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Ekelund, Magnus

2010

[Link to publication](#)

Citation for published version (APA):

Ekelund, M. (2010). *Genetic and Metabolic Markers for the Development of Diabetes after Gestational Diabetes Mellitus*. [Doctoral Thesis (compilation), Translational Muscle Research]. Department of Clinical Sciences, Lund University.

Total number of authors:

1

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Genetic and Metabolic Markers for the Development of Diabetes after Gestational Diabetes Mellitus

Doctoral Thesis

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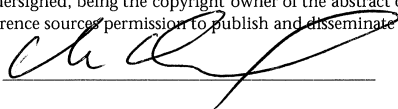
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Organization LUND UNIVERSITY Faculty of Medicine Department of Clinical Sciences Diabetes and Endocrinology Skåne University Hospital, Malmö	Document name DOCTORAL DISSERTATION	
	Date of issue May 20, 2010	
Author(s) Magnus Ekelund	Sponsoring organization	
Title and subtitle Genetic and Metabolic Markers for the Development of Diabetes after Gestational Diabetes Mellitus		
Abstract The aims of this work were to determine the prevalence of postpartum diabetes among women with gestational diabetes mellitus (GDM) in a prospective study 5 years postpartum; to characterize these women according to insulin secretion, insulin sensitivity, autoimmunity and genetics; and to identify possible markers and predictors for the development of manifest diabetes. The subjects were recruited through a regional screening programme in which oral glucose tolerance tests (OGTTs) are routinely offered to all pregnant women. The prevalence of MODY mutations among women with GDM and a family history of diabetes was found to be 5%. Arabian women were more insulin resistant than Scandinavian women with the same BMI, and showed impaired beta cell compensation for their degree of insulin resistance. GDM might share some genetic features with type 1 diabetes in Scandinavian women. The prevalence of manifest diabetes 5 years after GDM was 30% in our population. Antenatal levels of fasting glucose and HbA1c were identified as biochemical predictors of diabetes following GDM. These are easy to measure, and are relatively cheap, and could help identify women at particularly high risk of developing diabetes postpartum. Counselling regarding future risk of diabetes could then be initiated during pregnancy in high-risk individuals. The rs8050136 variant in the FTO (fat mass and obesity associated) gene was associated with an increased risk of developing postpartum diabetes, probably due to its effect on increasing obesity . Thus, genetic testing may provide a means of identifying pregnant women at high risk of developing postpartum diabetes.		
Key words: Gestational diabetes mellitus, Insulin secretion, Insulin sensitivity, Autoimmunity, Genetics, Oral glucose tolerance tests, HbA1c, Fasting glucose, Predictors, FTO-gene, Postpartum diabetes		
Classification system and/or index terms (if any):		
Supplementary bibliographical information:		Language English
ISSN and key title: 1652-8220		ISBN 978-91-86443-72-6
Recipient's notes	Number of pages 125	Price
	Security classification	

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*To Charlotte,
Caroline and Sophie*

ACKNOWLEDGEMENTS

I would like to thank everyone who has helped me by contributing to this work or by providing much-appreciated support in other ways. I would especially like to thank the following people:

Docent Kerstin Berntorp, my supervisor, for all her time, effort, advice and enthusiasm.

Professor Leif Groop, my assistant supervisor, for all his support and constructive criticism.

Dr Nael Shaat and Dr Jianping Weng, my co-authors and close collaborators, for their help and support, especially with the genetic aspects of the studies.

Peter Almgren for helping me with the statistics.

My other co-authors: Eva Anderberg, Dr Göran Ekberg, Dr Anders Frid, Professor Sten Ivarsson, Professor Mona Landin-Olsson, Dr Markku Lehto, Professor Åke Lernmark, Dr Haiyan Li, Docent Valeriya Lyssenko, Dr Anna Nilsson, Roland Perfekt and Dr Anders Åberg.

Docent Per Katzman and Docent Thomas Kjellström, my mentors, for their encouragement and inspiration, and for introducing me to this field of research.

Vera Gunnarsson and Ylva Wessman for technical assistance and help.

Docent Olle Melander and Docent Pål Wölner-Hansen for valuable comments.

My colleagues in Helsingborg: Dr Johan Kalén, Dr Peter Kalén, Dr Jerker Nederman, Dr Helene Nilsson and Dr Henrik Olsen for their friendship, support and understanding.

All other colleagues, past and present, in Helsingborg and Malmö for their friendship and support.

And last, but not least, my family: Charlotte, Caroline and Sophie.

This research was financed by grants from Thelma Zoega's Foundation, Stig and Ragna Gorthon's Foundation and the County of Skåne.

PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals. The papers are appended at the end of the thesis.

- I. *Screening for MODY mutations, GAD antibodies, and type 1 diabetes-associated HLA genotypes in women with gestational diabetes mellitus*
J Weng*, M Ekelund*, M Lehto, H Li, G Ekberg, A Frid, A Åberg, LC Groop, K Berntorp
Diabetes Care: Jan;25(1):68-71 (2002)
*These authors contributed equally.
- II. *Genotypic and phenotypic differences between Arabian and Scandinavian women with gestational diabetes mellitus*
N Shaat, M Ekelund, Å Lernmark, S Ivarsson, A Nilsson, R Perfekt, K Berntorp, L Groop
Diabetologia: 45:878-884 (2004)
- III. *Prediction of postpartum diabetes in women with gestational diabetes mellitus*
M Ekelund, N Shaat, P Almgren, L Groop, K Berntorp
Diabetologia: 53:452-457 (2010)
- IV. *The FTO rs8050136 variant predicts postpartum diabetes in women with gestational diabetes mellitus*
M Ekelund*, N Shaat*, P Almgren, E Anderberg, M Landin-Olsson, V Lyssenko, L Groop, K Berntorp
Manuscript
*These authors contributed equally.

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ABBREVIATIONS

BMI	Body mass index
<i>CDKN2A/2B</i>	Cyclin-dependent kinase inhibitor-2A and -2B gene
CI	Confidence interval
CRP	C-reactive protein
CV	Coefficient of variation
DM	Diabetes mellitus
EASD	European Association for the Study of Diabetes
<i>FTO</i>	Fat mass and obesity associated gene
GAD65Abs	Glutamic acid decarboxylase 65 antibodies
GCK	Glucokinase
GDM	Gestational diabetes mellitus
GIGT	Gestational impaired glucose tolerance
GNGT	Gestational normal glucose tolerance
HLA	Human leukocyte antigen
<i>HNFI1A</i>	Hepatocyte nuclear factor 1 homeobox A gene
HOMA	Homeostasis model assessment
HOMA-IR	Homeostasis model assessment of insulin resistance
HR	Hazard ratio
IA-2Abs	Tyrosine phosphatase antibodies
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
<i>INS VNTR</i>	Insulin gene, variable number of tandem repeats
<i>IPF1</i>	Insulin promoter factor 1 gene
<i>KCJN11</i>	Potassium inwardly rectifying channel, subfamily J, member 11 gene
MODY	Maturity onset diabetes of the young
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
OR	Odds ratio
P _c	Corrected p-value

<i>PPARG</i>	Peroxisome proliferator activated receptor, gamma 2 gene
SD	Standard deviation
SEM	Standard error of the mean
SNP	Single nucleotide polymorphism
<i>TCF7L2</i>	Transcription factor 7-like 2 gene

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POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Graviditetsdiabetes (GDM) definieras som ett diabetestillstånd som upptäcks under graviditet. Ofta orsakas detta tillstånd av de hormonförändringar som uppträder under graviditet vilka medför en ökad insulinresistens, dvs. ett ökat motstånd mot insulinets effekter. Diagnosen GDM utesluter dock inte diabetes av annan orsak som upptäcks under graviditet.

Efter förlossning försvinner oftast GDM eftersom hormonförändringarna då går tillbaka. Det är dock ett välkänt faktum att GDM innebär en kraftigt ökad risk för kvinnan att senare i livet drabbas av typ 2 diabetes, s.k. vuxendiabetes. Denna risk uppgick i vår studie till 30% inom 5 år. GDM medför också risker för barnet. Obehandlad GDM eller annan diabetes med högt blodsocker hos modern, ger i sin tur högt blodsocker hos fostret. Detta medför en ökad insulininsöndring hos fostret vilket ger risk för alltför kraftig fostertillväxt och förlossningskomplikationer. Det är också visat att GDM för barnets del medför risk för försämrad utveckling under uppväxten.

GDM och typ 2 diabetes har ofta likartade uppkomstmekanismer, dvs. insulinresistens och bristande insulinproduktion. Släktskapet mellan GDM och typ 2 diabetes är därför tydligt. Maturity Onset Diabetes of the Young (MODY) betecknar andra diabetestillstånd som är monogent nedärvda, har stark ärftlighet och ofta debut före 25 års ålder. I vår studie påvisades MODY-mutationer hos 5% av GDM kvinnor med diabetes i släkten.

Arabiska kvinnor med GDM befanns i vår studie ha en högre grad av insulinresistens och en lägre grad av kompensatorisk ökning av insulinproduktionen jämfört med skandinaviska kvinnor med GDM, vilka å andra sidan uppvisade vissa drag som är gemensamma med typ 1 diabetes, s.k. ungdomsdiabetes.

Eftersom GDM medför risk för framtida diabetes, särskilt typ 2 diabetes, hos drabbade kvinnor vore det önskvärt att finna markörer för framtida diabetesutveckling för att kunna ge individuella råd angående grad av risk och rekommendationer om livsstil. Vår studie visade att högt fasteblodglukos under graviditet, komplicerad av GDM, är förenat med ökad risk för framtida diabetes,

vilket andra grupper tidigare också har visat. Vad som däremot var ett nytt fynd i vår studie var att HbA_{1c}, glykosylerat hemoglobin, som används som ett mått på långtidsblodsockret, kan användas på likartat vis. Både fasteblodglukos och HbA_{1c} är lättillgängliga och relativt billiga test, som således skulle kunna tjäna som markörer för framtida utveckling av manifest diabetes efter GDM.

FTO-genen har tidigare visats ha samband med typ 2 diabetes. Vår avslutande studie påvisade en association mellan varianter i *FTO*-genen och utveckling av diabetes efter GDM, sannolikt beroende på *FTO*-genens effekt att ge ökad övervikt. Genetisk testning kan alltså komma att bli ett sätt att identifiera kvinnor med särskilt hög risk att utveckla diabetes efter GDM.

1. BACKGROUND AND DEFINITION OF GDM

1.1 History

The first recorded case of gestational diabetes mellitus (GDM) was described in 1823 by the German physician Heinrich Bennewitz, who presented his thesis, “*De Diabete Mellito Graviditatis Symptomate*”, in Berlin the following year. He described symptoms of diabetes, i.e. thirst and polyuria, in a pregnant woman. These symptoms disappeared after pregnancy, and Dr Bennewitz therefore believed that her diabetes was actually the result of pregnancy [1].

Increased risk of foetal death in pregnancies complicated by diabetes was first reported in 1882 [2], but the concept of GDM as a prediabetic state was not proposed until the 1940s. Studies during the 1940s also reported significantly increased perinatal mortality in infants born to mothers who subsequently developed diabetes [3-6]. The first prospective study of glucose metabolism during pregnancy was initiated in 1954 in Boston, USA, using an oral glucose tolerance test (OGTT). The glucose concentration in blood was measured 1 hour after the ingestion of 50 g glucose. This test has since been widely adopted in the USA to screen for diabetes during pregnancy [7]. The name gestational diabetes mellitus was introduced by O’Sullivan in 1961 [8].

1.2 Definition of GDM

When we talk about GDM, we actually mean hyperglycaemia due to the pregnancy itself. It is, however, often impossible to know whether the patient already had diabetes before pregnancy or not. For practical reasons, GDM is therefore defined as: “*Carbohydrate intolerance of varying severity with onset or first recognition during pregnancy*” [9]. This definition encompasses pre-existing diabetes revealed during pregnancy, and does not take into account whether hyperglycaemia persists after pregnancy or not [10].

1.3 Prevalence

The prevalence of GDM is about 2% in Sweden [11], but reported values vary between 1 and 17% worldwide, depending on the population studied and the diagnostic tests and criteria that have been used. In certain high-risk Arabian populations, a prevalence of almost 40% has been reported [12-18]. The glucose tolerance of women with GDM usually reverts to normal after delivery. However, GDM often returns in subsequent pregnancies, with recurrence rates between 17 and 70% [12, 19-27]. The considerable differences in prevalence, as well as in recurrence rates may, to some extent, reflect dissimilarities between populations; but are also the result of a lack of consensus regarding screening, i.e. which patients should be screened, and which diagnostic methods and diagnostic criteria should be used.

2. PATHOPHYSIOLOGY

2.1 Insulin resistance

2.1.1 What happens during normal pregnancy?

In early human pregnancy, insulin secretion increases while insulin sensitivity may be unchanged, decreased or increased. Adipose tissue accretion is promoted during this phase. In late gestation, maternal adipose tissue depots decline, while postprandial free fatty acid levels increase and glucose tolerance deteriorates [28].

Kühl studied women without GDM during early, mid- and late pregnancy and also after delivery [29]. He observed no significant changes in the fasting serum glucose concentration during pregnancy, but the fasting serum insulin level gradually increased. The mean glucose concentration following an OGTT was not changed until the second half of pregnancy, when the level was significantly elevated. The serum insulin response to glucose was significantly increased at all stages of gestation, indicating a reduction in insulin sensitivity during pregnancy. These results were supported by the findings reported by Buchanan et al. who also studied women without GDM, and found that their insulin sensitivity was reduced to only one third of that in nonpregnant women matched for age and weight. They also reported that pregnant women showed a threefold increase in first- and second-phase insulin responses compared with nonpregnant women in order to compensate for the increased insulin resistance [30].

Catalano et al. performed prospective longitudinal studies in which a hyper-insulinaemic-euglycaemic clamp model was used to investigate obese and non-obese women with normal glucose tolerance (NGT) before and during planned pregnancies [31-33]. Insulin sensitivity showed a 47% decrease in obese women and a 56% decrease in non-obese women during pregnancy.

The result of these physiological changes is that glucose tolerance gradually deteriorates, even during a pregnancy that is not complicated by GDM [29, 32]. In a review, Catalano concluded that women with NGT show a 30% increase in basal hepatic glucose production, a progressive decrease in insulin sensitivity, and an associated 3.0- to 3.5-fold increase in insulin response by weeks 34 to 36 of pregnancy [34].

2.1.2 Why do these changes occur?

The process of gradually deteriorating glucose tolerance starts during mid-pregnancy, and progresses through the third trimester due to the increased production of several insulin antagonising hormones from the placenta, i.e. human placental growth hormone and human placental lactogen. Corticotropin-releasing hormone is also produced in the placenta, which leads to increased production of cortisol. The increased production of these hormones results in increased insulin resistance at postreceptor level in skeletal muscle [30, 32, 35-41].

2.1.3 What are the special characteristics of GDM?

The traditional, and most often reported, risk factors for GDM are previous delivery of a macrosomic infant, high maternal age, weight and parity, and a family history of diabetes [12]. As mentioned above, insulin resistance increases during pregnancy, even in women who remain normoglycaemic, whereas women who develop GDM also often have a more chronic form of insulin resistance, present already before pregnancy. This insulin resistance deteriorates during pregnancy due to the physiological changes mentioned above [33]. Most women with GDM thus have a combination of acquired and chronic insulin resistance, and are therefore, as a group, slightly more insulin resistant than pregnant women without GDM [39]. Women with a history of GDM also show evidence of subclinical inflammation, with elevated levels of inflammatory markers, e.g. tumour necrosis factor- α . Tumour necrosis factor- α and other proinflammatory mediators suppress the transcription of adiponectin, which is a key insulin sensitizing hormone produced by adipose tissue [42-45]. Subclinical inflammation may thus, in addition to the above mentioned hormonal changes, contribute to the increased insulin resistance in women with GDM [28].

2.2 Insufficient beta cell function

Compared with normoglycaemic women, those with GDM exhibit lower insulin secretion for their degree of insulin resistance, mainly due to impaired early-phase insulin secretion [30, 32, 46-50]. Available evidence suggests that beta cell defects in GDM result from autoimmune disease, monogenic causes and insulin resistance, i.e. the same factors that cause beta cell defects in general [51].

In summary, the cause of GDM is in most cases considered to be inadequate insulin secretion that cannot compensate for the physiologically increased insulin resistance that occurs during pregnancy; commonly associated with chronic insulin resistance, starting during mid-pregnancy and progressing through the third trimester.

3. GENETICS AND GDM

3.1 Introduction

GDM often represents diabetes in evolution, which provides an excellent opportunity to study the pathogenesis of the disease. Most women who develop GDM show signs of beta cell dysfunction related to chronic insulin resistance, but an important minority does not [39]. Some of these women appear to have autoimmune beta cell dysfunction [39]. A pregnancy can also reveal maturity onset diabetes of the young (MODY) [52]. All these, and other cases, are covered by the broad definition of GDM if the diabetic state is first detected during pregnancy [9]. A genetic component in GDM is strongly suggested since women with a family history of diabetes have an increased risk of GDM, and since GDM clusters in families [53-56].

The genetics of GDM have been described in review articles by Watanabe et al. [57] and by Shaat and Groop from our own group who, in their systematic review concluded that GDM shares genetic features with type 1 diabetes, type 2 diabetes and MODY which will be discussed in this chapter [14]. Apart from the factors discussed below, there have been reports indicating possible associations between GDM and a number of other genes including a certain genotype of the insulin gene, variable number of tandem repeats (*INS VNTR*) [58] and the genes encoding: sulphonylurea receptor 1 [59], insulin receptor and insulin-like growth factor 2 [60], leptin [61], visfatin [62, 63], lectin [64], ectonucleotide pyrophosphatase/phosphodiesterase 1 [65], insulin receptor substrate 1 [66-68], glucose transporter 4 [69], adiponectin [42, 70-73], mannose-binding calpain-10 [66, 74], haemochromatosis [75] and beta 3-adrenergic receptor [67, 76-78]. There have also been similar reports regarding GDM and mutations in mitochondrial DNA [79, 80]. Some of these studies have reported contradictory results, and the findings have not always been reproducible.

3.2 Type 1 diabetes genes and GDM

The HLA (Human Leukocyte Antigen) region on chromosome 6 was early identified as a major group of susceptibility genes for type 1 diabetes [81-85]. The possible association between GDM and HLA has also been studied, but with

somewhat contradictory results. Many studies have failed to show increased frequencies of any *HLA II* alleles in GDM patients [86-88]; whereas the *HLA-DR3* and *HLA-DR4* alleles were found more often in GDM patients in another study, but only in a subgroup of black patients [89].

Associations between *HLA-DR3*, *HLA-DR4* and *HLA-DQB1*0302* and auto-antibodies have been demonstrated in GDM women [86, 90]. A study in Skåne the southernmost region of Sweden, showed that GDM women testing positive for at least one autoantibody, had a significantly higher frequency of *HLA-DR3-DQ2/X*, or *DR4*, or *DR4-DQ/X* genotypes compared with healthy controls, whereas antibody negative GDM patients in the same study exhibited an increased frequency of *HLA-DR7-DQ2/X*, *DR9-DQ9/X* and *DR14-DQ5/X* genotypes [91].

Our own group has previously examined 764 women with GDM, negative for glutamic acid decarboxylase 65 antibodies (GAD65Abs), tyrosine phosphatase antibodies (IA-2Abs) and insulin autoantibodies, in a population-based diabetes prediction study in Skåne [92]. The frequency of type 1 diabetes high-risk *HLA-DQ* alleles (*DQB1*0201*, *DQB1*0302*) did not differ between women with GDM and 1191 randomly selected non-diabetic control mothers, also testing negative for these autoantibodies. In contrast, the low-risk *DQB1*0602* allele was less prevalent in women with GDM than in controls. It was therefore suggested that this allele may offer protection not only from type 1 diabetes but also from GDM [92].

3.3 Type 2 diabetes genes and GDM

The mechanisms underlying type 2 diabetes are complex [93], and the traditional genetic detection process employing linkage analyses and candidate gene studies was slow until 2007. Only the three genes peroxisome proliferator activated receptor, gamma 2 (*PPARG*), potassium inwardly rectifying channel, subfamily J, member 11 (*KCJN11*) and transcription factor 7-like 2 (*TCF7L2*) could be consistently associated with type 2 diabetes [94]. The situation then changed dramatically in 2007 with the introduction of whole genome-wide association studies (WGAS), resulting in the detection of many novel genes for type 2 diabetes [95-103]. The transcription factor gene *TCF7L2* was found to be at the top of the list in every WGAS [94]. It is also noteworthy that a common denominator for several of the recently discovered genes, i.e. *KCJN11*, *TCF7L2* and many others, is that many of them seem to be involved in the secretion of insulin [93]. These findings emphasize the importance of beta cell dysfunction in the pathogenesis of type 2 diabetes. Beta cell dysfunction, resulting in an inability

to compensate for the increased insulin demand during pregnancy, is also a distinctive feature of GDM [14]. GDM and type 2 diabetes also share some traditional risk factors, such as age, obesity and high-fat diet [12, 104]. These findings, together with the fact that GDM is a well-known risk factor for developing type 2 diabetes later in life [105], support the theory that in many cases GDM is type 2 diabetes in evolution, and that the two conditions share the same background. In agreement with this, GDM has been shown to be associated with genetic variants connected to type 2 diabetes susceptibility loci. In a study by our group, published in 2005, 588 Scandinavians with GDM and 1,189 pregnant controls without diabetes were examined for polymorphisms in genes that had previously been associated with type 2 diabetes. It was concluded that the E23K polymorphism of *KCNJ11* seemed to predispose Scandinavian women to GDM [66]. However, it has been difficult to replicate many genetic variants associated with type 2 diabetes susceptibility loci in GDM studies, apart from the *TCF7L2* variants, which have been shown to be the genetic variants most strongly associated with GDM in most populations studied so far [106-108]. On the other hand, to the best of the author's knowledge only one study has been conducted to investigate the ability of these genetic variants to predict type 2 diabetes postpartum. This study by Lauenborg et al., showed an association between the variant rs10811661 in the cyclin-dependent kinase inhibitor-2A and -2B (*CDKN2A/2B*) gene and the rs10010131 variant in the Wolfram syndrome 1 (*WFS1*) gene with incident type 2 diabetes up to 2 years postpartum [109].

3.4 MODY and GDM

MODY is the term used to describe monogenic autosomal dominant forms of diabetes with impaired beta cell function presenting usually before the age of 25 years. The genes underlying susceptibility to MODY types 1 to 6 have been identified. All forms of MODY, except MODY 2, are caused by mutations in transcription factors [110-113]. MODY 2 is caused by mutations in the gene encoding the glycolytic enzyme glucokinase (GCK), and leads to mild chronic hyperglycaemia [52, 112, 114, 115]. MODY 2 accounts for approximately 20 to 30% of all MODY subtypes [116, 117]. Beta cell dysfunction makes MODY genes potential candidate genes also for GDM, since both conditions are characterized by impaired beta cell function [111, 112]. Several studies have identified mutations in *GCK* genes in GDM patients, with an estimated prevalence of about 5% in women with GDM [118-120]. Our own group has shown that common variants in MODY genes, i.e. *GCK*- and *HNF1A* (Hepatocyte nuclear factor 1 homeobox A) genes, increase the risk of GDM [121].

4. THE CONSEQUENCES OF GDM

4.1 For the child...

It has long been known that manifest diabetes is associated with significant risks for the foetus. In 1952, Jørgen Pedersen postulated that diabetic foetopathy in a maternal diabetic state is caused by increased foetal insulin secretion, which in turn is caused by foetal hyperglycaemia due to maternal hyperglycaemia [122]. GDM is also associated with increased risk of obstetric complications due to the high birth weight of the infant, with increased risk of complications both at delivery and later [123-126].

Despite the fact that it has long been clear that maternal hyperglycaemia is harmful to the foetus, the maternal glucose level at which the risk of adverse pregnancy outcome increases is still controversial. In a Swedish study conducted in Skåne, the rate of caesarean delivery and infant macrosomia was found to be increased in the group with capillary blood glucose values of >7.8 mmol/l 2 hours after the ingestion of 75 g glucose [127]. The international Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study was initiated in 1999, aimed at determining the degree of glucose intolerance, short of diabetes, that leads to a clinically important risk of adverse perinatal outcome. The study was blinded and 25,505 pregnant women at 15 centres in nine countries underwent 75 g OGTTs. The results showed strong continuous relations between maternal glucose levels, even below those diagnostic of diabetes, and increased birth weight and increased cord-blood serum C-peptide levels [128].

It has thus now been clearly demonstrated that GDM increases the risk to the child, but does treating the mother reduce these risks? In a large randomized Australian study, Crowther et al. showed that treating GDM significantly reduced serious neonatal morbidity compared with routine prenatal care [129]. These results have been supported by a retrospective study by Langer et al., who compared pregnancy outcome between treated and non-treated women with GDM [126]. More recently, in a randomized multicentre study by Landon et al., it was shown that treatment of even milder forms of GDM improved the outcome of pregnancy [130].

4.2 For the mother...

From the mother's point of view, GDM is a well-known risk factor for developing diabetes, especially type 2 diabetes, later in life. The cumulative incidence of diabetes increases markedly within the first five years after delivery, but then appears to plateau [131]. However, the reported prevalence of postpartum diabetes after GDM varies considerably due to differences in diagnostic criteria, follow-up time and the population studied [12, 105, 131-138]. Kjos and Buchanan reported a 17 to 63% risk of type 2 diabetes developing 1 to 16 years after GDM [139]. Löbner et al. found an 8-year postpartum diabetes risk of 52.7%. In women testing positive for GAD65Abs and/or IA-2Abs the risk was 96% by 8 years. Furthermore, the risk also increased in women who required insulin during pregnancy, had a body mass index (BMI) $>30 \text{ kg/m}^2$ or had had more than two prior pregnancies [134].

The progression to diabetes following GDM appears to be even more rapid among ethnic groups with a high background prevalence of type 2 diabetes [13]. The prevalence of the metabolic syndrome has also been shown to be three times higher in women previously treated for GDM through diet, than in age-matched control subjects in a Danish study [140].

According to a systematic review by Golden et al. published in 2009, elevated levels of fasting glucose, 2-hour OGTT glucose and the OGTT glucose area under the curve were predictors of subsequent type 2 diabetes after GDM [141]. The prevalence of type 2 diabetes after GDM has also been described in a recent systematic review and meta-analysis by Bellamy et al., who investigated the development of type 2 diabetes 6 weeks or more after the end of the index pregnancy. After the exclusion of studies without a control group, 20 prospective or retrospective cohort studies from 1960 to 2009 that fulfilled the authors' criteria remained. The meta-analysis showed an at least seven-fold increased risk of developing type 2 diabetes in women with previous GDM, compared with women who had been normoglycaemic during pregnancy. GDM and type 2 diabetes share many risk factors, i.e. family history of diabetes, increased BMI, increased age and Asian or black ethnic origin, suggesting an overlapping cause between GDM and type 2 diabetes [105]. One of the studies included in the meta-analysis was the study conducted by our own group, in which Åberg et al. concluded that maternal age over 40, a high 2-hour OGTT glucose value during pregnancy and insulin treatment during pregnancy predicted impaired glucose tolerance (IGT) or diabetes at follow-up one year after GDM [132].

Progression to diabetes may be even more rapid today than previously, because of the increased prevalence of obesity and physical inactivity. Lauenborg et al.

retrospectively studied risk factors for the incidence of diabetes in 2 cohorts of women in Denmark with diet-treated GDM, who were delivered from 1978 to 1985 and from 1987 to 1996. The incidence of diabetes had more than doubled over ten years, from 18.3% to 40.9%, and seemed to be due to a significant increase in BMI [142].

It has also been shown that among women with a family history of type 2 diabetes, those with prior GDM were even more likely, not only to have risk factors for cardiovascular disease, including metabolic syndrome and type 2 diabetes, but also to have experienced cardiovascular events, which also occurred at a younger age [143].

5. SCREENING AND DIAGNOSIS

5.1 To screen or not to screen

Despite the increasing amount of evidence, from both randomized and retrospective studies, that untreated GDM is associated with risks to the child, and that GDM signifies a future risk of diabetes in the mother, controversy remains regarding screening tests, diagnostic tests and the level of hyperglycaemia that is diagnostic of GDM [144]. Some have recommended universal screening of all pregnant women, while others are of the opinion that, although universal screening maximizes screening sensitivity, it might be less cost-effective than selective screening focusing on women with the most often reported risk factors for GDM, such as older maternal age, family history of diabetes, previous delivery of a macrosomic infant, high prepregnancy weight, high pregnancy weight gain, high parity and high-risk ethnicity [12, 136, 139, 145-147].

5.2 Screening tests

Many of the tests traditionally used for screening for GDM suffer from considerable weaknesses.

- *Anamnestic criteria* have often been used. However, a history of previous GDM, macrosomic infant, stillbirth or diabetes mellitus in the family only reveals some GDM cases [147]. A study by Östlund and Hanson at Uppsala University in Sweden showed that traditional anamnestic risk factors indicating an OGTT identified less than half of the total GDM group. The sensitivity increased when ethnicity was introduced as a risk factor [148].
- *Glucosuria* may occur during pregnancy, even in the absence of diabetes, due to an increase in glomerular filtration rate and a decrease in the tubular reabsorptive capacity for glucose [149].
- *Fasting glucose level* does not detect mild forms of GDM [150]. However, in another Swedish low-risk population, fasting capillary glucose measurements were found to be an acceptable and useful screening test for GDM [151].

- *Repeated random blood glucose measurements* during pregnancy were suggested during the early 1980s as a cheap and efficient screening method for GDM [149, 152]. Stangenberg et al. performed a study in which a blood glucose value ≥ 6.5 mmol/l was used as the selection criterion for OGTT [152]. Based on these results Persson and Hanson initiated routine screening for the detection of GDM in Sweden in the middle of the 1980s, by random measurements of blood glucose levels 4 to 6 times during pregnancy. Later results have also shown that repeated random blood glucose measurements give useful information and also increase the sensitivity of the screening procedure [153, 154].

5.3 OGTT for screening and diagnosis

Diagnostic tests differ from the above mentioned screening tests. GDM is usually diagnosed using some kind of OGTT. O'Sullivan and Mahan proposed the first criteria for the diagnosis of GDM in 1964 [155]. These early criteria have been widely used, especially in the USA, and were later modified by Carpenter and Coustan [156]. The mother's postpartum risk of developing diabetes mellitus was considered the main issue during this period, since at that time it was not obvious that GDM was associated with adverse perinatal events. Evidence for this was found later [124, 125, 127, 145, 157, 158]. The details of the different diagnostic criteria discussed in this section are given in Table 1.

The criteria recommended by the National Diabetes Data Group (NDDG) or the American Diabetes Association (ADA) are most commonly used in the USA today [159, 160]. The ADA recommendations are based on the Carpenter-Coustan 100 g OGTT criteria [160]. The ADA also supports a 75 g OGTT [161], as well as a 50 g OGTT. The 50 g OGTT is, however, only recommended for screening, but is used for this purpose very frequently in the USA. In 1990, more than 75% of all pregnant women in the USA were screened for GDM [162].

The Diabetic Pregnancy Study Group of the European Association for the Study of Diabetes (EASD) previously suggested a 75 g OGTT, but with different diagnostic values from the ADA criteria mentioned above, and the criteria proposed by the World Health Organization (WHO) mentioned below. According to these EASD criteria, GDM is confirmed by a 2-hour capillary blood glucose concentration ≥ 9 mmol/l, which corresponds to the 95th percentile [163]. This criterion was accepted in Sweden and Denmark, but in no other countries [164, 165]. Since this was generally adopted in clinical praxis in Sweden at the time of this study, the

diagnosis of GDM in the present papers is based entirely on a 2-hour capillary blood glucose concentration ≥ 9 mmol/l.

The WHO criteria from 1985 are widely used throughout the world for the diagnosis of GDM [166]. In 1999 the WHO suggested new criteria for the diagnosis of GDM, recommending the diagnostic limit of IGT in nonpregnant women as diagnostic of GDM [167]. These later criteria issued by the WHO mean a significant lowering of the cut-off value for diagnosing GDM compared with many other criteria, which seems appropriate since results, including those of our group, emphasize the significance of even moderately increased 2-hour OGTT blood glucose values (7.8 to 8.9 mmol/l) [127].

Based on the results of the HAPO study mentioned above [168], new recommendations were suggested at the ADA meeting in New Orleans, USA, in June 2009 (Buchanan, Kitzmiller and Metzger). Fasting glucose, 1-hour and 2-hour levels were all considered important. If the fasting glucose level exceeded the limit given in Table 1 the risk of adverse pregnancy outcome is 8%. The risk increases to 14% with 1-hour glucose levels above the limit given, and is 16% if the 2-hour value is also exceeded.

Table 1

Criteria used for screening and diagnosis of GDM. The criteria used throughout the present work are given in column 5, (EASD 1991) and suggestions for new criteria based on the results of the HAPO study are given in the last column.

	ADA 1995-1998		NDDG 1979	WHO 1985	WHO 1999	EASD 1991	New recommendations, ADA, June 2009
	100	75					
OGTT, g glucose		50 ^a	100	75	75	75	75
Overnight fasting	Yes	No	Yes	Yes	Yes	Yes	Yes
Fasting glucose level mmol/l (mg/dl)	≥5.3 (95)	≥5.3 (95)		≥7.8 * (140)			≥5.1
1-hour glucose level mmol/l (mg/dl)	≥10.0 (180)	≥10.0 (180)	≥10.6 (190)		-	-	≥10.0
2-hour glucose level mmol/l (mg/dl)	≥8.6 (155)	≥8.6 (155)	≥9.2 (165)	≥11.1* (200)	≥7.8 (140)	≥9.0 (162)	≥8.5
3-hour glucose level mmol/l (mg/dl)	≥7.8 (140)	-	≥8.1 (145)		-	-	-
Type of sample	Venous plasma	Venous plasma	Venous plasma	Venous plasma	Venous plasma	Capillary blood	Venous plasma

^a For screening purposes only.

* Fasting and/or 2-hour values should be met or exceeded for the diagnosis of GDM, according to the WHO, 1985.

5.4 Screening in Skåne

Physicians in the county of Skåne questioned the screening procedure based on random glucose measurements around 1990. A prerequisite for such a method is that the random measurements are truly random with respect to the intake of food and drink. That would not be the case if the woman, for example, always had her antenatal appointment at the same time of day. Åberg et al. found that infants previously born of women who were subsequently diagnosed as having GDM in a later pregnancy were heavier than the controls. The rate of intrauterine deaths was also significantly higher than in the control group. They concluded that GDM might have been undetected in previous pregnancies [123]. Another possible shortcoming of a screening method based on random glucose measurements is that a pathological random glucose value must be followed by another, diagnostic, test. Many women could be unnecessarily worried by this, since many pathological values obtained with random glucose tests would be followed by a normal OGTT result.

A Danish study has shown that a selective screening method based on pre-screening including an interview to identify clinical risk factors, two fasting capillary blood glucose measurements and a urine test for glucosuria, failed to predict GDM and adverse foetal outcome [169]. The traditional method of screening for GDM, by offering an OGTT only in selected cases, has thus also been questioned in Denmark.

Members of our research group, initiated a screening programme offering direct diagnostic OGTTs to all pregnant women in order to overcome the above mentioned problems. The programme started in 1991 in the district around the Skåne University Hospital in Lund, with a population of approximately 200,000. In 1995, the procedure was extended to the whole county of Skåne, with approximately 1.2 million inhabitants. The OGTT programme is now part of the clinical routine in the official antenatal care programme, with a compliance of more than 95% [170]. Since data at the time the programme started had shown that fasting glucose did not increase during normal pregnancies, the initial fasting glucose measurement has been omitted [11, 171], and a standard 2-hour, 75 g OGTT is performed after an overnight fast.

All pregnant women in Skåne are thus offered this kind of OGTT in week 27 or 28 of their pregnancy. For women with first-degree family history of diabetes, or GDM in previous pregnancies, an OGTT is also performed in weeks 10 to 12 of pregnancy and, if normal, repeated in week 28.

6. AIMS OF THIS WORK

The overall aims of the present work were to determine the prevalence of postpartum diabetes mellitus among GDM women in a prospective study, 5 years postpartum; to characterize these women according to insulin secretion, insulin sensitivity, autoimmunity and genetics; and to identify possible markers and predictors for the development of manifest diabetes.

The specific aims of the individual studies are given below.

- I. To investigate whether genetic susceptibility to type 1 diabetes or MODY increases susceptibility to GDM.
- II. To determine whether autoimmunity and genetic variations affecting insulin secretion and action contribute to the development of GDM, and whether the pathogenesis of GDM differs between women of Scandinavian and Arabian descent.
- III. To study the prevalence of postpartum diabetes 5 years after GDM, and to investigate biochemical and clinical predictors of postpartum diabetes.
- IV. To ascertain whether genetic variants that predispose individuals to type 2 diabetes could predict the development of diabetes in women with GDM after a median follow-up of 59 months postpartum.

7. SUBJECTS AND METHODS

7.1 Subjects

The subjects were recruited from among pregnant women in the region given an OGTT as part of the clinical routine in antenatal care. The OGTT was performed at local antenatal clinics, in week 27 or 28 of pregnancy. If the patient had previously had GDM, or had a first-degree family history of diabetes, the risk of GDM was considered particularly high. These women were therefore offered OGTTs already in weeks 10 to 12 of their pregnancy. If these early OGTTs showed normal values, a second OGTT was offered in week 27 or 28.

The study populations were recruited from the following four groups.

1. Women from the region of Malmö, who were pregnant in 1996 to 1999. Extended evaluation including biochemical and genetic testing was performed during pregnancy. OGTTs were offered to the women in this group 1, 2 and 5 years postpartum.
2. Women from the region of Lund, who were pregnant in 1996 to 2000 and from the region of Malmö who were pregnant in 1995 and 2000. Women in this group were offered genetic testing, but not the extensive clinical follow-up programme.
3. Women from four of five maternity departments in Skåne, who were delivered in 2002 to 2005, were invited to participate. Extended evaluation including biochemical and genetic testing, and OGTT after 1 to 2 and 5 years postpartum was offered to women in this group.
4. Women participating in the Diabetes Prediction in Skåne study.

7.1.1 Paper I

The study population was recruited from Group 1. 110 consecutive cases of GDM (women of Swedish descent) were recruited in the period March 1996 to June 1999. To increase the likelihood of finding MODY mutations, family history of diabetes was used as an additional selection criterion. 66 of the 110 GDM subjects (60%), aged 31.4 ± 4.5 years (mean \pm SD) had at least one first- or second-degree relative with diabetes. These 66 subjects thus formed the study population for the study presented in Paper I.

7.1.2 Paper II

The study population was recruited from Groups 1, 2 and 4. 500 consecutive GDM cases (400 women of Scandinavian and 100 women of Arabian descent) and 550 non-diabetic pregnant controls (428 of Scandinavian and 122 of Arabian descent) were recruited. The Arabian women were immigrants from most of the Arab countries (Iraq, Lebanon, Morocco, Palestine, Syria, etc.). The reason for the different sample sizes was the limited number of women of Arabian descent living in the region.

7.1.3 Paper III

The study population was recruited from Group 1. 182 consecutive women with GDM agreed to participate. However, 8 of them were not included in the study because OGTTs could not be performed on more than one occasion. The study population used for the study presented in Paper III thus consists of 174 women with GDM.

7.1.4 Paper IV

The study population was recruited from Groups 1, 2 and 3. 174 women were recruited from Group 1 and 350 women from Group 2. 164 women from Group 3, fulfilling the same diagnostic criteria for GDM as Groups 1 and 2 were also recruited. Furthermore, 324 women with glucose levels within the lower range of IGT were recruited from Group 3. This category was designated gestational impaired glucose tolerance (GIGT) in order to differentiate it from women fulfilling the criteria for GDM, and from those with gestational normal glucose tolerance (GNGT). Finally, 174 women with GNGT were recruited from Group 3 to form a control group.

7.2 Methods

7.2.1 OGTT

A simplified 75 g OGTT, omitting the initial fasting glucose measurement, was performed after an overnight fast. Seventy-five grams anhydrous glucose was given, dissolved in 300 ml water. After 2 hours, duplicate blood samples were taken for the determination of glucose concentrations. Five μ l capillary blood was collected in each microcuvette and immediately analysed in a Hemocue device

(HemoCue, Ängelholm, Sweden). If the difference between the two samples exceeded 0.3 mmol/l a third sample was taken. If the difference again exceeded 0.3 mmol/l the results of the test were rejected, and a second OGTT was offered [11, 171].

In the earlier part of these studies, the clinical routine in Sweden was to analyse glucose levels in whole blood, however, in 2004 the recommendation was changed to plasma levels; these values were therefore used during the later part of the work. The relation:

plasma glucose = 1.11 x blood glucose

has been used when required to allow comparisons [172].

The recommendations of the WHO (1999) regarding the definition, diagnosis and classification of diabetes mellitus have been used throughout this work, and are summarized in Table 2 [167].

Table 2

Values used for the diagnosis of diabetes mellitus and other kinds of hyperglycaemia, according to the WHO (1999).

	Glucose concentration mmol/l (mg/dl)			
	Venous blood	Capillary blood	Venous plasma	Capillary plasma
<i>Diabetes mellitus (DM)</i>				
Fasting	≥6.1 (≥110)	≥6.1 (≥110)	≥7.0 (≥126)	≥7.0 (≥126)
<i>or</i>				
2 h after 75 g glucose load	≥10.0 (≥180)	≥11.1 (≥200)	≥11.1 (≥200)	≥12.2 (≥220)
<i>Impaired glucose tolerance (IGT)</i>				
Fasting (if measured)	<6.1 (<110)	<6.1 (<110)	<7.0 (<126)	<7.0 (<126)
<i>and</i>				
2 h after 75 g glucose load	≥6.7 (≥120)	≥7.8 (≥140)	≥7.8 (≥140)	≥8.9 (≥160)
<i>Impaired fasting glucose (IFG)</i>				
Fasting	≥5.6 (≥100) and <6.1 (<110)	≥5.6 (≥100) and <6.1 (<110)	≥6.1 (≥110) and <7.0 (<126)	≥6.1 (≥110) and <7.0 (<126)
<i>and, if measured</i>				
2 h after 75 g glucose load	<6.7 (<120)	<7.8 (<140)	<7.8 (<140)	<8.9 (<160)

7.2.2 Metabolic measurements

BMI was calculated as weight/height² (kg/m²). Insulin resistance was estimated as the homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) index: (fasting serum insulin x fasting plasma glucose)/22.5. Beta cell function was evaluated by both the HOMA-beta cell index ([fasting serum insulin (μU/ml) x 20] / [fasting plasma glucose (mmol/l)-3.5]) [173], and the insulinogenic index, defined as the ratio of the increment in insulin to the increment in glucose during the first 30 minutes of the OGTT ([insulin 30 min–insulin 0 min]/[glucose 30 min–glucose 0 min]) [174]. The disposition index was used to adjust insulin secretion for the degree of insulin resistance (insulinogenic index/HOMA-IR).

7.2.3 Assays

Blood glucose was analysed using a HemoCue device (HemoCue, Ängelholm, Sweden; coefficient of variation (CV) =3.1 to 3.7% [11]), or a glucose oxidation method on a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA; CV <1%). Serum insulin was measured using an enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark; interassay CV =7%). C-peptide concentrations were measured with a radioimmunoassay (Linco Research, St. Charles, MO, USA; interassay CV =9%). HbA_{1c} was measured by ion-exchange chromatography [175]; normal range 3.6 to 5.0%. Cholesterol, HDL cholesterol, triacylglycerol and urate were measured on a Beckman Coulter LX20 Analyzer (Beckman Counter, Brea, CA, USA). LDL cholesterol concentration was calculated using the Friedewald formula [176]. The presence of GAD65Abs was determined by radioimmunoassay [177].

7.2.4 Statistical analyses

- In Paper I, the results are presented as means ±SEM. The significance of differences between group frequencies was tested with the χ^2 or Fisher's exact test, and inter-group means were tested with the Mann–Whitney test or Kruskal–Wallis test.
- In Paper II, the results are presented as means ±SEM. The significance of differences between group means was tested by ANOVA or the Mann–Whitney test. Logarithmic transformation was used for data with a right-skewed distribution. The HOMA-IR index was adjusted for BMI or *PPARG* genotype using ANCOVA. Group frequencies were compared using the χ^2 or Fisher's exact test.

- In Paper III, the results are presented as means \pm SD, or as medians and interquartile ranges. ANOVA was used to test for differences between group means (symmetrically distributed variables), and the Kruskal–Wallis test was used to identify differences between medians (non-symmetrically distributed variables). χ^2 tests (Pearson) or Fisher’s exact test were used for comparisons of group frequencies. Multivariate logistic regression with backward elimination and a retention p–value of <0.05 was used to identify possible correlations between the development of future manifest diabetes and the risk factors assessed during the pregnancy. These risk factors were elevated levels of fasting blood glucose and HbA_{1c}, high BMI, first-degree family history of diabetes, high-risk ethnicity, previous pregnancies, and older age. Odds ratios (ORs) are expressed per standard deviation (SD) increment in the respective continuous covariate.
- In Paper IV, the variables are presented as medians (and interquartile ranges). χ^2 tests were used for comparisons of group frequencies. Survival analysis was used to estimate the effect of genetic variants (risk and non-risk genotypes defined from previous studies) on the risk of developing postpartum diabetes and illustrated by Kaplan–Meier survival curves. The risk of developing postpartum diabetes was expressed as a hazard function (the negative slope divided by the survival curve) using an age- and ethnicity-adjusted Cox proportional hazards regression model [178]. The hazard function is the (conditional) probability of the development of postpartum diabetes during a time interval divided by the length of that time interval, for an individual who does not have diabetes at the start of the time interval. The relative effect is presented as the ratio between the hazard functions (hazard ratio [HR]) of the two groups. HRs quantify the size of the effect of discrete variables (carriers versus non-carriers). The genetic models were predefined (an additive and a dominant model) to test the effect of the genetic variants on postpartum development of diabetes. Individuals with missing data were excluded from the analyses.

The Bonferroni correction was used to correct for multiple testing, where significant p–values were multiplied by 13 (i.e. the number of tested single nucleotide polymorphisms (SNPs)). A two-sided p–value <0.05 was considered statistically significant.

Power calculations were performed using the Genetic Power Calculator [179]. The prevalence of GDM was assumed to be 2%. The present study has, under a multiplicative model, $>80\%$ power to detect an effect size of 1.35 in Europeans and 2.1 in non-Europeans (as measured in terms of genotypic relative risk) when the frequency of the predisposing allele equals 30% (for $\alpha = 0.05$).

8. RESULTS

8.1 Paper I. Screening for MODY mutations, GAD antibodies, and type 1 diabetes associated *HLA* genotypes in women with gestational diabetes mellitus

8.1.1 Clinical characteristics

From a group of 110 Swedish women with GDM, those at highest risk of having MODY mutations were identified by using family history of diabetes as a selection criterion. Mutations in MODY genes 1 to 4, the presence of GAD65Abs, and *HLA-DQB1* risk genotypes were studied in the 66 GDM patients who had a family history of diabetes. An OGTT was repeated in 43 women, 1 year postpartum.

8.1.2 Genetics

No increase in type 1 diabetes associated *HLA-DQB1* alleles or GAD65Abs was found when compared with a group of patients with type 2 diabetes (n=82) or healthy control subjects (n=86). Mutations in known MODY genes were identified in 3 of the 66 subjects: 1 MODY 2, A303R (CGG-TGG) in the *GCK* gene, 1 MODY 3, A203H (CGT-CAT) in the *HNF1A* gene, and 1 MODY 4, P239Q (CCG-CAG) in the insulin promoter factor 1 (*IPF1*) gene (see Figure 1). Of the 46 GDM subjects followed up, 2 had diabetes and 17 had IGT 1 year postpartum (see Figure 2). Of the two subjects who developed manifest diabetes, one carried a MODY 3 mutation (A203H) in the *HNF1A* gene. No increase in high-risk *HLA* alleles or GAD65Abs was found in the women who had manifest diabetes or IGT 1 year postpartum.

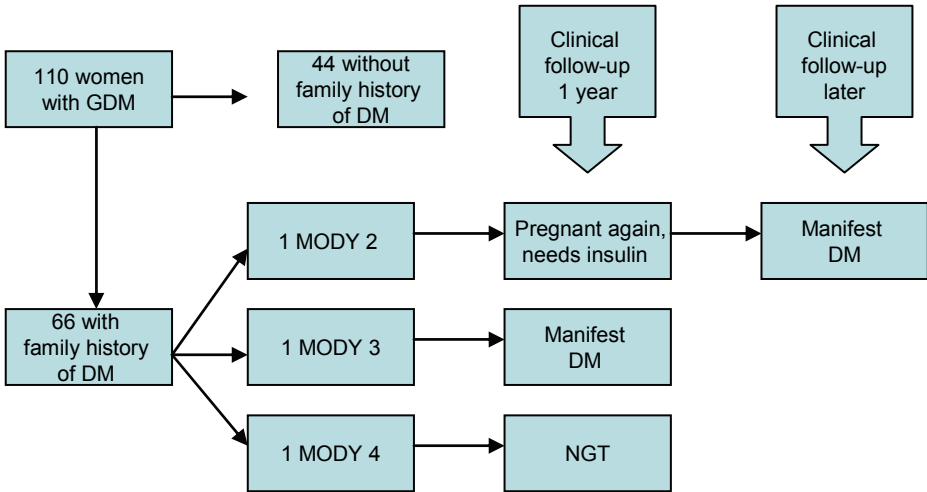


Figure 1. Screening for MODY genes 1 to 4 showed mutations in 3 out of 66 patients with a family history of diabetes.

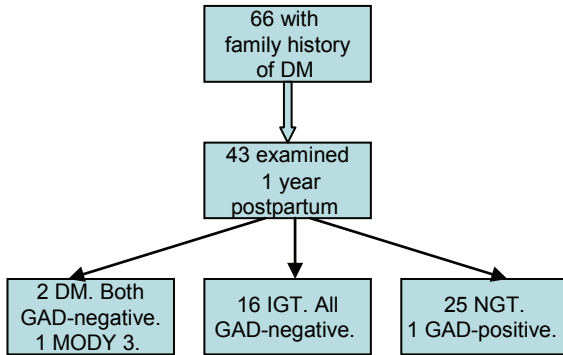


Figure 2. Clinical data at the 1-year follow-up.

8.2 Paper II. Genotypic and phenotypic differences between Arabian and Scandinavian women with gestational diabetes mellitus

8.2.1 Clinical characteristics

400 Scandinavian and 100 Arabian women with GDM were studied. 428 Scandinavian and 122 Arabian pregnant women without diabetes served as ethnicity-matched control groups. The women with GDM and the controls were investigated with respect to GAD65Abs, *HLA-DQB1* risk genotypes, *INS* VNTR, *mitochondrial tRNA^{Leu} A3243G* mutation, and *PPARG* Pro12Ala polymorphism.

The Arabian GDM women had a higher HOMA-IR index (3.2 ± 0.3 vs. 2.2 ± 0.2 , $p=0.02$) and a lower disposition index than Scandinavian GDM women after adjustment for BMI, i.e. the Arabian GDM women were more insulin resistant (50%). They also showed impaired beta cell compensation for their degree of insulin resistance.

8.2.2 Autoimmunity

The presence of GAD65Abs was associated with GDM among both Scandinavian and Arabian women. The frequency of GAD65Abs was 4.2% among Scandinavian women with GDM, compared with 0.9% in the Scandinavian controls ($p=0.008$). Similar frequencies were observed in Arabians, where 4.6% of GDM women tested positive for GAD65Abs compared with none (0.0%) in the corresponding control group ($p=0.03$).

8.2.3 Genetics

The frequency of *HLA-DQB1* risk genotypes was slightly higher in Scandinavian women with GDM (46.3%) than in the Scandinavian controls (38.8%), $p=0.03$, whereas no significant differences were found between the Arabian women with GDM (47%) and the Arabian controls (51.6%), $p=0.47$.

There were no significant differences in the frequency of the *INS* VNTR alleles or *PPARG-Pro12Ala* polymorphism between GDM women and controls, in the Arabian or the Scandinavian groups.

8.3 Paper III. Prediction of postpartum diabetes in women with gestational diabetes mellitus

8.3.1 Clinical characteristics

174 women with GDM were given OGTTs during pregnancy, as well as 1, 2 and 5 years postpartum. Women who developed impaired fasting glucose (IFG), IGT or diabetes were compared with women who remained normoglycaemic at 5 years. The insulinogenic index, disposition index and HOMA beta cell index were used to assess beta cell function, and insulin resistance was estimated by HOMA-IR. Baseline characteristics of the participants at 5 years and non-participants are presented in Table 3. A flow chart of the study population and the results is presented in Figure 3.

Table 3

Baseline characteristics during pregnancy comparing participants and non-participants at 5 years.

	Participants	Non-participants	p-value
n	144	30	
Age (years)	31.4 (5.2)	33.2 (5.7)	0.0998
BMI during pregnancy (kg/m ²)	28.1 (25.9-31.8)	28.9 (25.9-31.4)	0.9947
Diagnostic B-glucose (2 h) at OGTT (mmol/l)	9.5 (9.2-10.2)	9.7 (9.3-10.3)	0.3702
Diagnosed early in pregnancy	26 (18)	5 (17)	1.000
HbA _{1c} (%)	4.2 (3.9-4.6)	4.1 (3.8-4.4)	0.4894
GAD65Abs-positive	5 (3)	2 (7)	0.336
Swedish descent	67 (47)	16 (53)	0.550
First-degree relative(s) with diabetes	81 (56)	17 (57)	1.000
Insulin treatment during pregnancy	19 (15)	1 (3)	0.128
Previous pregnancies			
None	55 (39)	15 (52)	0.177
1	39 (27)	7 (24)	
2	17 (12)	5 (17)	
>2	32 (22)	2 (7)	

Data are given as n (%), means (SD) or median (interquartile range).

The percentages were calculated from all non-missing data for each phenotype within each group. Differences in means (medians) were tested using a t-test (Mann–Whitney test).

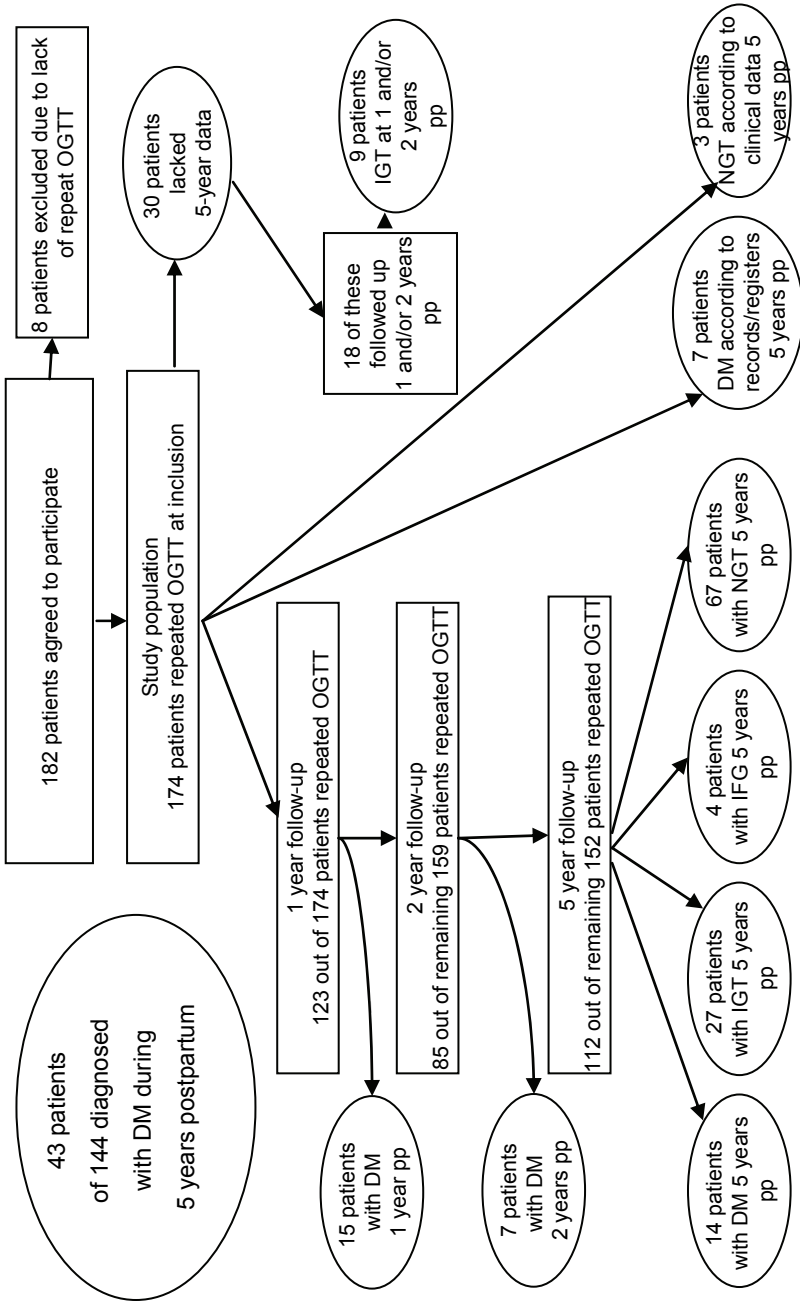


Figure 3. Flow chart of the study population and results regarding disturbances in glucose metabolism during the 5-year follow-up.

pp = postpartum

Differences in frequency were tested using Fisher's exact test. The study population was divided into three groups with respect to glucose tolerance after 5 years: (1) NGT, (2) IGT and/or IFG, and (3) diabetes. The characteristics of the women during pregnancy are presented in Table 4. A comparison of baseline data was made between the NGT group and the diabetes group, as well as between the NGT group and IGT and/or IFG group. Women who developed diabetes during follow-up had higher fasting glucose and HbA_{1c} levels at inclusion than those who remained normoglycaemic. They also had a lower HOMA beta cell index, insulinogenic index and disposition index, and required insulin more frequently during pregnancy than those who remained normoglycaemic (30% vs. 1%). There were more women of non-Nordic than Swedish origin (65% vs. 41%) in the groups with abnormal glucose tolerance.

Multivariate logistic regression analysis showed that the variables independently associated with diabetes postpartum were: previous pregnancies (OR 4.3, p=0.018), first-degree family history of diabetes (OR 4.1, p=0.025), HbA_{1c} (OR 2.6, p=0.012) and fasting glucose level (OR 2.1, p=0.038) during pregnancy. In addition to a model including only the main effects, the possibility of an interaction between HbA_{1c} and fasting glucose level was investigated. However, this interaction was non-significant (OR 1.0, p=0.985). Previous pregnancies (OR 4.1, p=0.025) and ethnicity (OR 3.1, p=0.047) were the variables independently associated with IGT and/or IFG postpartum.

The women were grouped into quartiles according to HbA_{1c} and fasting glucose levels during pregnancy. The medians (range) of HbA_{1c} (%) in the respective quartiles were: 3.8 (2.6-3.9) (n=48), 4.1 (4-4.2) (n=27), 4.4 (4.3-4.6) (n=29) and 5.0 (4.7-5.4) (n=32). The corresponding values for fasting blood glucose level (mmol/l) were: 3.8 (3-3.95) (n=30), 4.2 (4-4.35) (n=29), 4.7 (4.4-5.1) (n=26) and 5.45 (5.15-7.4) (n=28). A logistic regression analysis testing the value of HbA_{1c} and fasting glucose quartiles in predicting diabetes 5 years later showed that individuals with HbA_{1c} or fasting glucose in quartile 4 had a 4.8-fold (p=0.00014) and 6.8-fold (p=0.00002) increased risk of developing postpartum diabetes, respectively, compared with women in quartiles 1 to 3, see Figure 4.

Table 4

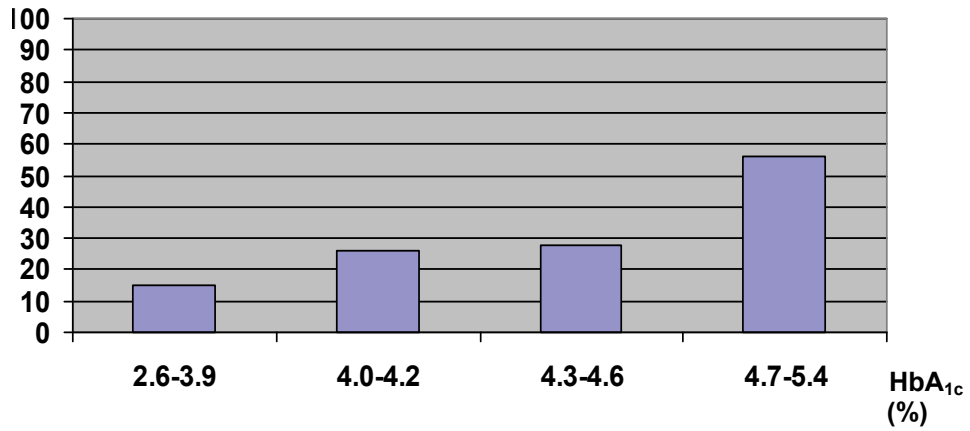
Comparison of baseline characteristics in relation to glucose tolerance at 5-year follow-up.

	Values 5 years postpartum			p-value
	NGT	IGT-IFG	DM	
n	70	31	43	
Age at delivery, (years)	31.0 (4.6)	32.0 (5.9)	31.6 (5.8)	0.6
BMI during pregnancy, (kg/m ²)	27.0 (25.8-29.9)	29.3 (26.2-32.0)	30.9 (27.1-32.9)	0.02
Swedish descent, n (%)	41 (59)	8 (26)	18 (42)	0.008
First-degree relative(s) with diabetes, n (%)	34 (49)	17(55)	30 (70)	0.09
One or more previous pregnancies, n (%)	33 (47)	24 (80)	31 (72)	0.002
Diagnosis early in pregnancy, n (%)	3 (4)	2 (6)	21 (49)	<0.0005
HbA _{1c} (%)	4.1 (0.48)	4.2 (0.52)	4.5 (0.51)	0.0001
Infant birth weight, (kg)	3.6 (0.62)	3.7 (0.51)	3.7 (0.56)	0.4
Pregnancy >37 weeks, n (%)	62 (89)	30 (97)	36 (84)	0.064
Insulin treatment during pregnancy, n (%)	1 (1)	5 (16)	13 (30)	<0.0005
GAD65Abs-positive at any time, n (%)	4 (6)	2 (6)	2 (5)	1
Diagnostic OGTT, 2 h glucose (mmol/l)	9.4 (9.1-9.8)	9.5 (9.2-10.1)	9.8 (9.3-10.8)	0.004
Repeat OGTT glucose (mmol/l)				
Fasting glucose	4.3 (3.9-4.7)	4.3 (3.9-4.9)	5.2 (4.4-5.6)	0.0004
1-h glucose	9.2 (8.2-10.1)	9.2 (8.1-10)	9.9 (8.8-10.8)	0.19
2-h glucose	7.8 (6.9-9.2)	7.9 (7.4-8.7)	8.9 (8-10.1)	0.005
Repeat OGTT serum insulin (pmol/l)				
Fasting glucose	54 (39-81)	62 (38-114)	61 (39-72)	0.76
1-h glucose	402 (288-492)	369 (272-455)	246 (204-312)	0.0004
2-h glucose	457 (272-564)	456 (329-608)	324 (263-450)	0.09
HOMA beta cell	143.4 (104.2-245.6)	134.6 (98.9-219.1)	92.4 (66.2-135.5)	0.0012
HOMA-IR	1.8 (1.4-3.0)	2.4 (1.3-3.8)	2.4 (1.5-3.2)	0.45
Insulinogenic index	4.4 (3.0-6.0)	2.9 (2.1-4.7)	2.6 (1.7-3.4)	0.0012
Disposition index	2.2 (1.1-3.4)	1.8 (1.0-2.4)	1.0 (0.7-1.7)	0.01

Data are given as n (%), means (SD) or medians (interquartile range).

a)

DM(%)



b)

DM(%)

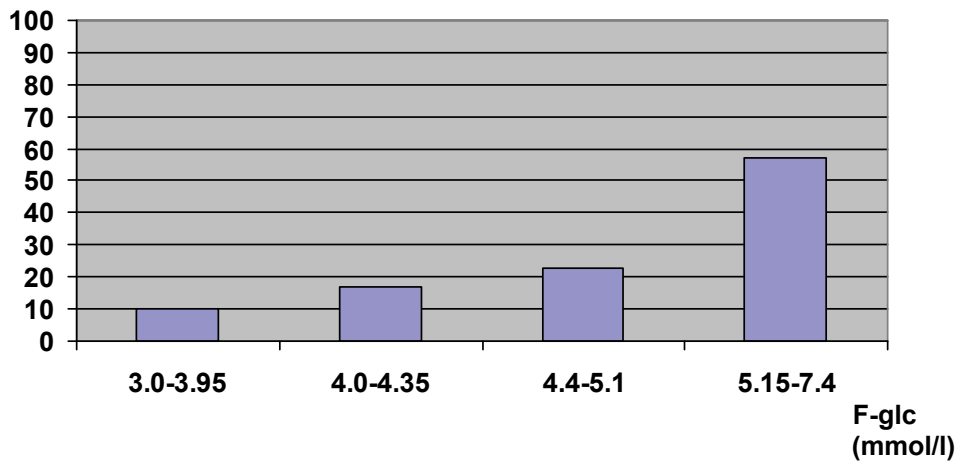


Figure 4. HbA_{1c} (a) and fasting glucose (F-glc) (b) in the upper quartile during pregnancy predicts postpartum diabetes.

Results from the 1- and 2-year follow-up are given in Table 5. Women with abnormal glucose tolerance after 5 years had a higher BMI and waist to hip ratio than those with NGT. The HOMA beta cell index did not differ between the groups, but the insulinogenic index was lower among women who had developed diabetes after 5 years, especially when adjusted for degree of insulin resistance. Cholesterol levels were similar in all three groups, while urate concentrations were higher in the diabetes group than in the other groups. The median (interquartile range) BMI at the end of the study was 24.3 (21.4-28.1) in the NGT group, 26.6 (23.6-30.7) in the IGT and/or IFG group and 28.7 (24.2-33.1) in the diabetes group ($p=0.0053$).

Table 5

Comparison of follow-up data 1 and 2 years postpartum in relation to glucose tolerance 5 years postpartum.

		Values 5 years postpartum			p-value
		NGT	IGT-IFG	DM	
n	1 year	57	23	30	
	2 years	41	24	19	
BMI (kg/m ²)	1 year	24.6 (4.6)	27.2 (4.6)	29.1 (4.8)	0.0001
	2 years	23.0 (20.8-25.7)	27.0 (23.1-30.5)	29.8 (25.8-34.2)	0.0001
WHR	1 year	0.80 (0.74-0.83)	0.81 (0.77-0.85)	0.84 (0.79-0.89)	0.033
	2 years	0.78 (0.06)	0.81 (0.06)	0.85 (0.07)	0.0009
HbA _{1c}	1 year	4.1 (0.26)	4.2 (0.40)	4.8 (0.56)	<0.00005
	2 years	4.1 (0.33)	4.4 (0.37)	4.6 (0.46)	<0.00005
S-Cholesterol (mmol/l)	1 year	4.3 (3.6-5.0)	4.4 (4.1-5.1)	4.8 (4.2-5.5)	0.06
	2 years	4.4 (4.1-4.6)	3.9 (3.6-5.0)	4.8 (4.5-5.2)	0.06
HDL (mmol/l)	1 year	1.3 (1.1-1.5)	1.2 (1.1-1.4)	1.2 (1.1-1.5)	0.67
	2 years	1.3 (0.2)	1.2 (0.2)	1.2 (0.3)	0.09
LDL (mmol/l)	1 year	2.8 (0.7)	2.8 (0.7)	3.0 (0.9)	0.53
	2 years	2.7 (0.7)	2.8 (0.9)	3.1 (0.6)	0.25
Triacylglycerol (mmol/l)	1 year	0.8 (0.6-1.1)	1.0 (0.7-1.4)	1.1 (0.8-1.8)	0.04
	2 years	0.8 (0.6-1.0)	0.8 (0.7-1.2)	1.4 (1.0-2.0)	0.0008
Urate (μmol/l)	1 year	258 (221-278)	229 (206-282)	276 (240-333)	0.02
	2 years	261 (225-288)	245 (225-285)	303 (287-324)	0.002
Fasting glucose (mmol/l)	1 year	5.0 (0.48)	5.2 (0.60)	5.8 (1.0)	<0.00005
	2 years	5.0 (4.8-5.1)	5.5 (4.85-5.8)	5.7 (5.4-6.2)	0.0001
2-h glucose (mmol/l)	1 year	6.1 (1.4)	7.0 (1.2)	9.8 (3.3)	<0.00005
	2 years	6.2 (1.3)	6.5 (1.1)	10.1 (3.7)	<0.00005
IGT and/or IFG	1 year	15/3	16/2	11/0	
	2 years	13/1	11/5	3/1	
HOMA beta cell	1 year	56 (42-86)	62 (49-104)	70 (46-107)	0.62
	2 years	64.8 (44.4-99.8)	83.4 (39.2-102.0)	72.1 (48.4-96.4)	0.96
HOMA-IR	1 year	1.6 (1.1-2.1)	2.0 (1.4-2.9)	2.6 (1.7-3.7)	0.0003
	2 years	1.7 (1.1-2.2)	3.2 (1.5-4.4)	3.8 (2.3-4.3)	0.03
Insulinogenic index	1 year	9.1 (4.9-13.8)	8.3 (5.1-16.8)	5.5 (4.3-7.7)	0.08
	2 years	9.2 (6.3-15.7)	10.8 (5.5-15.2)	5.5 (3.8-8.5)	0.02
Disposition index	1 year	6.1 (3.7-8.4)	3.9 (3.4-5.1)	1.7 (1.2-4.7)	0.0003
	2 years	6.1 (3.7-8.2)	3.5 (2.4-6.6)	1.7 (1.0-2.3)	0.0001

Data are given as n, means (SD) or median (interquartile range).

Differences in means (medians) were tested using ANOVA (Kruskal–Wallis test).

8.4 Paper IV. The *FTO* rs8050136 variant predicts postpartum diabetes in women with gestational diabetes mellitus

8.4.1 Clinical characteristics

A total of 514 women (68% Europeans and 32% non-Europeans) with GDM were included. Of these, 471 (92%) were followed up with postpartum OGTTs for a median period of 59 months (interquartile range 14 to 62 months). Based on the results of the OGTTs, diabetes was diagnosed in 23%, IGT in 22% and NGT in 56% of the cases. For further details see Table 6.

Table 6
Clinical characteristics of the study population in Paper IV.

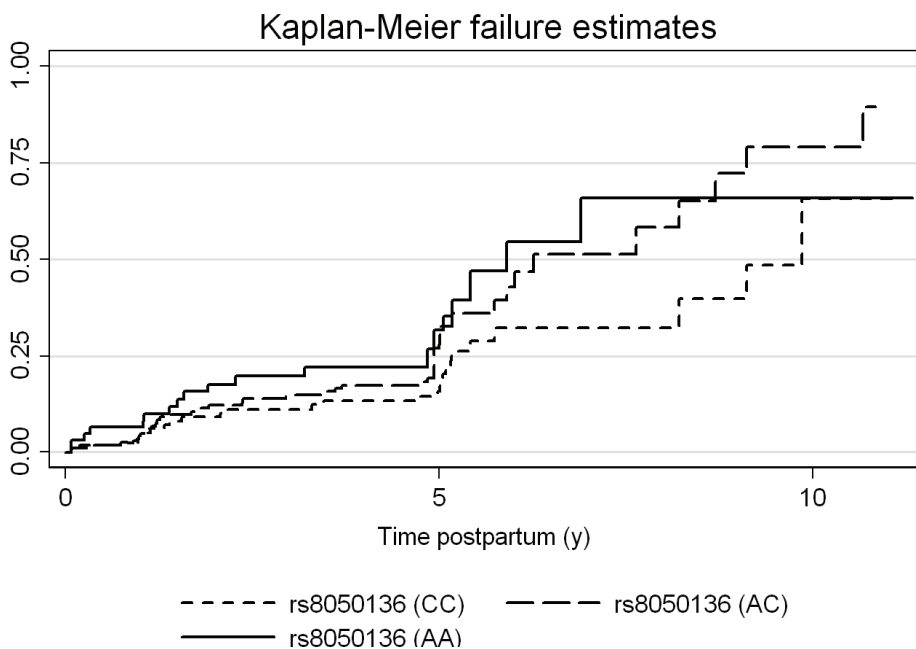
Characteristic	Malmö	Skåne		
	1995-2000	2002-2005		
Diagnosis during pregnancy	GDM	GDM	GIGT	GNGT
n	350	164	324	174
Ethnicity				
European	234 (67%)	118 (72%)	271 (84%)	154 (89%)
Non-European	114 (33%)	46 (28%)	53 (16%)	20 (11%)
Unknown	2 (<1%)			
OGTT postpartum				
Time postpartum, months	28 (12-61)	61 (31-63)	44 (14-61)	17 (14-24)
DM	84 (24%)	22 (13%)	23 (7%)	4 (2%)
IGT	61 (17%)	42 (26%)	53 (16%)	28 (16%)
NGT	165 (47%)	97 (59%)	246 (76%)	139 (80%)
Missing clinical follow-up	40 (11%)	3 (2%)	2 (<1%)	3 (2%)

Data are given as n (%) and medians (interquartile range).

8.4.2 Genetics

The HR for developing postpartum diabetes in women with the *FTO* rs8050136 variant was 1.53 (95% CI 1.15-2.03, $p=0.003$; corrected p -value [P_c] for multiple testing =0.039) for the A-allele under the additive model, and 1.77 (95% confidence interval 1.14–2.76, $p=0.011$; $P_c=0.14$) under the dominant model after adjustment for age and ethnicity [see Figure 5]. After adjustment for postpartum BMI and correction for multiple testing, the significance did not persist ($p=0.055$; $P_c=0.715$ under the dominant model and $p=0.008$; $P_c=0.104$ under the additive model). The overall increase in risk could be mainly attributed to the women of non-European descent, who had a HR of 2.2 (95% CI 1.37–3.5, $p=0.001$; $P_c=0.013$) for the development of postpartum diabetes. The other SNPs studied were not associated with a significant increase in risk of developing postpartum diabetes. The HR for developing diabetes in different *FTO* allele carriers obtained from Cox proportional hazards regression adjusted for age and ethnicity is given in Table 7.

5a) Additive model



5b) Dominant model

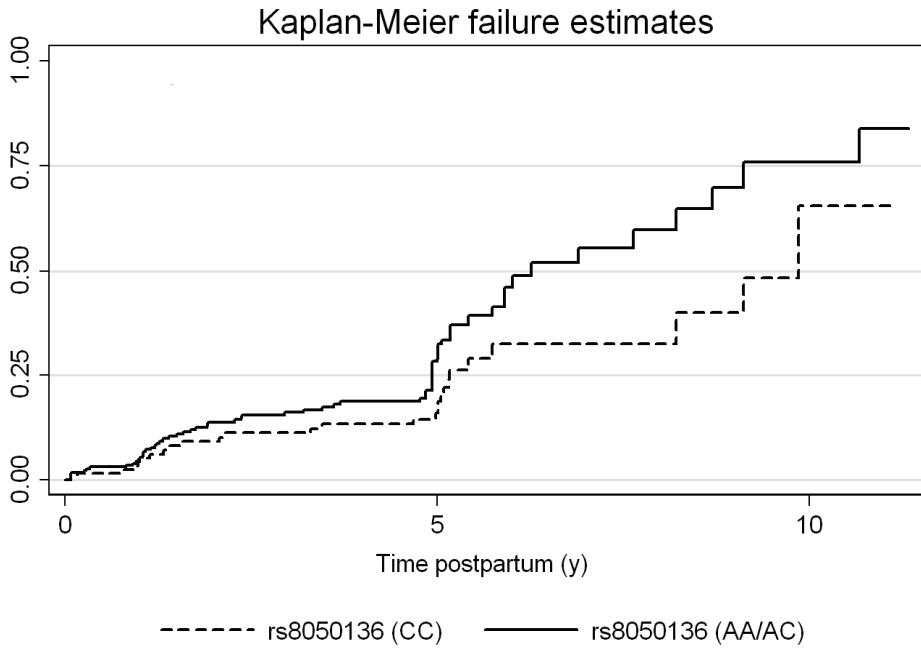


Figure 5. Kaplan-Meier diabetes-free survival probability curves for the *FTO* rs 8050136 variant under: a) the additive model, and b) the dominant model. 1 minus survival probability (i.e. failure) is plotted vs. the follow-up time in years.

Table 7

The effect of the genetic variants studied on the risk of developing postpartum diabetes.

SNP	Dominant model		Additive model	
	HR (95% CI) for development of postpartum diabetes	p-value	HR (95% CI) for development of postpartum diabetes	p-value
<i>CDKAL1</i> rs7754840	0.94 (0.63–1.40)	0.77	0.98 (0.75–1.30)	0.91
<i>CDKAL1</i> rs7756992	0.94 (0.64-1.40)	0.77	0.94 (0.72-1.24)	0.68
<i>CDKN2A/2B</i> rs10811661	0.55 (0.08-4.02)	0.56	1.14 (0.70-1.86)	0.60
<i>HHEX</i> rs1111875	1.07 (0.61-1.88)	0.80	1.26 (0.94-1.68)	0.12
<i>IGF2BP2</i> rs1470579	1.16 (0.77-1.74)	0.48	1.27 (0.94-1.71)	0.12
<i>IGF2BP2</i> rs4402960	1.02 (0.67-1.53)	0.94	1.14 (0.84-1.55)	0.41
<i>TCF7L2</i> rs7903146	1.42 (0.95-2.14)	0.09	1.23 (0.94-1.62)	0.13
<i>PPARG</i> rs1801282	0.51 (0.12-2.10)	0.35	0.92 (0.58-1.47)	0.74
<i>SLC30A8</i> rs13266634	0.75 (0.38-1.51)	0.43	1.16 (0.82-1.63)	0.40
<i>GCK</i> rs1799884	1.36 (0.90-2.07)	0.15	1.25 (0.90-1.74)	0.19
<i>HNF1A</i> rs1169288	1.04 (0.67-1.60)	0.86	0.99 (0.75-1.32)	0.97
<i>KCNJ11</i> rs5219	0.95 (0.62-1.44)	0.81	1.09 (0.81-1.45)	0.58
<i>FTO</i> rs8050136	1.77 (1.14-2.76)	0.01 (Pc=0.14)	1.53 (1.15-2.03)	0.003 (Pc=0.039)

9. DISCUSSION

9.1 Prevalence of diabetes after GDM

The prevalence of diabetes 5 years postpartum was 30% in the study presented in Paper III, which is in line with findings by other groups. It is, however, important to bear in mind that the prevalence of diabetes after GDM is closely related to the cut-off value applied for the diagnosis of GDM. A lower cut-off value includes those who do not exhibit major disturbance in glucose metabolism, thus, the prevalence of manifest diabetes postpartum can be assumed to be lower. Ethnic factors are also of great importance in the prevalence of postpartum diabetes. The prevalence of GDM and type 2 diabetes are correlated in a given population, and the progression to diabetes following GDM appears to be more rapid among ethnic groups with a high background prevalence of type 2 diabetes [12, 13].

9.2 Prevention and prediction of diabetes after GDM

To date, a number of prospective studies have indicated that it is possible to lower the incidence, or delay the onset, of diabetes in individuals at high risk of developing the disease by changing their lifestyle and/or pharmacological treatment. Changes in lifestyle have been found to be effective in preventing diabetes in patients with IGT in several previous studies [180-183].

Other studies have shown that pharmacological treatment with troglitazone or pioglitazone have diabetes-preventing or diabetes-delaying effects in women with previous GDM [184-188]. In the Troglitazone in Prevention of Diabetes (TRIPOD) study, treatment with troglitazone reduced the incidence of diabetes by 55% in women with prior GDM [188]. Beneficial effects were also found in the Diabetes Prevention Program (DPP), where both lifestyle changes and treatment with metformin were found to be highly effective in delaying or preventing diabetes in women with IGT and a history of GDM. Metformin was also found to be more effective in women with a history of GDM than in those without [189].

Women with GDM represent a high-risk group, showing signs of diabetes in evolution already at childbearing age. They are thus an ideal group for primary diabetes prevention, which should preferably be introduced while they are still pregnant, highly motivated and under close medical care. Recommendations concerning increased physical activity, maintenance of a normal weight and healthy diet should be included.

The risk of developing diabetes following GDM is high. However, not every woman with GDM develops diabetes. The ability to prospectively identify women with GDM who are at the highest risk of developing diabetes would be important from a preventive point of view. Genetic, as well as clinical and biochemical, variables have therefore been examined in GDM patients. It would be particularly interesting if markers for postpartum diabetes could be easily determined during pregnancy, at a reasonable cost. In the study presented in Paper III, it was concluded that fasting blood glucose ≥ 5.2 mmol/l and HbA_{1c} $\geq 4.7\%$ according to the Mono S-method (equivalent to $\geq 5.7\%$ according to the method by the National Glycohemoglobin Standardization Program) are associated with a 4- to 6-fold increase in the risk of developing diabetes within 5 years after GDM.

High fasting glucose level has been extensively studied as a predictor of diabetes after GDM. In a systematic review by Kim et al., an elevated fasting glucose level during pregnancy was found to be the risk factor most commonly associated with future risk of type 2 diabetes, in addition to measures of pancreatic beta cell function [131]. Schaefer-Graf et al. found that the highest fasting plasma glucose level during pregnancy was the best predictor of diabetes in early puerperium [190]. Similarly, Metzger et al. reported that in Chicago approximately three-quarters of women with GDM developed diabetes within 6 months of delivery, when their fasting plasma glucose level during pregnancy was > 7.2 mmol/l, compared with 10% of women who had a fasting glucose level < 5.8 mmol/l [191]. In the present study, a fasting blood glucose concentration ≥ 5.2 mmol/l, corresponding to a plasma glucose level of 5.8 mmol/l [172], was associated with a 6-fold increase in the risk of postpartum diabetes.

To the best of the author's knowledge, this is the first report of HbA_{1c} as a possible predictor of diabetes after GDM. This finding is supported by a recent study by Kwong et al., where postpartum hyperglycaemia was also significantly associated with higher HbA_{1c} [192]. HbA_{1c} has long been a valuable test in monitoring the effects of treatment of diabetes, but its use has previously not been advocated for diagnostic purposes due to a lack of standardization of its measurement. These problems have, however, now been addressed, and programmes for the standardization of HbA_{1c} assays have been established [193].

9.3 GDM and genetics

GDM often represents diabetes in evolution, which provides an excellent opportunity to study the pathogenic mechanisms underlying the development of diabetes. A genetic component of GDM is strongly suggested, and GDM shares genetic features with type 1 diabetes, type 2 diabetes and MODY [14, 57].

The studies presented here have been performed over several years. In the study described in Paper I, which was carried out in 2002, an association between GDM and MODY was demonstrated. Approximately 5% of the studied GDM subjects with a family history of diabetes carried a MODY mutation, in line with other reports [118-120]. No correlations between GDM and autoimmunity or high-risk *HLA* genotypes were found in this study, which, however, was quite small. It is not surprising to find correlations between GDM and MODY since beta cell dysfunction characterizes both conditions. Such correlations have also been found by others. Mutations in the *GCK* gene (MODY 2) have been studied by Ellard et al., who detected these mutations in 80% of GDM patients selected on the basis of clinical phenotype. They therefore suggested that patients with GDM who have a persistently elevated fasting glucose level, but show a small increment during OGTT, require insulin in pregnancy and have a first-degree relative with mild hyperglycaemia, should be considered for mutation analysis of the *GCK* gene [194].

In the study presented in Paper II Scandinavian and Arabian women were tested and compared regarding variations in genes affecting insulin secretion and action. An association was found between *GAD65Abs* and *HLA-DQB1* risk genotypes in Scandinavian women, indicating that GDM might share some genetic features with type 1 diabetes. Arabian women with GDM were more insulin resistant than Scandinavian women with the same BMI. The findings in the studies presented in Paper II, as well as Paper IV, support previous evidence that pathogenic mechanisms may differ between different ethnic groups.

The primary aim of the study presented in Paper IV was to investigate whether genetic variants that predispose individuals to type 2 diabetes could predict the development of diabetes postpartum in women with GDM. The key finding was that the *FTO* rs8050136 variant is associated with an increased risk of developing postpartum diabetes. Adjusting for BMI clearly attenuated the effect of the *FTO* variant on the risk of postpartum diabetes, suggesting that most of its effect is mediated through its effect on BMI. The effect was also mainly attributed to non-European ethnicity. These results are supported by previous studies showing that the common *FTO* rs9939609 variant increased the risk of diabetes through an effect on BMI [195], and that *FTO* rs8050136 polymorphism is associated with

measures of obesity [196]. However, in a meta-analysis in an Asian population the A-allele of rs805136 was found to be associated with an increased risk of type 2 diabetes, independent of measures of BMI and obesity [197]. It is interesting to note that the rs9939609 variant is in strong linkage disequilibrium with the rs805136 and rs7193144 variants in the *FTO* gene [198]. The finding that the *FTO* rs805136 variant increases the risk of postpartum diabetes is not surprising, since most women with GDM are obese, which is also the case in type 2 diabetes. Interestingly, a recent study has shown that the *FTO* rs805136 variant is associated with obese type 2 diabetes, whereas the *TCF7L2* rs7901695 variant was found to be the predominant genetic risk factor in non-obese type 2 diabetes subjects [199]. A Japanese study has shown that the *FTO* rs1121980 variant was strongly associated with the plasma immunoreactive insulin level and HOMA-IR, even after adjusting for age, gender and BMI. Moreover, a haplotype of three SNPs, rs9939609, rs1121980 and rs1558902 in the *FTO* gene, has also been reported to be significantly associated with plasma immunoreactive insulin and HOMA-IR [200]. The mechanism by which *FTO* polymorphism increases the risk of postpartum diabetes is thus thought to be its effect on BMI and insulin resistance. This is consistent with the pathogenesis of GDM and type 2 diabetes, where insulin resistance is one of the predominant features of both disorders [12].

Only a few studies have been conducted to specifically investigate the effect of *FTO* variants in women with GDM. Lauenborg et al. examined 11 established type 2 diabetes susceptibility variants in women with GDM, and showed that the *CDKN2A/2B* rs10811661 and the *WFS1* rs10010131 polymorphisms were associated with incident type 2 diabetes up to 2 years postpartum [109]. However, their study revealed no effect of the *FTO* rs9939609 variant, either on the risk of GDM or postpartum diabetes. The short follow-up period (up to 2 years) could explain the discrepancy between their study and the present one. Interestingly, the effect of the *FTO* rs805136 polymorphism on the risk of postpartum diabetes in our study was mainly attributed to non-European ethnicity. This is in agreement with previous observations that non-European ethnicity is a risk factor for GDM, as well as for diabetes [12].

Further examination of other genetic variants will hopefully increase our understanding of GDM. Genetic testing may become an appropriate approach to identifying women at high risk of developing postpartum diabetes after GDM.

10. CONCLUSIONS

The conclusions drawn from the results of this work are summarized below.

- The prevalence of MODY mutations in the study population was 5% among women with GDM and a family history of diabetes.
- Arabian women were more insulin resistant, than Scandinavian women with the same BMI, and exhibited impaired beta cell compensation for their degree of insulin resistance.
- In Scandinavian women, GDM may have genetic features in common with type 1 diabetes.
- The prevalence of manifest diabetes 5 years postpartum in women with GDM was 30%.
- Fasting glucose level and HbA_{1c} are possible predictors of diabetes following GDM. These tests are easy to perform during pregnancy and relatively cheap. They could help identify women at particularly high risk of developing diabetes postpartum. Counselling and education regarding the risk of future diabetes and treatment could then be initiated during pregnancy in high-risk individuals.
- Variants of the *FTO* gene were associated with an increased risk of developing postpartum diabetes, probably due to its effect of increasing obesity. Thus, genetic testing could be an appropriate means of identifying pregnant women at high risk of developing postpartum diabetes.

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