatocyte nuclear factor; IGT, impaired glucose tolerance; IPF1, insulin promoter factor-1; MODY, maturity-onset diabetes of the young; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

In Western society, ~1–3% of pregnant women are diagnosed with gestational diabetes mellitus (GDM) (1,2). This figure is even higher in populations with a high prevalence of type 2 diabetes (3), but does not seem to be different in countries with a high prevalence of type 1 diabetes (4). In a population study in southern Sweden, the prevalence of GDM was rather low (1.2%), using a 2-h capillary glucose value ≥ 9.0 mmol/l during a 75-g oral glucose tolerance test (OGTT) at gestational week 27–28 for the diagnosis of GDM. Importantly, if the 2-h value exceeded 7.8 mmol/l, there was an increased frequency of cesarean section and infant macrosomia (5).

GD may also be heterogeneous, and the relative contribution of impaired β -cell function and insulin resistance could differ. A genetic component seems established because GDM clusters in families (6). However, since GDM women often have mothers with diabetes (7), it has also been suggested that the intrauterine environment may contribute to GDM (8). Obesity is also associated with an increased risk for GDM, but its contribution may differ between different populations. In most women glucose intolerance during pregnancy is temporary and reverses to normal after delivery. However, it is noteworthy that women with a history of GDM have an increased risk of developing diabetes later in life (9,10).

Maturity-onset diabetes of the young (MODY) is a monogenic form of type 2 diabetes that is inherited in an autosomal-dominant fashion and expressed at an early age, usually before the age of 25 years. Genetic defects in five genes are known to cause MODY, i.e., the hepatocyte nuclear factor-4 α (HNF-4 α) (MODY1), glucokinase (GCK) (MODY2), the HNF-1 α gene (MODY3), insulin promoter factor-1 (IPF1) (MODY4), and HNF-1 β (MODY5) (11). All known forms of MODY are primarily linked with pancreatic β -cell dysfunction. Previous

Table 1—Clinical characteristics of patients with GDM, type 2 diabetes, and control subjects with NGT

	GD	Type 2 diabetes	NGT
n (M/F)	66 (0/66)	82 (41/41)	86 (39/47)
Age (years)	31.4 ± 4.5	48.5 ± 6.5	43.9 ± 9.2
BMI (kg/m ²)	26 ± 6.2	29.4 ± 4.6	25.3 ± 3.5
Fasting blood glucose (mmol/l)	4.3 ± 0.5	8.2 ± 2.5	4.7 ± 0.5
Fasting serum insulin (mU/l)	10 ± 5	12.8 ± 8.9	7.0 ± 4.0
HbA _{1c} (%)	4.2 ± 0.4	6.9 ± 1.5	5.2 ± 0.5
GAD antibody-positive (%)	3	12	3

Data are means ± SD, unless otherwise indicated.

studies have shown that mutations in the GCK (MODY2) gene could explain ~5–6% of GD in different populations (12–14). In our previous study of early onset diabetes, 11 of 29 diabetic women with MODY3 (38%) had had GDM (15). These previous findings prompted us to investigate whether MODY1–4 gene mutations increase susceptibility to GDM in Swedish subjects or whether the presence of such mutations is associated with an increased risk of subsequent diabetes.

RESEARCH DESIGN AND METHODS

During the period of March 1996 to June 1999, 110 Swedish women attending antenatal care centers in the geographical area of Malmö and Lund were identified with GDM. To increase the likelihood of finding MODY mutations, we also used a family history of diabetes as selection criteria. All 66 (66 of 110 [60%]) of the selected GDM subjects, aged (mean ± SD) 31.4 ± 4.5 years at diagnosis had at least one first- or second-degree relative with diabetes. The diagnosis of GDM was based on a 75-g OGTT at week 27–28 of pregnancy or at week 12 in women at risk (previous GDM or a family history of diabetes). GDM was defined as a 2-h capillary glucose concentration of at least 9 mmol/l. Of the GDM subjects, 11 (17%) were treated with insulin, and the others were treated with diet alone.

The frequency of HLA-DQB1 alleles [02/0302, 0302/X, 02/X, and 0602(3)/X] was tested by dot-blotting as previously described (16). A total of 82 unrelated subjects with type 2 diabetes and 86 subjects with normal glucose tolerance (NGT) served as controls for HLA typing (Table 1). GAD autoantibodies were tested by radioimmunoassay as described previously (16).

Mutation screening of MODY1–3

genes was performed by using a fluorescent single-strand conformation polymorphism (SSCP) technique. The first-strand polymerase chain reaction (PCR) synthesis was performed by using tailed primers. The second-strand PCR synthesis was performed by using fluorescent primers specific to tails: CAC GAA TTC CCA GAG TCC (forward) and CAA CTG CAG ACA CGT ACC (reverse). The mutation screening of the IPF1 (MODY4) gene was performed by radioactive-SSCP analysis. The amplified fragments ranged in size between 187 and 424 bp. For fluorescent SSCP (Applied Biosystems, Foster City, CA) analyses, 1 μl (FAM), 2 μl (TET), and 4 μl (HEX) were pooled and adjusted with water to a total volume of 10 μl. An aliquot of the pool (0.8 μl) was mixed with 1.4 μl denaturation buffer (G-500TAMRA, formamide and loading buffer). Before loading onto the gel (36-cm plates; 6% acrylamide/bisacrylamide, 50:1 dilution) samples were denatured at 90°C for 5 min. The gels were run in two nondenaturing conditions including 5% glycerol or 10% sucrose. Electrophoresis was performed with an ABI377 DNA sequencer (Applied Biosystems, Foster city, CA) by using power (60 W) as a constant limiting factor for 10 h. An external cooling system (Neslab Instruments, Portsmouth, NH) was used to maintain constant gel temperature (30°C) during electrophoresis. The gels were analyzed with a GeneScan 2.1.1a and Genotyper 2.0 software (Perkin Elmer). After SSCP analysis, each sample with mobility shift was sequenced with original primers on both strands, as previously described (17). The sequences of the primers and the PCR conditions are available from the authors upon request.

The statistical analysis was carried out using Biomedical Data Processing (BMDP, Los Angeles, CA) statistical soft-

ware. The significance of the difference between group frequencies was tested with χ^2 or Fisher's exact tests, and between-group means were tested with Mann-Whitney *U* or Kruskal-Wallis tests.

RESULTS— In 66 Swedish GDM subjects with a family history of diabetes, the mutation screening of MODY1–4 genes resulted in the identification of three amino acid substitutions: A303R (CGG-TGG) in the GCK (MODY2) gene, A203H (CGT-CAT) in the HNF-1 α (MODY3) gene, and P239Q (CCG-CAG) in the IPF-1 (MODY4) gene. The woman who carried the MODY3 gene mutation developed diabetes 1 year postpartum. However, she did not require insulin during the pregnancy. The woman who carried the MODY2 gene mutation was pregnant again 1 year postpartum. She also developed GDM during this pregnancy, with insulin requirements from gestational week 25. The patient with the MODY4 gene mutation required insulin during pregnancy but had NGT after 1 year of follow-up. All three women with MODY mutations were GAD antibody-negative.

An OGTT was repeated in 46 GDM subjects 1 year postpartum to study the subsequent incidence of diabetes. Of these subjects, 2 (4%) had developed diabetes, 17 (37%) had impaired glucose tolerance (IGT), and 27 (59%) had NGT according to World Health Organization criteria.

Only two GDM women (3%) were GAD antibody-positive (Table 1). One of them had NGT and the other one IGT at the 1-year follow-up. There were no significant differences in the HLA-DQB1 risk (02/0302, 0302/X, and 02/X) or protective [0602(3)/X] genotype frequency among GDM subjects, type 2 diabetic patients, and NGT subjects. However, the frequency of the 02/X genotype was significantly higher in GDM group than in NGT subjects (26 vs. 13%; $P = 0.036$), but when corrected for the number of genotypes tested, this difference was no longer statistically significant (Table 2). The prevalence of the HLA-DQB1 risk genotype among 44 GDM women without a family history of diabetes was similar to the prevalence in women with GDM and a family history of diabetes (19 of 43 [44%]). Of these 44 women, 2 (4.5%) were GAD antibody-positive. There was no difference in DQB1 risk genotype fre-

Table 2—HLA-DQB1 genotype frequency in patients with GDM, type 2 diabetes, and control subjects with NGT

	GDM	Type 2	NGT
n	65	82	86
HLA DQB1 genotype			
Risk	29 (45)	37 (45)	27 (31)
02/0302	4 (6)	3 (4)	4 (5)
0302/X	8 (12)	19 (23)	12 (14)
02/X	17 (26)*	15 (18)	11 (13)
Protective 0602(3)/X	11 (17)	16 (20)	22 (16)

Data are n (%). X, either a homozygous allele or any allele other than 02, 0302, or 0602(3). **P* = 0.036 compared with NGT group.

quency between patients who developed diabetes or IGT (8 of 18 [44%]) and those who maintained NGT 1 year postpartum (12 of 27 [44%]).

CONCLUSIONS— Because MODY5 is rare in Scandinavia and usually associated with severe cystic kidney disease (18), we screened only MODY1–4 genes for mutations, which resulted in the identification of mutations in 3 of 66 women, i.e., one MODY2, one MODY3, and one MODY4. Previous studies indicate that women with MODY mutations often present with GDM (12–15,19). It is important to identify these women because they have a predictable clinical course, and the autosomal-dominant inheritance means that their children have a 50% risk of being affected. All amino acids at codons 303 (Arg) of GCK, 203 (Arg) of HNF-1 α , and 239 (Pro) of IPF1 are conserved between the human, mouse, and rat protein sequences. The A203H of HNF-1 α has been associated with MODY in one previously described Japanese family (11). Expression of the IPF-1 variant (P239Q) in Nes2y cells showed a ~50% reduction in their ability to activate insulin gene transcription compared with wild-type IPF-1, and carriers of these variants also had a reduced insulin/glucose ratio during the OGTT (20). It is possible that the IPF-1 and GCK variants are less diabetogenic than the HNF-1 α variant, because the woman with the IPF-1 variant maintained NGT 1 year postpartum, and the sister to the woman with the MODY2 mutation developed GDM, despite being negative for the GCK mutation.

A number of studies have addressed the presence of immunological predictive markers of type 1 diabetes, such as islet cell antibodies, insulin autoantibodies, and GAD autoantibodies in women with

GDM. Recent studies applying more specific and sensitive assays suggest a low prevalence of such markers in GDM (21). We found that 3% of our women with GDM were GAD antibody-positive, which was not different when compared with control subjects. Similarly, a Danish follow-up study found that 2.2% of women with previous GDM were GAD antibody-positive (22).

Women with GDM have an increased lifetime risk of developing manifest diabetes (9,10). However, the prevalence figures differ depending on differences in diagnostic criteria and study populations. In a population study from southern Sweden, using the same diagnostic criteria as in the present study (5), 9% of the GDM women had manifest diabetes and 22% were IGT at 1 year postpartum (23). The figures were even higher in the present study group, with 41% having abnormal glucose tolerance at 1 year follow-up. This discrepancy could be explained by differences in selection criteria because all women in the present study had a family history of diabetes.

Two Danish follow-up studies of women with previous GDM found that women who stayed glucose-tolerant or developed type 2 diabetes had the same frequency of HLA-DR antigens as found in the background population, whereas women who developed type 1 diabetes had a pattern similar to that of other Danish type 1 diabetic patients (24,25). It is generally believed that patients with type 2 diabetes do not have specific HLA types (21). We found no increase in high-risk HLA alleles or GAD antibodies in women who had manifest diabetes or IGT at 1 year postpartum. This is in keeping with the findings that most women with previous GDM develop type 2 rather than type 1 diabetes (24).

Taken together, ~5% of the Swedish GDM subjects with a family history of diabetes carried a MODY mutation. Because the prevalence of GAD antibody-positive and HLA risk genotypes in the GDM subjects did not differ from that in the control subjects, autoimmunity is not likely to play a major role in the development of GDM in these women.

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References

- Gabbe SG: Gestational diabetes mellitus. *N Engl J Med* 315:1025–1026, 1986
- Guttorm E: Practical screening for diabetes mellitus in pregnant women. *Acta Endocrinol Suppl (Copenh)* 182:11–24, 1974
- World Health Organization Ad Hoc Diabetes Reporting Group: Diabetes and impaired glucose tolerance in women aged 20–39 years. *World Health Stat Q* 45:321–327, 1992
- Tuomilehto J, Zimmet P, Mackay IR, Koskela P, Vidgren G, Toivanen L, Tuomilehto-Wolf E, Kohtamäki K, Stengård J, Rowley MJ: Antibodies to glutamic acid decarboxylase as predictors of insulin-dependent diabetes mellitus before clinical onset of disease. *Lancet* 343:1383–1385, 1994
- Åberg A, Rydström H, Frid A: Impaired glucose tolerance associated with adverse pregnancy outcome: a population-based study in southern Sweden. *Am J Obstet Gynecol* 184:77–83, 2001
- Martin AO, Simpson JL, Ober C, Freinkel N: Frequency of diabetes mellitus in mothers of probands with gestational diabetes: possible maternal influence on the predisposition to gestational diabetes. *Am J Obstet Gynecol* 151:471–475, 1985
- Karter AJ, Rowell SE, Ackerson LM, Mitchell BD, Ferrara A, Selby JV, Newman B: Excess maternal transmission of type 2 diabetes: the Northern California Kaiser

- Permanente Diabetes Registry. *Diabetes Care* 22:938–943, 1999
8. Pettitt DJ, Aleck KA, Baird HR, Carraher MJ, Bennett PH, Knowler WC: Congenital susceptibility to NIDDM: role of intra-uterine environment. *Diabetes* 37:622–628, 1988
 9. Dornhorst A, Bailey PC, Anyaoku V, Elkeles RS, Johnston DG, Beard RW: Abnormalities of glucose tolerance following gestational diabetes. *Q J Med* 77:1219–1228, 1990
 10. Damm P, Kuhl C, Bertelsen A, Molsted-Pedersen L: Predictive factors for the development of diabetes in women with previous gestational diabetes mellitus. *Am J Obstet Gynecol* 167:607–616, 1992
 11. Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN: Mutation in hepatocyte nuclear factor-1 gene (TCF2) associated with MODY. *Nat Genet* 17:384–385, 1997
 12. Stoffel M, Bell KL, Blackburn CL, Powell KL, Seo TS, Takeda J, Vionnet N, Xiang KS, Gidh-Jain M, Pillis SJ, Ober C, Bell GI: Identification of glucokinase mutations in subjects with gestational diabetes mellitus. *Diabetes* 42:937–940, 1993
 13. Zouali H, Vaxillaire M, Lesage S, Sun F, Velho G, Vionnet N, Chiu K, Passa P, Permutt A, Demenais F, Cohen D, Beckman JS, Froguel P: Linkage analysis and molecular scanning of glucokinase gene in NIDDM families. *Diabetes* 42:1238–1245, 1993
 14. Saker PJ, Hattersley AT, Barrow B, Hammersley MS, McLellan JA, Lo Olds RJ, Gillmer MD, Holman RR, Turner RC: High prevalence of a missense mutation of the glucokinase gene gestational diabetic patients due to a founder-effect in a local population. *Diabetologia* 39:1325–1328, 1996
 15. Lehto M, Tuomi T, Mahtani MM, Widen E, Forsblom C, Sarelin L, Gullstrom M, Isomaa B, Lehtovirta M, Hyrkko A, Kaninen T, Orho M, Manley S, Turner RC, Brettin T, Kirby A, Thomas J, Duyk G, Lander E, Taskinen MR, Groop LC: Characterization of MODY3 phenotype: early-onset diabetes caused by an insulin secretion defect. *J Clin Invest* 99:582–590, 1997
 16. Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A, Nissen M, Ehrnstrom BO, Forsen B, Snickars B, Lahti K, Forsblom C, Saloranta C, Taskinen MR, Groop LC: Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. *Diabetes* 48:150–157, 1999
 17. Lehto M, Wipemo C, Ivarsson SA Lindgren C, Lipsanen-Nyman M, Weng J, Wibell L, Widen E, Tuomi T, Groop LC: High frequency of mutations in MODY and mitochondrial genes in Scandinavian patients with familial early-onset diabetes. *Diabetologia* 42:1131–1137, 1999
 18. Weng JP, Lehto M, Forsblom C, Huang X, Li H, Groop LC: Hepatocyte nuclear factor-1 β (MODY5) gene mutations in Scandinavian families with early-onset diabetes or kidney disease or both. *Diabetologia* 43:131–132, 2000
 19. Ellard S, Beards F, Allen LIS, Shepherd M, Ballantyne E, Harvey R, Hattersley AT: A high prevalence of glucokinase mutations in gestational diabetic subjects selected by clinical criteria. *Diabetologia* 43:250–253, 2000
 20. Weng JP, Macfarlane WM, Lehto M, Gu HF, Ivarsson SA, Wibell L, Smith T, Groop LC: Functional consequences of mutations in the MODY4 gene (IPF1) and coexistence with MODY3 mutations. *Diabetologia* 44:249–258, 2001
 21. Kühl C: Etiology and pathogenesis of gestational diabetes (Review). *Diabetes Care* 21 (Suppl. 2):B19–B26, 1998
 22. Petersen JS, Dyrberg T, Damm P, Kühl C, Molsted-Pedersen L, Buschard K: GAD65 autoantibodies in women with gestational or insulin dependent diabetes mellitus diagnosed during pregnancy. *Diabetologia* 39:1329–1333, 1996
 23. Åberg A: *Gestational diabetes, screening, diagnosis and prognosis*. PhD thesis. Lund, Sweden, University of Lund, 2001
 24. Damm P, Kuhl C, Buschard K, Jakobsen BK, Svejgaard A, Sodoyez-Goffaux F, Shattock M, Bottazzo GF, Mølsted-Pedersen L: Prevalence and predictive value of islet cell antibodies and insulin autoantibodies in women with gestational diabetes. *Diabet Med* 11:558–563, 1994
 25. Møller-Jensen B, Buschard K, Buch I, Mølsted-Pedersen L, Kühl C, Jakobsen BK, Svejgaard A: HLA association in insulin dependent diabetes mellitus diagnosed during pregnancy. *Acta Endocrinol* 116:387–389, 1987