



Systematic Genetic Study of Youth With Diabetes in a Single Country Reveals the Prevalence of Diabetes Subtypes, Novel Candidate Genes, and Response to Precision Therapy

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Identifying gene variants causing monogenic diabetes (MD) increases understanding of disease etiology and allows for implementation of precision therapy to improve metabolic control and quality of life. Here, we aimed to assess the prevalence of MD in youth with diabetes in Lithuania, uncover potential diabetes-related gene variants, and prospectively introduce precision treatment. First, we assessed all pediatric and most young-adult patients with diabetes in Lithuania ($n = 1,209$) for diabetes-related autoimmune antibodies. We then screened all antibody-negative patients ($n = 153$) using targeted high-throughput sequencing of >300 potential candidate genes. In this group, 40.7% had MD, with the highest percentage (100%) in infants (diagnosis at ages 0–12 months), followed by those diagnosed at ages >1–18 years (40.3%) and >18–25 years (22.2%). The overall prevalence of MD in youth with diabetes in Lithuania was 3.5% (1.9% for *GCK* diabetes, 0.7% for *HNF1A*, 0.2% for *HNF4A* and *ABCC8*, 0.3% for *KCNJ11*, and 0.1% for *INS*). Furthermore, we identified likely pathogenic variants in 11 additional genes. Microvascular complications were present in 26% of those with MD. Prospective treatment change was successful in >50% of eligible candidates, with C-peptide >252 pmol/L emerging as the best prognostic factor.

Monogenic forms of diabetes are estimated to account for 1–2% of all diabetes cases, with a higher prevalence in the pediatric population (1–6). Monogenic diabetes (MD) includes all diabetes arising from a single gene defect, such as neonatal diabetes mellitus (NDM), defined as onset of diabetes within the first 6 months of life; maturity-onset diabetes of the young (MODY); and syndromic diabetes (7). Because of the complexity of the clinical phenotypes, a genetic test is mandatory to formally diagnose MD. Thanks to next-generation sequencing techniques, genetic testing has advanced considerably even as costs have dropped, yet most cases of MD remain undiagnosed or misdiagnosed worldwide (4). Recent data from a pediatric multinational registry show that 38% of *HNF4A* and *HNF1B* diabetes are misclassified as type 1 or 2 diabetes (8). Among young people in the U.K., 50% of MD cases may be misdiagnosed as type 1 diabetes. The diagnosis of MD is crucial because treatment tailored to the underlying gene defect optimizes metabolic control and quality of life.

The main aim of this study was to assess the prevalence and clinical manifestations of MD in youth with diabetes in Lithuania, a genetically quite homogenous population, and to search for additional relevant gene variants. For this purpose, we systematically screened a registry covering

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100% of the pediatric and 70% of the young-adult population with diabetes, using high-throughput sequencing of 307 genes associated with diabetes, including *MODY*, *NDM*, and syndromic diabetes. We further prospectively assessed the predictive factors for successful treatment change from insulin to sulfonylurea according to the diabetes subtype.

RESEARCH DESIGN AND METHODS

Study Participants

This study included participants from a joint Lithuanian-Swiss project, consisting of the whole Lithuanian pediatric diabetes population (ages 0–18 years) and 70% of those ages >18–25 years with diabetes (the coverage was only 70% because many had left the country and some refused to participate). At study entry, all participants were screened for autoimmune markers for type 1 diabetes (antibodies to GAD65, tyrosine phosphatase [IA-2], and insulin [IAA]), as described earlier (9).

Diabetes Onset Data

Data on diabetes manifestation (age at onset, glycated hemoglobin [HbA_{1c}], fasting C-peptide, glycemia levels), treatment at diabetes onset, birth weight, and gestational age were collected retrospectively from medical files. Ketosis was defined by the presence of diabetic ketoacidosis or urine ketone bodies $\geq 1+$ at diabetes diagnosis.

Study Entry Data

All patients were investigated in a single research center at the Department of Endocrinology of the Lithuanian University of Health Sciences Kauno Klinikos. Clinical data and blood samples were collected during the first visit, and microvascular complications were assessed.

Laboratory Analyses

All autoimmune markers (GAD65, IA-2, IAA) were measured by radioimmunoassay. The cutoffs for positivity were >1.0 units/mL for GAD65 and IA-2 and ≥ 0.4 units/mL for IAA.

Genetic Analysis

Genetic analyses were performed by high-throughput sequencing of DNA and selected for all coding and splicing region of 307 genes (14 *MODY* and 28 *NDM* genes, genes involved in glucose homeostasis, pancreas development, and β -cell function [Supplementary Table 1]). The DNA regions were captured by bait using the HaloPlex technology (Agilent Technologies, Santa Clara, CA) (for detailed variant analysis, see Supplementary Data).

The mitochondrial mutation (m.3243A>G) was analyzed by real-time PCR and melting curve analysis.

Data After Molecular Diagnosis

After molecular diagnosis, patients with variants in known *MODY* genes were invited to the clinic for a follow-up. Clinical data and blood samples were taken to measure fasting C-peptide levels and HbA_{1c} . In the context of the

treatment change trial, patients with *HNF1A*, *HNF4A*, *KCNJ11*, and *ABCC8* diabetes were switched from insulin injections to oral sulfonylurea therapy. Three months after the treatment change, glycemic control was again assessed.

Statistical Analyses

SPSS software, version 22.0, was used for all statistical analyses. Data are presented as mean \pm SD, with minimal and maximal values expressed in parentheses, unless stated otherwise. For normally distributed data, Student two-tailed *t* test (for continuous variables) or the χ^2 test (for categorical variables) was used. For nonnormal distributions, we used the Mann-Whitney *U* test. Fisher exact test was applied for contingency tables. $P < 0.05$ was defined as statistically significant, and all tests were two tailed.

Bioethics

The study was approved by the local and national ethics committees (no. BE-2–5), and all patients and their parents or legal guardians gave informed consent. The study was carried out in accordance with the 1964 Declaration of Helsinki and its later amendments.

Data and Resource Availability

The data sets generated during or analyzed during the current study are not publicly available due to patient confidentiality. Data sets will be available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during the current study.

RESULTS

Participant Selection and General Characteristics of the Cohort

We screened 1,209 patients with diabetes for autoimmune diabetes antibodies (GAD65, IA-2, and IAA) because antibody positivity is a good discriminatory marker for MD (10) and selected 153 patients for further genetic testing (Fig. 1). The selected patients were autoantibody negative ($n = 86$) or positive only for IAA ($n = 65$ [if they had been tested after the introduction of insulin treatment]). In addition, we included patients for whom we had a strong suspicion of MD ($n = 2$), regardless of positivity for antibodies (11) (Fig. 1). Basic clinical characteristics are represented in Table 1. A higher prevalence of variants in known MD genes was found in the antibody-negative group versus the IAA⁺ group (40.7% vs. 9.2%, $P < 0.001$) (Table 1).

Genetic Results

We identified variants in known diabetes-associated genes in 42 participants, who then were classified as having MD. Of all participants with MD, 16.7% had positive antibodies (7 of 42). *GCK* diabetes was the most common form of all diabetes subtypes in the cohort, present in 54.8% (23 of 42) of the MD patients and in 25.6% (22 of 86) of those who were antibody negative (Supplementary Tables 2 and 3). *HNF1A* diabetes was the second most common form identified, with 19.0% (8 of 42) in the MD group and 8.1%

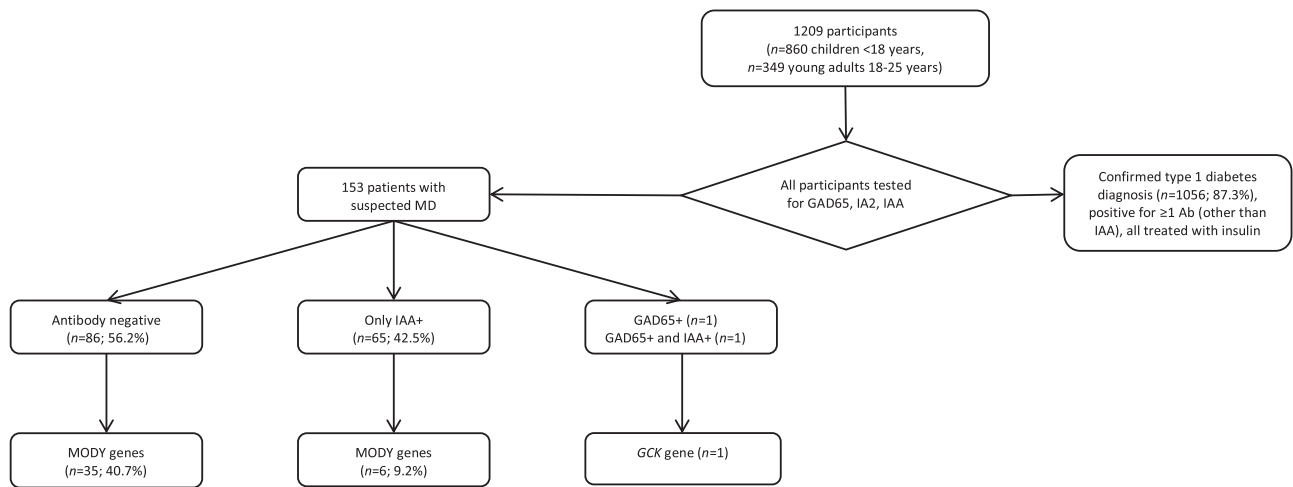


Figure 1—Outline of the study. All 1,209 participants were tested for GAD65, IA-2, and IAA antibodies (Ab). A total of 153 individuals were selected for genetic testing with suspected MD based on one of the following criteria: antibody negative, IAA⁺ after introduction of insulin treatment, no insulin requirement, positive family history (one first-degree relative or diabetes in three generations), syndromic diabetes.

(7 of 86) in the antibody-negative group (Supplementary Table 2). *HNF4A* variants were much rarer, identified in only 7.1% (3 of 42) of all patients with MD and in 1.2% (1 of 86) of the antibody-negative group. Four patients in the cohort had permanent NDM (Supplementary Table 2), all resulting from *KCNJ11* changes (9.5% [4 of 42]) (Supplementary Table 3). We found two novel *ABCC8* variants, representing 4.8% (2 of 42) of the patients with MD and 1.2% (1 of 86) of the antibody-negative group. A novel *INS* variant located in the A chain and introducing a stop codon was discovered in one patient (Supplementary Table 2). We

also identified a novel *KLF11* variant in one child with a mild developmental delay (Supplementary Table 2). We did not detect the mitochondrial mutation 3243A>G in any patients.

Additional Potential Diabetes Genes

In this study, we detected likely pathogenic variants in 11 additional potential diabetes genes, with all variants classified according to the American College of Medical Genetics and Genomics (12). Six of the genes are expressed in human β-cells at the RNA level (13) (Table 2). Among

Table 1—Basic clinical characteristics of the cohort at diabetes diagnosis and comparison according to autoimmunity status

	All	Ab ⁻ group	Only IAA ⁺ group	Other*	P (comparing Ab ⁻ and only IAA ⁺ groups)
% (n)	153	56.2 (86)	42.5 (65)	4.8 (2)	NA
Female, % (n)	47.7 (73)	53.5 (46)	40 (26)	50 (1)	0.1
Age at diabetes diagnosis, months	106 ± 68	124 ± 71	82 ± 56	135 ± 16	<0.001
Glycemia, mmol/L	15.8 ± 9.4	14.6 ± 10.9	18.3 ± 6.5	9.4 ± 2.2	0.028
HbA _{1c} , % (mmol/mol)	8.2 ± 2.3 (66 ± 2)	7.9 ± 2.9 (63 ± 9)	9.1 ± 2.9 (76 ± 9)	7.1 ± 0.8 (54 ± 1)	0.98
C-peptide, pmol/L	800 ± 2,100†	1,000 ± 2,600‡	400 ± 400§	1,000	0.107
Ketosis, % (n)	51.4 (74 of 144)	44.6 (37 of 83)	62.7 (37 of 59)	0	0.033
OHA, % (n)	3.9 (5 of 129)	6.9 (5 of 72)	0	0	<0.001
Insulin, % (n)	65.1 (84 of 129)	47.2 (34 of 72)	90.9 (50 of 55)	0	<0.001
Insulin dose, units/kg/day	0.76 ± 0.46	0.7 ± 0.4	0.9 ± 0.5	—	0.135
Family history of diabetes, % (n)	47.7 (73 of 153)	55.8 (48 of 86)	36.9 (24 of 65)	50 (1 of 2)	0.021
Variants found in MODY genes, % (n)	27.5 (42 of 153)	40.7 (35 of 86)	9.2 (6 of 65)	50 (1 of 2)	<0.001

Data are mean ± SD unless otherwise indicated. The 153 individuals selected for genetic analysis were divided into three groups according to their autoimmunity status (antibody negative, only IAA⁺, and GAD65⁺), and clinical characteristics were compared. Ab, antibody; OHA, oral hypoglycemic agents. *One patient GAD65⁺ and one patient GAD65⁺ and IAA⁺. †Valid data for 57 of 153 cases. ‡Valid data for 37 of 86 cases. §Valid data for 20 of 65 cases. ||Valid data for 1 of 2 cases.

Table 2—Variants identified in additional genes

Patient no.	Gene	DNA change	Protein effect	PolyPhen-2	SIFT	Class	gnomAD allele frequency	Molecular function	RNA expression in human β -cells (RPKM)*
43	CASP10	c.104del	p.Asp35Valfs*10	—	—	4	—	Protease	0.2
44	DACH1	c.454_456dup	p.Ser152dup	—	—	4	—	DNA binding	6.9
45	GCKR	c.1484T>G	p.Val495Gly	0.96	0	4	—	Carbohydrate binding	0.1
6†	HGFAC	c.711C>G	p.Cys237Trp	1	0	4	0.00026	Protease	0
46	HGFAC	c.711C>G	p.Cys237Trp	1	0	4	0.00026	Protease	0
18†	KCNQ1	c.498C>A	p.Phe166Leu	0.967	0.05	4	—	Potassium channel	0.3
47	MC4R	c.230C>T	p.Ser77Leu	0.855	0	4	—	G-protein-coupled receptor	0
48	RFX2	c.1894G>A	p.Ala632Thr	0.999	0	4	—	DNA binding	3.9
49	RREB1	c.3218C>T	p.Ser1073Leu	0.988	0.02	4	—	DNA binding	9
50	SLC5A1	c.932A>T	p.Lys311Met	0.999	0	4	—	Glucose:sodium symporter	3.5
51	SLC5A1	c.1415T>C	p.Leu472Pro	0.892	0	4	—	Glucose:sodium symporter	3.5
52	SLC5A1	c.1415T>C	p.Leu472Pro	0.892	0	4	—	Glucose:sodium symporter	3.5
53	TMPRSS6	c.2263A>G	p.Lys755Glu	0.998	0.01	4	—	Peptidase	4.4
54	ZBED3	c.211C>G	p.Leu71Val	0.977	0	4	—	DNA binding	8.3

Summary of all class 4 (likely pathogenic) variants identified in genes not yet associated with MD. Classification of the variants according to the methodology of Richards et al. (12). PolyPhen-2 score considered pathogenic if >0.47 and SIFT <0.05. gnomAD, Genome Aggregation Database; RPKM, reads per kilobase per million mapped reads. *Nica et al. (13). †The variants in the HGFAC and KCNQ1 genes are not likely to be causative for diabetes because the two identified carriers also had a GCK variant and a classical GCK diabetes phenotype. The HGFAC variant may also be considered to be too frequent in the gnomAD database to be an MD variant (26).

these, four (*DACH1*, *RFX2*, *RREB1*, and *ZBED3*) encode transcription factors, *SLC5A1* encodes a sodium–glucose cotransporter, and *TMPRSS6* encodes a protease involved in iron metabolism.

Microvascular Complications

Microvascular complications were present in both patients with variants in the *ABCC8* gene, in three of four patients with *KCNJ11* variants, and in two of three patients with *HNF4A* variants (Supplementary Table 2). Surprisingly, 17.4% of patients with *GCK* diabetes had microalbuminuria.

Prospective Precision Treatment

Of 42 patients with variants in known MD-related genes, 16 were offered precision therapy after the genetic diagnosis. Six patients with *HNF1A* diabetes and three patients with *KCNJ11* NDM were successfully switched to sulfonylurea agents while the insulin substitution was stopped (Table 3).

DISCUSSION

In this study, we found that MD prevalence was 3.5% (42 of 1,209) and MODY prevalence was 3.14% (38 of 1,209) in the pediatric and young-adult population with diabetes in Lithuania. There was a high pickup rate of MD, with 40.7% (35 of 86) among patients who were antibody negative. These results are higher than reported in previous systematic studies, such as the Norwegian Childhood Diabetes Registry, where 6.5% of antibody-negative diabetes cases involved MODY (14). In that Norwegian study, however, patients diagnosed in an outpatient setting were largely missing from the registry, which was based only on hospital admissions.

The prevalence of MD identified here in the young population with diabetes in Lithuania also is higher than in other countries, including Norway (1.1%) (2), Germany (up to 2.3%) (3), the U.K. (2.5%) (4), New Zealand (2.5%) (5), and the U.S. (1.2%) (6). One explanation for the overall difference is the consistency of our cohort, which included all children with diabetes nationwide and most young adults. In addition, we performed genetic testing using a broad next-generation sequencing panel that included MODY and NDM genes for all patients with suspicion of MD and thus had a larger number of genes tested. Our results are consistent with a Polish nationwide study, however, that yielded a prevalence of MD of 3.1–4.2% (15), which possibly traces to similar ancestry among people living in these neighboring countries.

GCK diabetes was the most common MD form, followed by *HNF1A* and *KCNJ11* in our cohort.

The prevalence of permanent NDM in Lithuania was 0.33% (4 of 1,209), which is slightly higher than the reported prevalence of 0.22% in the SEARCH for Diabetes in Youth (SEARCH) study, 0.15% in Norway (2), and 0.16% in Italy. No patient had diabetes associated with the mitochondrial 3243A>G mutation, possibly because the mean age of diabetes onset is rather after the third decade (16).

Table 3—Comparison of patients with successful and unsuccessful treatment switch

	Patients with successful treatment change (n = 9)	Patients with unsuccessful treatment change (n = 7)	P*
Age at diabetes diagnosis, months	178 (1; 262)	120 (1; 159)	0.203
Duration of diabetes until treatment change, months	54 (16; 267)	221 (30; 336)	0.031
Age at treatment change, months	264 (17; 313)	321 (160; 347)	0.023
HbA _{1c} before treatment change, % [mmol/mol]	6.8 (5.5; 11.6) [51 (37; 103)]	8.7 (7.3; 13.1) [72 (56; 120)]	0.080
HbA _{1c} after treatment change, % [mmol/mol]	6.4 (5.6; 10.2) [46 (38; 88)]	11.6 (7.6; 12.8) [103 (60; 116)]	0.011
C-peptide before treatment change, pmol/L#	498 (220; 1,140)	65 (1; 252)	0.005
Insulin treatment, n (%)	8 (89)	7 (100)	1\$
Insulin dose before treatment change, units/kg/day	0.5 (0.2; 1.3)	0.9 (0.6; 1.1)	0.117

Data are median (minimum; maximum) unless otherwise indicated. Summary of the 16 patients who were offered treatment switch after genetic diagnosis. Nine patients (six with *HNF1A* and three with *KCNJ11* diabetes) could successfully be treated with sulfonylureas. Seven patients (three with *HNF4A*, two with *ABCC8*, one with *HNF1A*, and one with *KCNJ11* diabetes) had to restart insulin therapy. *Mann-Whitney *U* test. \$Fisher exact test. #One missing value in both groups.

We report several novel, likely pathogenic gene variants that may affect diabetes development. Four genes—*DACH1*, *RFX2*, *RREB1*, and *ZBED3*—are transcriptional regulators and expressed in human β -cells. *Dach1* gene deletion in mice reduces the numbers of β -cells and blocks perinatal β -cell proliferation (17). *RFX2* is a member of the RFX protein family, which includes *RFX3* and *RFX6*—both known diabetes-associated genes (18,19). *RREB1* is a transcription factor that binds specifically to the RAS-responsive elements of gene promoters and potentiates the transcriptional activity of *NEUROD1* (20), a known MODY-related gene. In general, RAS-responsive element binding augments RAS-mediated transcription response of that promoter. In addition, coding variants in *RREB1* are associated with type 2 diabetes, and recent data suggest a role in β -cell function and development (21). Thus, *RREB1* variants with more dramatic effects could be involved in childhood diabetes, and those with a smaller effect might have a role in type 2 diabetes. Such a role has been described for variants of *KCNJ11* and *HNF4A*, which have been associated with MODY or type 2 diabetes, depending on how the variant affects protein function (22,23).

In line with the proposed approach of precision medicine, we performed a prospective treatment change in participants with *HNF1A*, *HNF4A*, *KCNJ11*, and *ABCC8*

diabetes. The most significant predictor for a positive response to sulfonylurea treatment was a higher C-peptide level, followed by age at treatment change and diabetes duration. The latter two factors have recently been associated with treatment switch success (24), and our findings confirm this link.

The main strength of our study is the coverage of 100% of pediatric patients with diabetes and the majority of young-adult patients in Lithuania. Moreover, we evaluated autoimmunity status for all participants and recruited for genetic testing only those with negative GAD65 and IA-2 or with strong suspicion for MD. In our genetically analyzed cohort, GAD65 and IA-2 positivity was 2.4%, while others have reported <1% positivity in patients with MODY (10).

Our pickup rate of 40.7% justifies screening for MD in autoimmune antibody-negative pediatric patients, even if the analysis is done after diabetes onset. Of note, in the IAA⁺ subgroup, 6 (9.2%) had a positive genetic result, so children who are positive for IAA antibodies and treated with insulin should not be excluded from genetic testing.

Follow-up studies in patients in whom we could not identify any suggestive genetic variant will include a genetic risk score analysis for type 1 diabetes (25). To further evaluate the impact of possible mutations on novel genes, we plan to perform functional analysis in vitro for selected variants to reveal their contribution to diabetes development.

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Author Contributions. I.S. participated in data collection and database creation, carried out statistical analyses, and wrote the manuscript. R.V. participated in the conception, design, and coordination of the study. J.-L.B. carried out the study design for genetic analysis, supervised the genetic analyses, and carried out data interpretation. P.K. helped to set up the database and participated in manuscript editing. R.D. participated in the conception and design of the study and coordinated pediatric patient data collection. E.D. participated in the design of the study and coordinated adult patient data collection. M.D. participated in manuscript editing. F.S. programmed the pipeline for genetic data analysis and analyzed the genetic data. D.R.-V. participated in adult patient enrollment and in data collection and database creation. D.M. participated in the conception, design, and coordination of the study. E.J. participated in pediatric patient enrollment. G.M. participated in pediatric patient enrollment. V.M.S. participated in the conception

and design of the study, contributed to discussion, and edited and revised the manuscript. V.M.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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