Increased C282Y Heterozygosity in Gestational Diabetes

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Key Words
Gestational diabetes mellitus • Hemochromatosis gene • C282Y • H63D

Abstract
Background: Hereditary hemochromatosis is an autosomal recessive disorder of iron metabolism that is characterized by excess accumulation of iron in various organs and often leads to diabetes mellitus (DM). To study whether mutations in the hemochromatosis gene (HFE) could be a risk factor for the development of gestational diabetes mellitus (GDM), the prevalence of HFE mutations in patients with GDM was compared to that of healthy pregnant controls.

Methods: GDM was diagnosed in 208 of 2,421 pregnant women screened between the 24th and 28th week of gestation over a period of 18 months. Patients and 170 matched control subjects were screened for the HFE gene mutations C282Y and H63D.

Results: In North and Central European GDM patients, the allele frequency of the C282Y mutation (7.7%) was higher than in pregnant controls (2.9%; p = 0.04), while the frequency of the H63D mutation was not different (p = 0.45). Three patients with GDM were homozygous for H63D (3.1%), 1 patient was homozygous for C282Y (1.0%), 2 patients were compound heterozygous (2.0%) and 26 were heterozygous [11 C282Y (11.2%) and 15 H63D (15.3%)]. C282Y and H63D allele frequencies were not different between controls and GDM patients of Southern European or non-European origin. Irrespective of the HFE-mutation status, serum ferritin levels were increased in patients with GDM compared to healthy pregnant controls (p = 0.01), while transferrin saturation was similar in both groups.

Conclusions: In North and Central European patients with GDM, the C282Y allele frequency is higher than in healthy pregnant women, suggesting a genetic susceptibility to the development of GDM.

Introduction
Gestational diabetes mellitus (GDM) is defined as the appearance of hyperglycemia in a pregnant woman not previously known to be diabetic. Risk factors for the development of GDM include obesity, diabetes in first-degree relatives, a history of impaired glucose tolerance (IGT) and previous infants with macrosomia [1]. GDM is associated with a higher risk of subsequent development of maternal diabetes later in life [2–4].

The pre-diabetic state of GDM is characterized by impaired insulin secretion and insulin resistance [5]. Such abnormalities may be at least partly attributed to iron.
overload [6] in subjects with mutations in the hemochromatosis gene (HFE) and thus may increase their risk to develop GDM. The most common condition of primary iron overload is HFE-associated hereditary hemochromatosis (HH). Among Caucasians, the C282Y point mutation is the most common mutation [7], with a carrier frequency of 1:8 to 1:10 in persons of Northern-Western or Central European origin [8]. In contrast, the H63D mutation has a carrier frequency of up to 1:2 [9] and is found worldwide. The clinical relevance of this mutation and certain other rare mutations (S65C and Dooley) [10–13] is not fully understood. The primary defect of HH is the failure to regulate intestinal iron absorption. Increased iron uptake results in the deposition of iron in various organs including the pancreas. The clinical course of HH is highly variable, only about 50% of homozygotes will develop a symptomatic disease [14] that includes diabetes mellitus (DM) [15–17]. The risk factors associated with the occurrence of symptomatic HH are largely unknown. For liver disease, alcohol abuse and chronic viral hepatitis are well-known risk factors [18, 19].

To explore whether the HFE gene could be a risk factor for development of GDM, the prevalence of the two most common mutations, C282Y and H63D, was determined in a large cohort of pregnant women diagnosed with GDM and compared to that of healthy pregnant controls.

Subjects and Methods

All 2,421 pregnant women treated at this hospital from October 1999 to March 2001 were referred to our outpatient service for screening of GDM between the 24th and 28th gestational week. All women were tested for IGT by a 75 g oral glucose tolerance test (OGTT) according to the criteria of Weiss [20]. GDM was diagnosed in 208 patients (mean age: 30.2 ± 1.01, range: 17–41), of whom 26 (12.6%) had a family history of diabetes. All GDM patients and 170 consecutive pregnant women with normal fasting blood glucose concentration and normal OGTT (mean age: 29.16 ± 0.43; range: 18–43) were tested for HFE mutations. The later group served as non-diabetic controls. Control subjects were also screened with 75 g OGTT between the 24th and 28th gestational week in order to prove their ‘normal glucose tolerance’. The conventional risk factors for GDM (obesity, previous gestational diabetes or glucose intolerance, family history of diabetes, previous macrosomic infants, stillbirth or malformation and age older than 25) were not significantly different in GDM patients and controls.

Ninety-eight (47.1%) and 102 (60%) GDM patients and controls, respectively, were born in Northern or Central Europe; 96 (46.2%) and 62 (36.5%) in Mediterranean countries and 14 (6.7%) and 6 (3.5%) were of non-European origin (China, India, Philippines, Korea, and Egypt).

Laboratory Determinations

OGTT

All pregnant women, GDM and controls, between the 24th and 28th gestational week were tested for IGT by a 75 g oral OGTT. Seventy-five grams of glucose was administered orally in the morning after an overnight fast of at least 12 h. Glucose was measured in venous whole blood in the fasted state and at 60 min after the oral intake of glucose. The test was considered abnormal if the venous plasma glucose values exceeded ≥160 mg/dl or 8.9 mmol/l at 60 min [20].

A follow-up OGTT was carried out in GDM-diagnosed patients 3 months after delivery. Patients were asked to follow a normal diet 3 days before the test and to abstain from smoking on the morning of the test. The OGTT was considered abnormal if the venous plasma glucose values exceeded ≥140 mg/dl or 7.8 mmol/l after 2 h. Blood glucose concentrations between ≥140 mg/dl (7.8 mmol/l) and <200 mg/dl (11.1 mmol/l) were defined as IGT, while blood glucose concentrations ≥200 mg/dl (11.1 mmol/l) were considered as diagnostic for DM [21].

Iron Status

Iron status was evaluated by measuring serum iron concentrations (by the ferrozine method; normal range: 40–150 µg/dl), serum transferrin [by nephelometry (BNA; Behring, Marburg, Germany); normal range: 1.57–3.52 g/l] and serum ferritin [by turbidimetry on a BM-Hitachi automatic analyzer (Boehringer Mannheim, Germany); normal range: 10–150 µg/l]. Transferrin saturation was calculated using the equation: serum iron × 0.709/serum transferrin (normal range: 16–45%).

Gene Analysis

HFE gene analysis was performed in both patients and controls as described earlier [22]. Essentially, PCR-amplified DNA obtained from peripheral blood mononuclear cells was digested with the restriction enzymes SnapI (for detection of C282Y) and Bcl-I (for detection of H63D), and analyzed by PAGE.

Statistical Analysis

For statistical analysis, an unpaired two-sided Student’s t test or Fisher’s exact test was used. Statistical significance was considered at p < 0.05. All data are presented as mean ± standard error of the means.

Results

HFE Frequency in North and Central Europe

The prevalence of GDM reported in this study (8.6%) is comparable to that observed in another large pregnancy cohort, where the prevalence of GDM was calculated to be 8% [23]. Since the C282Y mutation of the HFE gene is common in subjects of Celtic origin (West, North, and Central Europeans) but rare in Mediterranean and non-European subjects, patients with GDM and healthy pregnant controls were divided into two groups according to their country of origin. The C282Y allele frequency in patients with GDM of Northern and Central European
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Origin was higher than in healthy pregnant controls (p = 0.04) from that region, while the allele frequency for H63D was not different. The distribution of HFE gene mutations is presented in Table 1.

**HFE Frequency in Mediterranean or Non-European Countries**

Within the group from Mediterranean or non-European countries, there was no difference in the allele frequencies for C282Y and H63D between GDM and healthy pregnant women (Table 1). Furthermore, none of the patients were homozygotes or compound heterozygotes for C282Y and H63D and there was no difference in the distribution of HFE mutations between primipara and pluripara patients with GDM.

One 35-year-old patient with good clinical health and normal liver function tests was found to be C282Y homozygous. The patient’s serum ferritin concentration was 288 μg/l and the transferrin saturation index was 33.1%. Screening of her parents revealed that the mother was also homozygote for C282Y and suffered from DM with severe nephropathy, while the father was a C282Y/H63D compound heterozygote with a history of liver damage of unknown origin.

One hundred and fifty-one patients (72.6%) returned for a follow-up visit 3 months after delivery. An OGTT revealed that one C282Y heterozygote was diabetic, while none of the other patients or controls had evidence of IGT.

**Iron Status**

The results of serum ferritin levels are shown in Table 2. Serum ferritin levels were higher in patients with GDM than in healthy pregnant women (p = 0.01), although only 3 patients with GDM (one was a C282Y homozygote) had elevated plasma ferritin values (>150 mg). Despite the observation of a significant increase of serum ferritin concentration, no correlation between ferritin concentration or transferrin saturation and the C282Y heterozygosity was detected.

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<tr>
<th>Table 1. Distribution of HFE mutations in GDM patients and in healthy pregnant women according to their countries of origin (patients and controls from North/Central European and Mediterranean countries)</th>
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<td>Country of origin</td>
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<tr>
<td>Mutation status</td>
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<tr>
<td>Wt/wt</td>
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<td>H63D/wt</td>
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<td>C282Y allele frequency</td>
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<td>H63D allele frequency</td>
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Wt/wt = Homozygous for wild type; H63D/wt = heterozygous for H63D; C282Y/wt = heterozygous for C282Y; C282Y/H63D = compound heterozygous; C282Y/C282Y = homozygous for C282Y; H63D/H63D = homozygous for H63D; GDM = gestational diabetes mellitus.

* p = 0.04; ** = from ref. 22.

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<th>Table 2. Serum ferritin concentration in GDM patients and healthy pregnant women (normal range: 10–150)</th>
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<td>Serum ferritin n (%)</td>
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<td>GDM (n = 208)</td>
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<td>Controls (n = 170)</td>
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<td>p value</td>
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n = Number of subjects; GDM = gestational diabetes mellitus.
Discussion

The pathogenesis of DM in general, and of GDM in particular, is multifactorial. GDM offers a unique possibility to study the role of HFE gene mutations for the occurrence of DM in previously healthy subjects exposed to the metabolic stress of normal pregnancy. To our knowledge, this is the first report on the role of HFE gene mutations in patients with GDM. In the present study, heterozygosity for HFE mutations appears to play a role in the development of GDM; at least in patients born in Northern or Central Europe, since the C282Y allele frequency was significantly higher in GDM (7.7%) than in healthy pregnant controls (2.9%) (p = 0.04). One patient was a homozygous carrier of the C282Y mutation; 2 were compound heterozygotes. A follow-up screening 3 months after delivery revealed that only 1 patient had postpartum DM. Although this patient was heterozygous for C282Y, the risk of C282Y carriers in the development of postpartum diabetes could not be assessed due to the small number of patients examined.

The C282Y allele frequency in the group of ‘healthy’ pregnant controls (2.9%) in this study was lower than in healthy subjects (blood donors or healthy nurses) who were assessed in our previous studies (4.9 and 5.1%) [22, 24], with the same genetic background. We cannot offer a clear explanation for this paradox. A direct selection or a referral bias appears to be unlikely, since all pregnant women treated in this hospital are routinely referred for diabetes screening. In general, 28.3% of all pregnant women of this area are referred to this Ob. Gyn. Dept. by their obstetricians for delivery. The Wilhelminen hospital is a district hospital which serves an area with 210,000 people. However, we cannot exclude that women with anemia are more frequently sent to the hospital because they have a higher incidence of pregnancy-related complaints (i.e. tiredness) or complications. Assuming that the higher tissue iron levels in HFE mutation carriers [24] contribute to the manifestation of GDM, a lower frequency for GDM is expected in women with iron deficiency. In the reproductive age, C282Y heterozygous women have increased levels of hemoglobin and serum iron [24] suggesting that the C282Y mutation protects against iron deficiency and iron deficiency anemia. Therefore in contrast to women without these gene mutations, a protection against iron deficiency and iron deficiency anemia is observed in women who carry the C282Y mutation. The most likely explanation is that the majority of C282Y carriers will develop GDM. Thus, non-GDM pregnant women are expected to have a lower mutation-carrier rate. The C282Y allele frequency in the whole group of 200 Northern/Central European patients (98 with GDM and 102 without) would be 5.25%, which is not different to the frequency in healthy control populations. The validity of this assumption should be studied prospectively, and if confirmed, determination of the transferrin saturation index should be checked in all women prior to a planned pregnancy.

Since the allele frequency of the C282Y mutation is much lower in South and Southeastern European and non-European populations [25–28] than in populations of Celtic origin, (such as in the alpine regions of Austria) the role of the C282Y mutation cannot be assessed in patients with GDM originating from these parts of the world. In contrast to the C282Y mutation, the H63D mutation is rarely associated with severe iron overload and thus does not appear to increase the risk of GDM or liver disease.

Unfortunately, common indices of iron overload failed to identify patients at risk of developing GDM. Overall, serum ferritin concentrations were higher in patients with GDM compared to nondiabetic healthy pregnant controls. However, neither serum ferritin nor the transferrin saturation index, correlated with the presence or absence of HFE mutations. Interestingly, high serum ferritin levels do not necessarily reflect high iron concentrations in tissues. Hyperferritinemia has been reported in many [29, 30], but not all [31, 32], patients with newly diagnosed DM. Three possible explanations may account for the raised serum ferritin concentrations in diabetic patients. First, ferritin may be a sign of iron overload that results in the occurrence of DM [33, 34]. Second, increased levels of ferritin may reflect the release of iron from damaged tissues in acute or chronic inflammation [29] or chronic liver disease [34–36] as a consequence of DM. Ferritin is an acute phase reactant and may be increased in diabetic women due to urinary tract infections [37], eclampsia [38] or preterm delivery [39, 40]. However, none of the investigated GDM patients had evidence of any infection at the time of testing. Finally, hyperferritinemia contributes to the development or progression of nonalcoholic steatohepatitis, a condition that is frequently associated with DM [41]. The delayed clearance of glycosylated ferritin in diabetic patients [42] may result in higher ferritin levels. The impact of raised ferritin levels on GDM is presently unknown, but it was observed that phlebotomy improves peripheral insulin sensitivity and resistance in DM [6].

In summary, compared to healthy pregnant women from Northern and Central Europe the C282Y allele fre-
quency is higher in GDM patients. These data therefore suggest that mutations in HFE gene contribute towards the development of GDM. C282Y mutation carriers should be closely followed throughout pregnancy to detect and to treat GDM as soon as possible. The exact role of the iron metabolism in the development of GDM requires further investigation.

References


