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Context: Gestational diabetes mellitus (GDM), defined as any degree of glucose intolerance with first recognition during pregnancy, is a heterogeneous form of diabetes characterized by various degrees of β-cell dysfunction.

Objectives: We aimed to estimate the prevalence of possibly pathogenic variants in the maturity-onset diabetes of the young genes *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, and *INS* among women with GDM. Furthermore, we examined the glucose tolerance status in variant carriers vs noncarriers at follow-up.

Design, Setting, and Patients: We sequenced the coding regions and intron/exon boundaries of *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, and *INS* using targeted region capture and next-generation sequencing in 354 Danish women with diet-treated GDM. Glucose tolerance was examined at follow-up 10 years after the index pregnancy.

Main Outcome Measures: The prevalence of possibly pathogenic variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, and *INS* was estimated, and differences in anthropometric traits, high-sensitivity C-Reactive Protein (CRP), and glucose metabolism were measured.

Results: At baseline, 17 possibly disease-causing variants were found in 21 women, revealing a combined *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, and *INS* variant prevalence of 5.9% (95% confidence interval: 3.5% to 8.4%). At follow-up, 15 out of 135 women with diabetes (11%) were carriers of variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, or *INS*.

Conclusions: Almost 6% of Danish women with diet-treated GDM have possibly pathogenic variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, or *INS*. These women are at high risk of developing diabetes after pregnancy. Thus screening for variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, and *INS* should be considered among women with GDM.

Abbreviations: BMI, body mass index; CI, confidence interval; GADA, glutamic acid decarboxylase antibody; GDM, gestational diabetes mellitus; hsCRP, high-sensitivity CRP; IFG, impaired fasting glycemia; IGT, impaired glucose tolerance; MODY, maturity-onset diabetes of the young; OHA, oral hyperglycemic agent.

Freeform/Key Words: monogenic diabetes, gestational diabetes, MODY, genes, genetic screening

Gestational diabetes mellitus (GDM) defined as any degree of glucose intolerance with first recognition during pregnancy [1] is a frequent pregnancy complication. The prevalence of GDM was 2.4% in 2000 among 5,235 women in Denmark [2] and is globally affecting between 1% and 28% of all pregnancies with large country-wise differences [3].

During a normal pregnancy, insulin resistance is observed during the first trimester. To maintain normoglycemia during the pregnancy, insulin release is increased significantly [4]. Women unable to adapt to such pregnancy-induced physiological changes in insulin sensitivity are at risk of developing GDM [5].

Despite normalization of glucose tolerance shortly after delivery, women with a history of GDM have increased risk of developing diabetes later in life. A systematic review and a meta-analysis including 675,455 women reported a sevenfold risk of developing diabetes among women with a history of GDM compared with women having a normoglycemic pregnancy in studies with follow-up times up to 30 years after index pregnancy [6]. In Denmark, 40% of 481 women with a history of diet-treated GDM had developed type 2 diabetes 10 years after their index pregnancy [7]. Apart from risk of diabetes later in life, there are additional complications for mothers having GDM such as increased risk of undergoing caesarean section and pre-eclampsia, whereas the child is at risk for macrosomia, shoulder dystocia, neonatal hypoglycemia, and development of obesity and prediabetes in young adulthood [5, 8].

A family history of diabetes has been established as a nonmodifiable risk for GDM [9]. Shared genetics with both type 1 diabetes, type 2 diabetes, and monogenic forms of diabetes has been suggested as the underlying genetic etiology for GDM [10].

Cross-sectional studies estimating the presence of mutations in maturity-onset diabetes of the young (MODY) genes among women with GDM have found prevalences between 0% and 5% of *GCK* mutations in studies including between 17 and 247 individuals, depending on inclusion criteria [11–17]. One study found a prevalence of 80% *GCK* mutations when stringent selection criteria were applied [18]. The prevalence of *HNF1A* and *HNF4A* mutations among GDM women have been estimated in a few studies including between 66 and 119 women with GDM. It was found that 0% to 1% of GDM can be attributed to deleterious *HNF1A* or *HNF4A* mutations in women having a positive family history of diabetes [13, 14].

The aim of the current study was to screen for variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, and *INS* and establish the prevalence of disease-associated variants in a large study cohort of 354 consecutively recruited Danish women diagnosed with diet-treated GDM. Furthermore, we also estimated the impact of *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, or *INS* mutations on glucose tolerance at follow-up examination on average 9.8 years after the index pregnancy to identify the risk of progression to diabetes among GDM women with and without disease-associated variants.

1. Methods

Women diagnosed with diet-treated GDM were recruited for the study between 1978 and 1985 and 1987 and 1996 at the Center for Pregnant Women with Diabetes, Department of Obstetrics, Rigshospitalet, Denmark [7]. The women were invited to a follow-up study between 2000 and 2002 investigating their glucose tolerance status. We included 354 Danish women with diet-treated GDM in the current study. The median follow-up time was 9.8 years after the index pregnancy [7]. All women were glutamic acid decarboxylase antibody (GADA) negative (Table 1).

Table 1. Clinical Description of the Total Study Population at Follow-Up

Traits	GAD Negative (n = 354)
Age (y)	43.0 (37.0–48.0)
BMI (kg/m^2)	27.6 (23.9–32.9)
Waist/hip ratio	0.82 (0.78–0.87)
HbA1C (%)	5.4 (5.0–6.1)
HbA1C (mmol/mol)	35.5 (31.1–43.2)
Fasting plasma glucose (mmol/L)	6.3 (5.69–7.50)
Fasting serum insulin (pmol/L)	54 (35–79)
GADAs (% of total population)	0%
Parental diabetes (yes/no/unknown)	161/150/43
Glucose-tolerant [% (n)]	17% (n = 60)
IFG [% (n)]	26% (n = 92)
IGT [% (n)]	19% (n = 67)
Diabetes [% (n)]	38% (n = 135)

Data are presented as median and interquartile range.
Abbreviation: GAD, glutamic acid decarboxylase.

A. Anthropometrical Measures and Biochemical Measures at Follow-Up

The women had two blood samples drawn from an antecubital vein for measurement of plasma glucose, serum insulin, and serum GADAs after 10 hours of fasting. Women without known diabetes underwent a 2-hour, 75-g oral glucose tolerance test. Diabetes at follow-up was classified according to American Diabetes Association criteria [19], and prediabetes was defined as either impaired fasting glycemia (IFG), impaired glucose tolerance (IGT), or both.

Plasma glucose was measured by the glucose oxidase method using an automated colorimetric method on a Cobas Mira analyzer. GADAs were detected by a radioimmunoassay [7, 20]. The cutoff limit was 9.5 units/mL, and the intra- and interassay coefficients of variation were 0.024 and 0.036, respectively. High-sensitivity CRP (hsCRP) was measured on a Roche/Hitachi MODULAR analyzer (Tina-quant cardiac C-reactive protein high sensitive, cobas, Roche) with a measuring range of 0.1 to 20 mg/L.

B. Diabetes Family History

Based on questionnaires, information on family history of diabetes was divided into three categories: parental diabetes, no parental diabetes, and unknown.

C. Sequencing

Genomic DNA was obtained from human leukocyte nuclei. DNA in the targeted region, which included the coding regions and exon/intron boundaries of *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, and *INS* genes, was captured and sequenced using the Illumina HiSeq2000 Analyzer as described [21]. All coding regions were covered with a minimum mean depth of 76×. Qualified reads were aligned to the reference human genome (UCSC hg19) using the Burrows-Wheeler Aligner tool (http://bio-bwa.sourceforge.net), and single-nucleotide polymorphisms and indels were identified using the Genome Analysis Toolkit (https://www.broadinstitute.org/gatk/).

D. Evaluation of Variant Pathogenicity

Variants located in coding regions, -10 nucleotides upstream of the transcription start site or 5 nucleotides into intron boundaries, were evaluated. Nonsynonymous variant pathogenicity was evaluated as described [22] (Supplemental Tables 1 and 2). This includes the previous establishment of the variant's deleterious effect, the frequency in public

databases, the level of computational evidence for functionality, and the patient's phenotype, among other criterions. The cutoff for frequency in public databases was defined as a minor allele frequency ≤ 0.05% in the ExAC database (http://exac.broadinstitute.org/) and absence of the variant among 1000 glucose-tolerant Danes [23]. The patient's phenotype was included (1) for *GCK* if carriers had a fasting plasma glucose above 5.5 mmol/L at follow-up, as elevated fasting glucose levels are a phenotypic characteristic for *GCK* MODY patients [24], and (2) for *HNF1A* variants if carriers had a plasma glucose increment after an oral glucose load [plasma glucose 120 minutes after an oral glucose load (fasting plasma glucose)] above 3 mmol/L [25] or a level of hsCRP below 1 mg/L [26], which are phenotypic characteristics of *HNF1A* patients. The criteria for computational evidence for functionality were applied if the variant was predicted to be damaging in three or more of the following programs: SIFT [27], PolyPhen2 HVAR [28], LRT [29], MutationTaster [30], MutationAssessor [31], and FATHMM [32]. Splice variants were classified as functional according to Human Splicing Finder (www.umd.be/HSF3/index.html) [33], and 5-untranslated region functionality was based on the effect on transcription factor binding sites using the JASPAR database (http://jaspar.genereg.net/).

E. Statistical Analysis

To test quantitative traits for differences between groups, a general linear model was used, adjusted for age. The difference in the prevalence of diabetes between groups was calculated using a χ^2 test, with a *P* value of 0.05 considered to be significant.

2. Results

Screening of *GCK*, *HNF1A*, *HNF4A*, *INS*, and *HNF1B* in 354 GDM women revealed a total of 50 different variants in the target region (Supplemental Table 1), of which 17 were classified as pathogenic or possibly pathogenic. The variants were found in 21 patients, resulting in a 5.9% (95% CI: 3.5% to 8.4%) prevalence of possible diabetes-predisposing gene variants (Fig. 1).

A. Variants Identified in GCK

Six possibly pathogenic *GCK* variants were found in seven individuals. Four carriers were diagnosed with diabetes previous to follow-up; yet, at follow-up, all carriers had overt diabetes or prediabetes (Supplemental Table 2). Of the four patients with diagnosed diabetes, one received insulin treatment. The remaining three diagnosed patients were either diet treated (n = 2) or treated with an oral hyperglycemic agent (OHA) (n = 1). When comparing the phenotypic characteristics, hemoglobin A1c (HbA1c) was significantly higher in women with
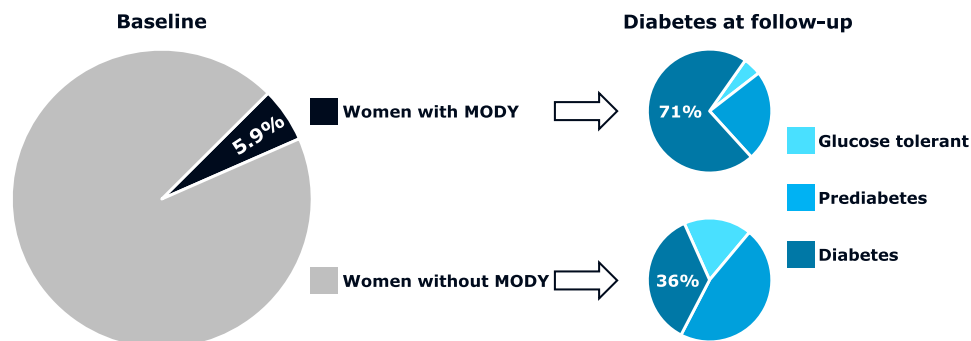
Figure 1. Schematic presentation of the prevalence of GDM patients with diabetes-predisposing variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, or *INS* in the total GDM population and the distribution of glucose tolerance status.

Table 2. Summarized Phenotypic Characteristics of Women With Diabetes-Predisposing Variants and Women Without

	GCK Variant Carriers (n = 7)	HNF1A Variant Carriers (n = 7)	HNF4A Variant Carriers (n = 6)	INS Variant Carrier (n = 1)	MODY Gene Variant Carriers (Group 1) (n = 21)	GAD-Negative Women With No Variants (Group 2) (n = 333)	P Value, Group 1 vs Group 2
Age (y)	47.0 (41.3–50.0)	42.0 (38.0–43.0)	47.5 (42.3–49.8)	41	43.0 (41.00–49.0)	43.0 (37.0–48.0)	0.9
Weight prepregnancy (kg)	68.0 (59.5–91.0)	71.0 (60.0–83.5)	72.0 (62.0–103)	73.9	72.0 (59.5–89.5)	68.00 (60.0–83.0)	0.6
Weight at follow-up (kg)	97.0 (68.4–102)	73.9 (62.1–87.4)	69.9 (62.1–83.5)	74.1	74.1 (63.5–97.0)	75.1 (65.2–89.0)	1.0
BMI prepregnancy (kg/m^2)	24.2 (21.9–33.6)	24.2 (23.4–24.4)	24.3 (22.5–37.8)	26.2	24.3 (22.6–30.8)	24.6 (21.6–29.8)	0.5
BMI at follow-up (kg/m^2)	36.2 (25.7–37.2)	25.6 (24.9–29.3)	25.8 (22.9–31.3)	27.6	27.5 (24.3–33.1)	27.6 (23.9–32.9)	1.0
Waist/hip ratio	0.83 (0.81–0.86)	0.83 (0.80–0.86)	0.89 (0.80–0.92)	0.85	0.84 (0.79–0.88)	0.82 (0.78–0.87)	0.5
HbA1C (%)	6.10 (6.10–6.75)[a]	5.40 (5.05–6.70)	7.25 (6.13–9.35)[a]	5.40	6.10 (5.40–7.10)	5.4 (5.00–6.10)	0.007
Fasting plasma glucose (mmol/L)	7.70 (6.85–8.95)	6.20 (6.00–7.65)	9.30 (7.92–13.4)[a]	5.90	7.68 (6.05–9.83)	6.20 (5.68–7.29)	0.04
Fasting serum insulin (pmol/L)	51.0 (29.3–72.3)	62.3 (41.5–78.1)	41.3 (20.5–74.0)	62.0	54.8 (31.0–80.1)	53.25 (35.5–76.0)	0.4
Fasting plasma HDL (mmol/L)	1.45 (1.23–1.67)	1.44 (1.38–1.52)	1.46 (1.11–1.85)	1.72	1.45 (1.23–1.72)	1.45 (1.22–1.75)	0.7
Fasting plasma triglycerides (mmol/L)	0.75 (0.73–1.18)	1.22 (1.06–1.29)	0.98 (0.73–1.24)	0.78	1.02 (0.74–1.25)	1.29 (0.92–1.90)	0.02
Fasting plasma FFA (mmol/L)	0.52 (0.43–0.70)	0.67 (0.48–0.88)	0.70 (0.61–0.77)	0.57	0.63 (0.51–0.77)	0.57 (0.42–0.75)	0.9
Serum hsCRP (mg/L)	4.90 (1.19–8.83)	0.61 (0.54–2.69)	1.75 (0.85–9.83)	7.58	1.74 (0.57–6.75)	1.86 (0.88–4.79)	0.6
Family history (n)	No parental: 3 Parental: 3 Unknown: 1	No parental: 3 Parental: 2 Unknown: 2	No parental: 1 Parental: 4 Unknown: 1	Parental diabetes	No parental: 7 Parental: 10 Unknown: 4	No parental: 154 Parental: 140 Unknown: 39	0.4
Treatment before follow-up (n)	Diet: 2 OHA: 1 Insulin: 1	Diet: 0 OHA: 0 Insulin: 1	Diet: 2 OHA: 4 Insulin: 0	NA	Diet: 4 OHA: 5 Insulin: 2	Diet: 19 OHA: 25 Insulin: 14	0.01
Diabetes status at follow-up (ADA)	Glucose-tolerant: 0 Prediabetes: 2 Diabetes: 5	Glucose-tolerant: 1 Prediabetes: 2 Diabetes: 4	Glucose-tolerant: 0 Prediabetes: 0 Diabetes: 6	Glucose-tolerant: 0 Prediabetes: 1 Diabetes: 0	Glucose-tolerant: 1 Prediabetes: 5 Diabetes: 15	Glucose-tolerant: 59 Prediabetes: 155 Diabetes: 120	0.002

Data are presented as median and interquartile range.
Abbreviations: ADA, American Diabetes Association; FFA, free fatty acids; NA, not applicable.
[a]$P < 0.05$.

GCK variants [6.1% (6.1 to 6.8)] compared with women without MODY gene variants [5.4% (5.0 to 6.1)]; $P = 0.04$), whereas no other clinical features at follow-up differentiated from those of women without diabetes-predisposing variants (Table 2).

B. Variants Identified in HNF1A

Five *HNF1A* diabetes-predisposing variants were found among seven carriers. The Gly288fs* variant was found in a carrier diagnosed with diabetes prior to follow-up and receiving insulin treatment (Supplemental Table 2). None of the remaining *HNF1A* variants carriers were diagnosed with diabetes prior to follow-up, despite three of them having overt diabetes and two being prediabetic. The phenotype of *HNF1A* variant carriers did not differ significantly from those of women not carrying diabetes-predisposing variants (Table 2).

C. Variants Identified in HNF4A

Five *HNF4A* diabetes-predisposing variants were found in a total of six individuals (Table 2). These individuals were all diagnosed with diabetes before follow-up. Two were diet treated, whereas the remaining four were treated with an OHA (Table 2); however, there is no information on the type of OHA selected. Despite diagnosing the patients before follow-up, fasting plasma glucose and HbA1c were significantly higher among the women with *HNF4A* variations [9.3 mmol/L (7.9 to 13.4) and 7.3% (5.7 to 7.3)] compared with women without [6.2 mmol/L (5.7 to 7.3) and 5.4% (5.0 to 7.3) $P = 0.03$ and $P = 0.01$, respectively] (Table 2).

D. Variants Identified in HNF1B

None of the two identified nonsynonymous variants in *HNF1B* were classified as diabetes predisposing (Supplemental Table 1).

E. Variants Identified in INS

Four variants were found in *INS*, of which one was classified as a diabetes-predisposing variant (Supplemental Table 1). This individual was 41 years of age and had prediabetes and a father with diabetes (Table 2; Supplemental Table 2).

F. Carriers vs Noncarriers

At follow-up, we compared the phenotypes of women with variants in the examined MODY genes to women without and found that women having variants in MODY genes had a higher level of fasting plasma glucose ($P = 0.01$) and HbA1c ($P = 0.007$) (Table 2). In addition, their levels of triglycerides were reduced compared with women without variants in the investigated genes ($P = 0.02$). None of the remaining biochemical or anthropometrical measured traits discriminated variant carriers from women with no MODY gene variants, including family history of diabetes (Table 2). Yet, despite an insignificant difference, 95% (n = 20) of women with diabetes-predisposing variants had IGT, IFG, or diabetes at follow-up compared with only 82% (n = 275) among women without variants ($P = 0.1$) (Table 2).

In total, 135 women had diabetes at follow-up either based on diagnosis before follow-up examination or based on the performed oral glucose tolerance test. Fifteen out of these 135 (11%) were carriers of MODY gene variants. Thus, the prevalence of diabetes at follow-up among individuals not carrying MODY gene variants was 36% (95% CI: 31% to 41%) (n = 120), in contrast to 71% (95% CI: 52% to 91%) (n = 15) in carriers, revealing a significant difference in diabetes prevalence between these two groups ($P = 0.002$) (Fig. 1; Table 2). However, the phenotypic characteristics of the 15 diabetic women having variants in MODY genes did not differ from the remaining 120 women with diabetes at follow-up (Supplemental Table 3).

3. Discussion

In the current study, we found a 5.9% prevalence of diabetes-predisposing variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, or *INS* among women diagnosed with diet-treated GDM. Furthermore, 71% of women with variants in the examined MODY genes had diabetes at 10-years

follow-up compared with only 36% of the women not having variants in these genes. Thus, 11% of the total number of diabetic women at follow-up had diabetes-predisposing variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, or *INS*. This result also reveals that a segment of the carriers do not develop diabetes within 10 years follow-up. Yet, 95% of carriers had a dysregulated glucose metabolism, with only one individual being normoglycemic at follow-up among variant carriers. A previous study found a 0.5% and 1.5% prevalence of likely diabetes-causing variants in *HNF1A*, *GCK*, *HNF4A*, and *HNF1B* in two community-based study populations [34], with a large proportion of carriers being normoglycemic. Thus, nondiabetic variant carriers have been encountered in previous studies of unselected populations.

Most previous studies have included fewer GDM women than the present, and no study has investigated the combined prevalence of mutations in common MODY genes among GDM patients. The prevalence of diabetes-predisposing *GCK* variants in the current study is consistent with previous studies of *GCK* variants among GDM women. In contrast, among the few previous studies investigating the prevalence of *HNF1A* and *HNF4A* variants in women with GDM, lower prevalences have been found. These studies were performed in GDM women with a positive family history. This may suggest that selecting patients based on a family history underestimates the prevalence of diabetes-predisposing variants and enriches for more penetrant mutations. This is in line with our finding that a positive family history of diabetes is not a marker for carriers of diabetes-predisposing variants in our study. The prevalence of *HNF1A* and *HNF4A* diabetes-predisposing variants is similar to what was reported in a population-based study of rare variants in genes for a dominant Mendelian form of diabetes [34]. Interestingly, a recent study of the genetic architecture of type 2 diabetes identified a widespread enrichment for type 2 diabetes association among rare coding alleles in genes that are causal for monogenic diabetes [35]. Age of diabetes diagnosis was no lower in variant carriers than noncarriers. In line with the studies of the genetic architecture of type 2 diabetes, the variants identified among GDM women in the current study do not likely have sufficient penetrance to drive familial segregation or early-onset diabetes, but still they increase the predisposition to diabetes. Furthermore, GDM *HNF1A* variant carriers have decreased hsCRP compared with carriers of variants in other genes, indicating that the identified *HNF1A* mutations are functional. Yet, hsCRP levels are higher than reported among *HNF1A* MODY patients [26]. This further indicate that the variants are diabetes predisposing but not of sufficient penetrance to cause clinical MODY.

New pregnancy-specific screening criteria have been suggested to identify women with *GCK* MODY. The criteria include a fasting plasma above 5.5 mmol/L during pregnancy and a prepregnancy body mass index (BMI) $<$25 kg/m^2 as the best discriminators between MODY and GDM [15]. However, we did not find that prepregnancy BMI below 25 kg/m^2 improved diagnosis of GDM women carrying *GCK* mutations (Table 2; Supplemental Table 2).

We establish that close to 6% of the women having diet-treated GDM have diabetes-predisposing variants in the examined MODY genes. Diagnosing such variants in women with GDM might be important in relation to treatment. Discontinuation of treatment after pregnancy in the two women carrying *GCK* variants and receiving insulin and OHA treatment, respectively, should be considered. However, this should be evaluated in the light of other phenotypic characteristic such as fasting plasma glucose of 12.1 mmol/L and a BMI of 37 kg/m^2, in which case, type 2 diabetes in addition to *GCK* MODY should be considered. Diagnosing diabetes-predisposing variants in MODY genes among patients with GDM might be important not only for treatment during pregnancy, but also for treatment and prognosis of GDM women after delivery.

A limitation to this study is the lack of direct functional investigation of all identified variants, which may result in the exclusion of truly functional variants and the inclusion of nondeleterious variants. However, using stringent selection criteria for the classification of pathogenic variants based on variant location, type of variant, allele frequency, absence in healthy controls, previous described involvement in MODY, functionality, and phenotypic presentation of carriers, we believe we have circumvented this issue to a large extent.

Women were selected only if they had been diagnosed with diet-treated GDM. We may have identified a higher prevalence of especially non-*GCK* MODY if women diagnosed with more

severe insulin-treated forms of GDM also been included in this study. Approximately 15% of GDM women were treated with insulin at the center from which the women in the current study were recruited [7]. Selecting only women with diet-treated GDM may also have introduced a bias toward identification of less pathogenic variants, as the effects of the variants as mentioned are likely a spectrum with various effect sizes.

4. Conclusion

With almost 6% of Danish women with diet-treated GDM having diabetes-predisposing variants in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, or *INS*, this study indicates that an increased focus on screening these MODY genes among women with GDM is warranted. Correct diagnosis is important to ensure optimal counseling and treatment during pregnancy as well as identifying women having a significant increased risk of developing diabetes only a few years after delivery. Furthermore, diagnosis is important in relation to the screening of children of MODY mutation carriers, which could help early treatment initiatives in the offspring generation. Further studies of the impact on disease history and treatment response of variants identified in *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, or *INS* among women with GDM are warranted.

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