ORIGINAL ARTICLE

Mutation search within monogenic diabetes genes in Polish patients with long-term type 1 diabetes and preserved kidney function

Jerzy Hohendorff[1,2*], Magdalena Kwiatkowska[1,2*], Dorota Pisarczyk-Wiza[3], Agnieszka Ludwig-Słomczyńska[4], Magdalena Milcarek[3], Przemysław Kapusta[4], Barbara Zapała[5], Beata Kieć-Wilk[1,2], Iwona Trznadel-Morawska[1,2], Magdalena Szopa[1,2], Dorota Zozulińska-Ziółkiewicz [3], Maciej T. Małecki[1,2]

1 Department of Metabolic Diseases, Jagiellonian University Medical College, Kraków, Poland
2 University Hospital in Krakow, Kraków, Poland
3 Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences, Poznań, Poland
4 Center for Medical Genomics OMICRON, Jagiellonian University Medical College, Kraków, Poland
5 Department of Clinical Biochemistry, Jagiellonian University Medical College, Kraków, Poland

KEY WORDS

maturity-onset diabetes of the young, microvascular complications, next-generation sequencing, type 1 diabetes

ABSTRACT

INTRODUCTION Some patients with type 1 diabetes (T1DM) are free from advanced complications despite long-standing disease. These patients may be carriers of gene mutations responsible for maturity-onset diabetes of the young and may have been misdiagnosed with T1DM.

OBJECTIVES We aimed to determine the clinical characteristics of patients with long-term T1DM, without advanced microvascular complications, and with well-preserved kidney function. A search for mutations in monogenic diabetes genes was performed.

PATIENTS AND METHODS Patients were recruited at 2 Polish university centers based on the following criteria: T1DM duration of 40 years or longer and absence of advanced complications defined as chronic kidney disease (estimated glomerular filtration rate [eGFR] <60 ml/min/1.73 m^2), overt proteinuria, blindness, and diabetic foot syndrome. Mutations in the 7 most frequent monogenic diabetes genes were identified using next-generation sequencing.

RESULTS We enrolled 45 patients with T1DM (mean [SD] age at examination, 59.2 [8.0] years; mean [SD] age at T1DM diagnosis, 14.6 [6.7] years). Mean (SD) hemoglobin A_{1c} levels were 7.6% (1.4%); daily insulin dose, 0.48 (0.17) U/kg; high-density lipoprotein (HDL) cholesterol levels, 1.9 (0.6) mmol/l; body mass index (BMI), 26.4 (5.0) kg/m^2; and eGFR, 82.2 (12.1) ml/min/1.73 m^2. Albuminuria and retinopathy were reported in 7 and 39 patients, respectively. We were not able to assign a causative role to any of 10 genetic variants identified by next-generation sequencing in this cohort.

CONCLUSIONS Patients with long-term T1DM and preserved kidney function have good glycemic control, elevated HDL cholesterol levels, low insulin requirements, near-normal BMI, and a rare occurrence of mutations in monogenic diabetes genes.

Correspondence to:
Maciej T. Małecki, MD, PhD, Jagiellonian University Medical College, Department of Metabolic Diseases, ul. Jakubowskiego 2, 30-688 Kraków, Poland, phone: +48 12 400 29 50, email: maciej.malecki@uj.edu.pl
Received: June 28, 2021.
Revision accepted: October 5, 2021.
Published online: November 26, 2021.
Pol Arch Intern Med. 2022; 132 (2): 16143
doi:10.20452/pamw.16143

* JH and MK contributed equally to this work.

INTRODUCTION The average life expectancy of patients with type 1 diabetes (T1DM) has increased significantly over the last decades.[1] However, recent studies have reported an estimated loss in life expectancy of 11 to 13 years in patients with T1DM as compared with the general population.[2,3] Moreover, cardiovascular disease remains the most common cause of death in these patients.[4-6] Risk factors for cardiovascular disease in this population include microvascular complications, age, diabetes duration, body mass index (BMI), hemoglobin A_{1c} (HbA_{1c}), hypertension, and dyslipidemia.[7,8] The number and severity of microvascular complications were shown to

WHAT'S NEW?

This study is the first to determine the clinical characteristics of Polish patients with long-term type 1 diabetes and preserved kidney function in comparison with data from the Joslin 50-year Medalist Study and the Golden Years Cohort. In addition, patients were screened for mutations in monogenic diabetes genes. The study revealed that this highly selected group of patients was characterized by elevated levels of high-density lipoprotein cholesterol, near-normal body mass index, and low insulin requirements, while mutations within monogenic diabetes genes seemed to be rare.

be associated with an increased rate of all-cause mortality and cardiovascular events. Among these complications, chronic kidney disease seems to be the greatest risk factor for excess mortality.[9,10]

Although the available evidence supports potential benefits from using new insulins and technologies in diabetes management, numerous patients with T1DM still experience chronic microvascular complications that adversely affect their life expectancy and quality of life.[11,12] However, not all patients with long-term T1DM develop advanced microangiopathy, as reported in the Joslin 50-years Medalist Study and Golden Years Cohort.[13,14] This suggests that there may be some genetic and environmental factors that protect patients against microvascular complications. One of the possible factors is an elevated level of high-density lipoprotein (HDL) cholesterol,[15,16] while the others include normal BMI, lack of hypertension, low insulin requirement, HbA_{1c} near the treatment target (about 7%), and a family history of longevity.[15] Another possible protective factor is preserved insulin secretion. It was also hypothesized that the population of patients with long-term T1DM without advanced complications may include individuals with monogenic diabetes, especially maturity-onset diabetes of the young (MODY),[16] misdiagnosed as T1DM. The percentage of undiagnosed MODY in this specific group of patients is higher than in the general population of patients with T1DM.[16] In support of this hypothesis, published estimates showed that monogenic diabetes was misdiagnosed as T1DM or type 2 diabetes (T2DM) in the vast majority of cases (90%).[17] Both patients with monogenic diabetes and those with T1DM are usually slim and young at the time of diagnosis. Another supporting piece of evidence is that chronic complications are almost absent in glucokinase MODY (*GCK*-MODY).[18,19] They are also less prevalent and less severe in the other forms of MODY, such as the most frequent MODY3 caused by a mutation in the hepatocyte nuclear factor-1α gene, *HNF1A*.[18,19] In fact, in the Medalist Study, almost 8% of T1DM patients with a disease duration of 50 years were suspected to have monogenic diabetes.[16]

The aim of the present study was to determine the clinical characteristics of patients with long-term T1DM without advanced microvascular complications, with a particular focus on individuals with well-preserved kidney function. Additionally, patients were screened for mutations within a set of monogenic diabetes genes.

PATIENTS AND METHODS

Patients Patients diagnosed with T1DM were recruited at 2 Polish university hospitals in Kraków and Poznań. The inclusion criteria were as follows: T1DM duration of at least 40 years and absence of advanced complications defined as chronic kidney disease with an estimated glomerular filtration rate (eGFR) lower than 60 ml/min/1.73 m^2, overt proteinuria or previous kidney transplant, blindness in at least 1 eye, and diabetic foot syndrome (currently or in the past). After completing a standard questionnaire, all patients underwent physical examination. We collected data on sex, age at examination, age of diagnosis, weight, height, waist-to-hip ratio, blood pressure, daily dose of insulin, medication use, family history of diabetes, presence of chronic microvascular and macrovascular complications as well as comorbidities, and history of smoking. Fasting blood and first-pass urine samples were obtained for laboratory tests, including the measurement of urinary albumin-to-creatinine ratio and the levels of HbA_{1c}, C-peptide, creatinine, lipids, and high-sensitivity C-reactive protein.

Clinical retinal examination was performed by a trained ophthalmologist. Peripheral polyneuropathy was assessed using a 10-gram monofilament for tactile sensation, 128-Hz tuning forks for vibration sensation, and a rod with 2 different ends for temperature sensation. Polyneuropathy was diagnosed if 2 or more of the following criteria were met: the presence of symptoms, lack of the ankle reflex, and impaired sensation of touch, temperature, and/or vibration.[20]

Genetic testing Next-generation sequencing was used for detecting mutations in a set of selected monogenic diabetes genes.[21] Genomic DNA was extracted, libraries prepared, and data processed as described in detail previously.[21] We evaluated 7 genes that are the most frequent causes of monogenic diabetes (*GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *ABCC8*, *KCNJ11*, and *INS*) for potentially pathogenic variants, in line with a recent French study.[22] Variant scoring was based on the American College of Medical Genetics and Genomics (ACMG) guidelines. To predict the pathogenicity of the variants, the VarSome engine was used.[23,24]

Ethical approval The study was approved by the Bioethics Committee of Jagiellonian University Medical College in Kraków, Poland, and conducted in accordance with the 1975 Declaration of Helsinki, with subsequent revisions. All patients gave written informed consent to participate in the study.

Statistical analysis The parametric *t* test or the nonparametric *U* test was performed, as applicable, to describe the clinical characteristics

of patients and differences between individuals with or without diagnosed proliferative retinopathy. For nominal variables, the Fisher exact test was used. A multivariable logistic regression analysis was performed to identify factors associated with the presence of proliferative retinopathy and / or albuminuria. The parameters used to build the multivariable model included sex, age at onset, duration of diabetes, BMI, daily insulin dose, HbA_{1c}, hypertension, smoking, family history of diabetes, and the levels of C-peptide, low-density lipoprotein (LDL) cholesterol, HDL cholesterol, and triglycerides. A separate analysis was performed to examine the factors associated with macrovascular complications, such as previous myocardial infarction and / or stroke. In addition to the parameters listed above, the multivariable model in this analysis included also a number of microvascular complications (proliferative retinopathy, albuminuria, and peripheral polyneuropathy). Statistical analysis was performed using Statistica, version 13 (TIBCO Software Inc, Palo Alto, California, United States). A *P* value of less than 0.05 was considered significant.

RESULTS The study included 45 patients with T1DM (29 women and 16 men) with a mean (SD) age at examination of 59.2 (8.0) years and a mean (SD) age at diabetes onset of 14.6 (6.7) years. The mean (SD) BMI in the study group was 26.4 (5.0) kg/m^2. Moreover, patients had good glycemic control with a mean (SD) HbA_{1c} level of 7.6% (1.4%) (mean [SD], 59.3 [15.1] mmol/mol) and a mean (SD) daily insulin dose of 0.48 (0.17) units/kg. The mean (SD) eGFR was 82.2 (12.1) ml/min/1.73 m^2. Albuminuria was reported in 7 patients. There were no cases of overt proteinuria. Retinopathy was found in 39 participants (nonproliferative in 7 and proliferative in 32). Consistent with the inclusion criteria, there were no cases of blindness. Peripheral polyneuropathy was present in 24 participants. Cardiovascular disease, defined as coronary artery disease, stroke, or peripheral artery disease, was diagnosed in 20 individuals based on medical records. The clinical and biochemical characteristics of patients are shown in **TABLE 1**. The independent risk factors for proliferative retinopathy and / or albuminuria, identified by a backward stepwise elimination procedure, included T1DM duration (odds ratio [OR], 1.25; 95% CI, 1.02–1.53) and LDL cholesterol levels (OR, 2.83; 95% CI, 1.05–7.65). The only independent factor associated with myocardial infarction and / or stroke was smoking (OR, 2.83; 95% CI: 1.05–7.65).

The next-generation sequencing analysis identified 9 patients as carriers of 10 variants in the 7 analyzed genes; 1 patient was a carrier of 2 variants. The identified variants are summarized in **TABLE 2**, while the detailed clinical characteristics of mutation carriers are presented in **TABLE 3**.

Five variants were found in the *ABCC8* gene, including 2 missense mutations classified as likely pathogenic. The first mutation was a new Ala1410Thr variant found in a woman aged 8 at diagnosis and 53 at the time of the examination. She was on a rather low dose of insulin (26 U/d), and her glycemic control was good with an HbA_{1c} level of 7.1% (54.1 mmol/l). Her BMI was 20.0 kg/m^2. She was free from diabetic complications. Both her parents (aged 82 at the time of the study) were diagnosed with T2DM; however, they refused genetic testing. The other missense mutation, Arg1530Cys, was found in a female patient diagnosed with T1DM at the age of 16. At the time of examination, she was 60 years old, and she received intensive insulin therapy (multiple daily injections), with a daily insulin requirement of 31 units combined with metformin due to obesity. She also developed proliferative retinopathy. The Arg1530Cys missense mutation is also present in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) with uncertain significance annotation. However, our patient and her family were unavailable for further evaluation.

There were 2 new null variants in the *ABCC8* gene in our cohort, one frameshift (Ser1051fs/c.3150_3151insCT) and one splicing (c.2117--1G>C). Finally, the *ABCC8* Val849Ile variant detected in a single patient was classified as a sequence difference of uncertain significance according to the ACMG criteria.

Next, we found 2 missense variants in the *GCK* gene classified as likely pathogenic. One of them, a newly identified missense mutation, Glu22Asp, was observed in a female patient who was also a carrier of the *HNF1B* His336Asp variant. The other one, the His380Gln mutation, was previously reported but without data on the frequency and clinical significance.[25] Both female carriers were characterized by an insulin requirement typical for T1DM (daily dose of insulin, 35 U/d and 40 U/d, respectively) and good glycemic control (HbA_{1c}, 7.4% [57.4 mmol/mol] and 5.8% [39.9 mmol/mol], respectively). They also developed proliferative retinopathy requiring laser therapy, and their C-peptide levels were barely detectable (<0.1 ng/ml) at the time of examination.

We also detected an rs138986885 sequence difference corresponding to the His336Asp missense mutation in the *HNF1B* gene in 2 unrelated participants. This was classified as a variant of uncertain significance.

Finally, an rs137853242 variant was found in the *HNF1A* gene corresponding to the missense mutation Arg583Gln in exon 9. The female carrier of the rare Gln variant was diagnosed with T1DM at the age of 19, and her age at examination was 59. Her current C-peptide levels were almost undetectable (0.1 ng/ml), and her HbA_{1c} level was 9.5% (80.3 mmol/mol).

DISCUSSION This study reports the clinical, biochemical, and genetic characteristics of a highly selected group of Polish patients with long-term T1DM. Because our population included only patients without advanced

TABLE 1 Clinical and biochemical characteristics of the study group

Parameter		Study group (n = 45)	Retinopathy		*P* value
			No/Nonproliferative (n = 13)	Proliferative (n = 32)	
Sex, n (%)	Male	16 (35.6)	4 (8.9)	12 (26.7)	0.74
	Female	29 (64.4)	9 (20.0)	20 (44.4)	
Age, y		59.2 (8.0)	58.7 (7.2)	59.4 (8.5)	0.78
Age of diabetes onset, y		14.6 (6.7)	16.0 (5.3)	14.1 (7.1)	0.38
Diabetes duration, y		44.5 (41.0–47.0)	42.7 (40.0–45.0)	45.4 (41.5–47.5)	0.12[a]
Family history of diabetes, n (%)	Yes	16 (35.5)	2 (4.4)	14 (31.1)	0.09
	No	29 (64.4)	11 (24.4)	18 (40.0)	
BMI, kg/m^2		26.4 (5.0)	24.9 (3.9)	27.1 (5.4)	0.19
Waist-to-hip ratio	Male	0.94 (0.07)	0.90 (0.07)	0.95 (0.06)	0.21
	Female	0.85 (0.09)	0.84 (0.05)	0.86 (0.10)	0.73
HbA_{1c}, %		7.3 (6.7–8.4)	7.1 (6.7–8.2)	7.4 (6.7–8.5)	0.80[a]
HbA_{1c}, mmol/mol		56.8 (50.0–67.8)	54.1 (49.7–66.1)	57.4 (50.8–68.3)	0.80[a]
DDI, U		33.0 (13.5)	32.5 (12.4)	34.6 (14.0)	0.63
DDI, U/kg		0.48 (0.17)	0.47 (0.15)	0.49 (0.18)	0.82
HDL cholesterol, mmol/l		1.7 (1.4–2.3)	2.0 (1.7–2.5)	1.7 (1.4–2.2)	0.25[a]
LDL cholesterol, mmol/l		2.6 (0.8)	2.3 (0.9)	2.7 (0.8)	0.14
Triglycerides, mmol/l		1.0 (0.7–1.3)	0.9 (0.6–1.3)	1.1 (0.8–1.3)	0.13[a]
Hs-CRP, ug/ml		1.5 (0.6–3.3)	3.3 (0.4–4.8)	1.4 (0.7–3.0)	0.70[a]
C-peptide, ng/ml		0.03 (0.01–0.05)	0.04 (0.02–0.06)	0.03 (0.01–0.04)	0.17[a]
eGFR, ml/min/1.73m^2		82.2 (12.1)	81.0 (12.2)	82.8 (2.2)	0.67
Albuminuria, n (%)	Yes	7 (15.5)	0	7 (15.5)	0.09
	No	38 (84.4)	13 (28.9)	25 (55.5)	
Peripheral polyneuropathy, n (%)	Yes	24 (53.3)	5 (11.1)	19 (42.2)	0.32
	No	21 (46.7)	8 (17.8)	13 (28.9)	
Smoking (current or past), n (%)	Yes	6 (13.3)	1 (2.2)	5 (11.1)	0.66
	No	39 (86.7)	12 (26.7)	27 (60.0)	
Hypertension, n (%)	Yes	31 (68.9)	8 (17.8)	23 (51.1)	0.50
	No	14 (31.1)	5 (11.1)	9 (20.0)	
SBP, mmHg		125 (120–136)	130 (123–138)	125 (119–135)	0.26[a]
DBP, mmHg		70 (66–80)	78 (70–80)	70 (65–75)	0.049[a]
CVD, n (%)	Yes	20 (44.4)	4 (8.9)	16 (35.6)	0.33
	No	25 (55.6)	9 (20.0)	16 (35.6)	
Stroke, n (%)	Yes	3 (6.7)	1 (2.2)	2 (4.4)	>0.99
	No	42 (93.3)	12 (26.7)	30 (66.7)	
CAD, n (%)	Yes	17 (37.8)	3 (6.7)	14 (31.1)	0.31
	No	28 (67.2)	10 (22.2)	18 (40.)	
MI, n (%)	Yes	7 (15.6)	1 (2.2)	6 (13.3)	0.65
	No	38 (84.4)	12 (26.7)	26 (57.8)	
PAD, n (%)	Yes	4 (9.1)	1 (2.3)	3 (6.8)	>0.99
	No	40 (90.9)	11 (25.0)	29 (65.9)	
ACEIs/ARBs, n (%)	Yes	34 (77.3)	8 (18.2)	26 (59.1)	0.13
	No	10 (22.7)	5 (11.4)	5 (11.4)	
ASA, n (%)	Yes	26 (59.1)	7 (15.9)	19 (43.2)	0.74
	No	18 (40.9)	6 (13.6)	12 (27.3)	
Statins, n (%)	Yes	35 (79.5)	11 (25.0)	24 (54.5)	0.70
	No	9 (20.5)	2 (4.5)	7 (15.9)	

a *P* values were derived from the *U* test. In the remaining cases, *P* values were derived from the Fisher exact test or the *t* test.

Data are presented as mean (SD) or median (interquartile range) unless indicated otherwise.

Abbreviations: ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid; BMI, body mass index; CAD, coronary artery disease; hs-CRP, high-sensitivity C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; DDI, daily dose of insulin; eGFR, estimated glomerular filtration rate; HbA_{1c}, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; PAD, peripheral artery disease; SBP, systolic blood pressure

TABLE 2 Summary of the identified variants in monogenic diabetes genes

Patient ID	Type	Gene	Exon	Codon_ change	aa_change	VarSome prediction	VarSome predicted pathogenicity
6KL	snp	ABCC8	35	Gcc/Acc	p.Ala1410Thr/c.4228G>A	PM1, PM2, PP2, PP3	Likely pathogenic
21WM	snp	ABCC8	38	Cgc/Tgc	p.Arg1530Cys/c.4588C>T	PM1, PM2, PP2, PP3	Likely pathogenic
11ZT	indel	ABCC8	25	tgc/tgcCT	p.Ser1051fs/c.3150_3151insCT	PVS1, PM1, PM2, PPS3	Pathogenic
20FJ	snp	ABCC8	15	N/A	c.2117–1G>C	PVS1, PM2, PP3, PP5	Pathogenic
19MZ	snp	ABCC8	21	Gtt/Att	p.Val849Ile/c.2545G>A	PM1, PM2, PP2, BP4	Uncertain significance
37JA	snp	GCK	9	caC/caA	p.His380Gln/c.1140C>A	PVS1, PM2, BP4	Likely pathogenic
23SM	snp	GCK	2	gaG/gaT	p.Glu22Asp/c.66G>T	PM1, PM2, PP2, PP3	Likely pathogenic
23SM	snp	HNF1B	4	Cac/Gac	p.His336Asp/c.1006C>G	PM1, PM5, PP2, PP3, BS1. BS2	Uncertain significance
30CH	snp	HNF1B	4	Cac/Gac	p.His336Asp/c.1006C>G	PM1, PM5, PP2, PP3, BS1. BS2	Uncertain significance
46SM	snp	HNF1A	9	cGg/cAg	p.Arg583Gln/c.1748G>A	PM5, PP2, PP3, BS1, BS2, BS3	Benign

microvascular complications, it is not representative for individuals with T1DM in general. In particular, all participants had an eGFR higher than 60 ml/min/1.73 m^2, and albuminuria (but not overt proteinuria) was present in only 7 of the 45 patients. Previous studies reported a potential association between genetic factors and the risk of all microvascular complications in patients with T1DM,[26,27] while the genetic background of diabetic nephropathy is well determined.[28-30] It seems that some patients with T1DM do not develop diabetic nephropathy despite long-term glycemic exposure. The German Diabetes Documentation System reported any retinopathy in more than 80% of individuals with diabetes duration longer than 40 years.[31] Although most of our study participants were diagnosed with either nonproliferative or proliferative retinopathy, no case of blindness was observed, which is consistent with the study entry criteria. In the Medalist Study, the diagnosis of retinopathy was reported in 53.4% of patients.[13] Peripheral polyneuropathy was also common, as it was diagnosed in more than half of participants. This is in line with findings from a population-based cohort study by Dyck et al.[32] Unlike diabetic nephropathy, both retinopathy and neuropathy seem to depend more on environmental factors, such as glycemic exposure, than on hereditary factors.

Interestingly, in our highly selected population of patients with T1DM and well-preserved kidney function, there was no association between the HbA_{1c} level and proliferative retinopathy or albuminuria. This may be explained by the fact that HbA_{1c} levels were measured at a single time point, and no long-term data on glycemic control were available. Moreover, the mean HbA_{1c} level was relatively close to the recommended target. Of note, the Medalist Study did not report such an association either.[13] Our findings of high HDL cholesterol levels, low insulin requirement, and near-normal BMI as potential factors protecting against advanced complications and premature death are also in line with the results of the Medalist Study and the Golden Years Cohort (**TABLE 4**).[13-15] In those studies, HDL cholesterol levels were higher by about 0.3 mmol/l than those reported in a population-based study by Eeg-Olofsson et al.[33] High HDL cholesterol levels are known to have a strong genetic background and to be associated with lower cardiovascular risk.[34-36] Of note, while most of our patients were on statins, this class of lipid-lowering drugs seems to have limited impact on the HDL cholesterol level in autoimmune diabetes.[37,38]

It was reported that patients with an established diagnosis of T1DM and a positive family history of diabetes are frequently misdiagnosed and the actual disease is MODY. For example, a study assessing participants in the Czech T1DM Prediction Programme revealed a significant proportion of MODY in families where at least 2 family members were affected by diabetes and the proband had an initial clinical diagnosis of T1DM. The authors reported MODY in 45% of families with multiple occurrences of diabetes.[39] Genetic testing performed within the expanded Joslin Medalist Study in a group of patients with long-duration T1DM showed that almost 8% of the population were carriers of a likely pathogenic variant in monogenic diabetes genes.[16] In our study, a positive family history of diabetes was reported by 16 of the 45 individuals. Overall, we identified 10 variants, but we were not able to confirm that any of them had a causative role in the disease. The Arg1530Cys variant was previously reported in the Norwegian cohort of children with a clinical diagnosis of T1DM but absence of T1DM-related autoantibodies.[40] Functional analyses performed in that study suggested that the variant was involved in the pathogenesis of diabetes in the carrier. However, sulfonylurea treatment was unsuccessful, most probably due to the fact that the patient developed autoimmune diabetes.[40]

Next, the Arg1530Cys missense mutation was present in the ClinVar database with an annotation of uncertain significance. The 2 null variants

TABLE 3 Clinical and biochemical characteristics of the carriers of variants in monogenic diabetes genes

Patient ID	Gene	Sex	Age at diagnosis, y	Age at examination, y	BMI, kg/m^2	HbA_{1c}, %	C-peptide, ng/ml	DDI, U/kg	eGFR, ml/min/1.73 m^2	Chronic complications	Family history of diabetes
6KL	ABCC8	F	8	53	20.0	7.1	0.029	0.58	80	None	Father, mother
21WM	ABCC8	F	16	60	34.0	7.4	0.039	0.36	86	PDR, peripheral polyneuropathy	Mother
11ZT	ABCC8	F	18	63	26.0	4.9	0.01	0.14	79	PDR, peripheral polyneuropathy	No
20FJ	ABCC8	F	14	60	20.4	7.0	0.038	0.42	62	NPDR, peripheral polyneuropathy	No
19MZ	ABCC8	M	19	64	21.3	6.1	0.035	0.55	83	PDR, peripheral polyneuropathy	Sibling
37JA	GCK	F	20	60	27.8	5.8	0.042	0.53	95	PDR	No
23SM	GCK	F	23	70	22.5	7.4	0.01	0.58	73	PDR	Sibling
23SM	HNF1B	F	23	70	22.5	7.4	0.01	0.58	73	PDR	Sibling
30CH	HNF1B	F	12	64	29.4	8.7	0.01	0.71	92	PDR, peripheral polyneuropathy	Sibling
46SM	HNF1A	F	19	59	24.7	9.5	0.1	0.67	99	NPDR, peripheral polyneuropathy	No

Abbreviations: F, female; M, male; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; others, see TABLE 1

in the *ABCC8* gene found in our patients were not the cause of diabetes, because the biallelic null variants in this gene cause hyperinsulinism, and not diabetes. Therefore, diabetic individuals heterozygous for the *ABCC8* null variant detected by next-generation sequencing were incidental carriers of hyperinsulinism.[41] Additionally, the presence of the Val849Ile variant in 4 heterozygotes in the gnomAD database (rs770722134) suggests that it is likely a benign or a recessive hyperinsulinism variant. Overall, it was an unlikely cause of diabetes in our patient. It is also unlikely that *GCK*-MODY was the only etiology of the disease in our carriers of the *GCK* variants. However, we cannot exclude that T1DM was superimposed on monogenic *GCK*-related diabetes. The rare His336Asp variant in the *HNF1B* gene was reported in a Spanish pediatric cohort with diabetes and negative autoimmunity.[42] This variant was also reported in patients with kidney disorders but not diabetes.[43] The Arg583Gln variant was initially described as a likely causative mutation in T2DM or MODY cohorts.[44-46] However, more recent studies did not confirm its pathogenicity—it was also present in a nondiabetic population at a frequency of 2% to 3%.[47,48]

Of note, half of the variants detected in our study occurred in the *ABCC8* gene. This large gene containing 39 exons was previously described as highly polymorphic, which makes it difficult to interpret the identified variants in the context of diabetes.[41] Unless there is clear evidence for the presence of neonatal diabetes, MODY-like diabetes with sensitivity to sulfonylurea treatment, or sufficient cosegregation with diabetes in the patient or family members, the novel *ABCC8* missense variants should not be reported as causative ones. Therefore, in the absence of additional supporting clinical information, the 3 missense *ABCC8* variants identified in our study should be considered as of uncertain significance and should not be reported as the cause of diabetes. The confirmation of monogenic diabetes in such patients might be important for possible modification of treatment and the introduction of sulfonylurea therapy.[49] Still, it is unlikely that switching from insulin therapy to oral hypoglycemic agents would be successful in patients with a diabetes duration of more than 40 years, even if monogenic diabetes was confirmed.[50] Detecting monogenic diabetes is also important for predicting the course of diabetes in subsequent generations, including the early institution of targeted therapy.

Our study has several limitations. First, the sample size was small in comparison with the 50-Years Medalist Study and the Golden Years Study. Second, diabetes duration in our cohort was shorter by 10 years compared with the 2 other studies. Third, our patients were not assessed for the presence of human leukocyte antigen genotypes for the risk of T1DM or the presence of T1DM autoantibodies. Fourth, the different types of cardiovascular disease were diagnosed on the basis of medical records and questionnaires.

TABLE 4 Clinical and biochemical characteristics of patients in the current study vs the Joslin 50-Year Medalist Study and the Golden Years Cohort

Parameter	Polish cohort	Joslin 50-Year Medalist Study[13]	Golden Years Cohort[14]
No. of participants	45	326	400
Male sex, %	55	45.3	54
Age at diagnosis, y	14.6	12.6	13.7
Age at examination, y	59.2	69.5	68.9
Diabetes duration, y	44.6	57.1	55.8
BMI, kg/m^2	26.4	24.5	25.0
HbA_{1c}, %	7.6	7.0[a]	7.6
DDI, U/kg	0.48	0.50	0.52
Triglycerides, mmol/l	1.1	–	1.49
HDL cholesterol, mmol/l	1.9	1.75	1.84
LDL cholesterol, mmol/l	2.6	–	–
Hypertension, %	69	51	–
Proliferative retinopathy, %	71.1	48.1	–
Creatinine, μmol/l	77	–	125
eGFR, ml/min/1.73 m^2	82.2	–	–
Albuminuria, %	16	–	35
Neuropathy, %	62.2	53.1	–
Smoking (current or past), %	13	–	64
CAD, %	38	–	34
MI/stroke, %	22.2	–	–

Data presented as means unless indicated otherwise.

a Median

Abbreviations: see **TABLE 1**

Finally, as there was no control group, the presence of variants in monogenic diabetes genes was not tested in the general T1DM population.

In conclusion, patients with long-term T1DM and well-preserved kidney function were characterized by good glycemic control, high HDL cholesterol levels, low insulin requirement, and near-normal BMI. Factors associated with proliferative retinopathy and/or albuminuria included diabetes duration and LDL cholesterol levels. The only factor associated with a composite cardiovascular end point (myocardial infarction and/or stroke) was smoking. Mutations in monogenic diabetes genes were rare in our population.

ARTICLE INFORMATION

ACKNOWLEDGMENTS We would like to thank Dr. Kevin Colclough (Royal Devon & Exeter Hospital) for his valuable comments.

FUNDING The study was funded by a grant from Jagiellonian University Medical College (no. K/ZDS/005596; to MS).

CONTRIBUTION STATEMENT MTM contributed to study concept and design. JH, MK, DP-W, MM, BZ, BK-W, MS, and DZ-Z contributed to data acquisition. JH, PK, and MTM contributed to data analysis and interpretation. JH and MTM drafted the manuscript. AL-S and DZ-Z were responsible for critical revision of the manuscript. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST None declared.

HOW TO CITE Hohendorff J, Kwiatkowska M, Pisarczyk-Wiza D, et al. Mutation search within monogenic diabetes genes in Polish patients with long-term type 1 diabetes and preserved kidney function. Pol Arch Intern Med. 2022; 132: 16143. doi:10.20452/pamw.16143

REFERENCES

1 Ioacara S, Lichiardopol R, Ionescu-Tirgoviste C, et al. Improvements in life expectancy in type 1 diabetes patients in the last six decades. Diabetes Res Clin Pract. 2009; 86: 146-151.

2 Huo L, Harding JL, Peeters A, et al. Life expectancy of type 1 diabetic patients during 1997-2010: a national Australian registry-based cohort study. Diabetologia. 2016; 59: 1177-1185.

3 Livingstone SJ, Levin D, Looker HC, et al. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. JAMA. 2015; 313: 37-44.

4 Petrie D, Lung TW, Rawshani A, et al. Recent trends in life expectancy for people with type 1 diabetes in Sweden. Diabetologia. 2016; 59: 1167-1176.

5 Lind M, Svensson AM, Kosiborod M, et al. Glycemic control and excess mortality in type 1 diabetes. N Engl J Med. 2014; 371: 1972-1982.

6 Rawshani A, Rawshani A, Franzén S, et al. Mortality and cardiovascular disease in type 1 and type 2 diabetes. N Engl J Med. 2017; 376: 1407-1418.

7 Shah VN, Bailey R, Wu M, et al. Risk factors for cardiovascular disease (CVD) in adults with type 1 diabetes: findings from Prospective Real-life T1D Exchange Registry. J Clin Endocrinol Metab. 2020; 105: e2032-e2038.

8 Bebu I, Schade D, Braffett B, et al. Risk factors for first and subsequent CVD events in type 1 diabetes: the DCCT/EDIC study. Diabetes Care. 2020; 43: 867-874.

9 Garofolo M, Gualdani E, Giannarelli R, et al. Microvascular complications burden (nephropathy, retinopathy and peripheral polyneuropathy) affects risk of major vascular events and all-cause mortality in type 1 diabetes: a 10-year follow-up study. Cardiovasc Diabetol. 2019; 18: 159.

10 Jørgensen ME, Almdal TP, Carstensen B. Time trends in mortality rates in type 1 diabetes from 2002 to 2011. Diabetologia. 2013; 56: 2401-2404.

11 Miller KM, Foster NC, Beck RW, et al. Current state of type 1 diabetes treatment in the U.S.: updated data from the T1D Exchange clinic registry. Diabetes Care. 2015; 38: 971-978.

12 Pease A, Lo C, Earnest A, et al. The efficacy of technology in type 1 diabetes: a systematic review, network meta-analysis, and narrative synthesis. Diabetes Technol Ther. 2020; 22: 411-421.

13 Keenan HA, Costacou T, Sun JK, et al. Clinical factors associated with resistance to microvascular complications in diabetic patients of extreme disease duration: the 50-Year Medalist Study. Diabetes Care. 2007; 30: 1995-1997.

14 Bain SC, Gill GV, Dyer PH, et al. Characteristics of type 1 diabetes of over 50 years duration (the Golden Years Cohort). Diabet Med. 2003; 20: 808-811.

15 Gale EA. How to survive diabetes. Diabetologia. 2009; 52: 559-567.

16 Yu MG, Keenan HA, Shah HS, et al. Residual β cell function and monogenic variants in long-duration type 1 diabetes patients. J Clin Invest. 2019; 129: 3252-3263.

17 Shields BM, Hicks S, Shepherd MH, et al. Maturity-onset diabetes of the young (MODY): how many cases are we missing? Diabetologia. 2010; 53: 2504-2508.

18 Szopa M, Wolkow J, Matejko B, et al. Prevalence of retinopathy in adult patients with GCK-MODY and HNF1A-MODY. Exp Clin Endocrinol Diabetes. 2015; 123: 524-528.

19 Isomaa B, Henricsson M, Lehto M, et al. Chronic diabetic complications in patients with MODY3 diabetes. Diabetologia. 1998; 41: 467-473.

20 Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010; 33: 2285-2293.

21 Szopa M, Ludwig-Gałęzowska A, Radkowski P, et al. Genetic testing for monogenic diabetes using targeted next-generation sequencing in patients with maturity-onset diabetes of the young. Pol Arch Med Wewn. 2015; 125: 845-851.

22 Donath X, Saint-Martin C, Dubois-Laforgue D, et al. Next-generation sequencing identifies monogenic diabetes in 16% of patients with late adolescence/adult-onset diabetes selected on a clinical basis: a cross-sectional analysis. BMC Med. 2019; 17: 132.

23 Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; 17: 405-424.

24 Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: the human genomic variant search engine. Bioinformatics. 2019; 35: 1978-1980.

25 Carmody D. Monogenic Diabetes: Gene Directed Therapy And Mutation Specific Phenotypes. Master's thesis. Royal College of Surgeons in Ireland; 2017. http://pdfs.semanticscholar.org/4d8b/328672c6e2e6f53f0126cc0ec3d31db80c84.pdf. Accessed January 19, 2022.

26 Monti MC, Lonsdale JT, Montomoli C, et al. Familial risk factors for microvascular complications and differential male-female risk in a large cohort of American families with type 1 diabetes. J Clin Endocrinol Metab. 2007; 92: 4650-4655.

27 The Diabetes Control and Complications Trial Research Group. Clustering of long-term complications in families with diabetes in the diabetes control and complications trial. Diabetes. 1997; 46: 1829-1839.

28 Pezzolesi MG, Poznik GD, Mychaleckyj JC, et al. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. Diabetes. 2009; 58: 1403-1410.

29 Mooyaart AL, Valk EJ, van Es LA, et al. Genetic associations in diabetic nephropathy: a meta-analysis [published correction appears in Diabetologia. 2014; 57: 650]. Diabetologia. 2011; 54: 544-553.

30 Sandholm N, Van Zuydam N, Ahlqvist E, et al. The genetic landscape of renal complications in type 1 diabetes. J Am Soc Nephrol. 2017; 28: 557-574.

31 Hammes HP, Kerner W, Hofer S, et al. Diabetic retinopathy in type 1 diabetes-a contemporary analysis of 8,784 patients. Diabetologia. 2011; 54: 1977-1984.

32 Dyck PJ, Kratz KM, Karnes JL, et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. Neurology. 1993; 43: 817-824.

33 Eeg-Olofsson K, Cederholm J, Nilsson PM, et al; Steering committee of the Swedish National Diabetes Register. Glycemic and risk factor control in type 1 diabetes: results from 13,612 patients in a national diabetes register. Diabetes Care. 2007; 30: 496-502.

34 Weissglas-Volkov D, Pajukanta P. Genetic causes of high and low serum HDL-cholesterol. J Lipid Res. 2010; 51: 2032-2057.

35 Schaefer EJ, Lamon-Fava S, Ordovas JM, et al. Factors associated with low and elevated plasma high density lipoprotein cholesterol and apolipoprotein A-I levels in the Framingham Offspring Study. J Lipid Res. 1994; 35: 871-882.

36 Cai A, Li X, Zhong Q, et al. Associations of high HDL cholesterol level with all-cause mortality in patients with heart failure complicating coronary heart disease. Medicine (Baltimore). 2016; 95: e3974.

37 Dogra GK, Watts GF, Chan DC, Stanton K. Statin therapy improves brachial artery vasodilator function in patients with type 1 diabetes and microalbuminuria. Diabet Med. 2005; 22: 239-242.

38 Duvnjak L, Blaslov K. Statin treatment is associated with insulin sensitivity decrease in type 1 diabetes mellitus: a prospective, observational 56-month follow-up study. J Clin Lipidol. 2016; 10: 1004-1010.

39 Petruzelkova L, Dusatkova P, Cinek O, et al. Substantial proportion of MODY among multiplex families participating in a type 1 diabetes prediction programme. Diabet Med. 2016; 33: 1712-1716.

40 Pörksen S, Laborie LB, Nielsen L, et al. Disease progression and search for monogenic diabetes among children with new onset type 1 diabetes negative for ICA, GAD- and IA-2 Antibodies. BMC Endocr Disord. 2010; 10: 16.

41 De Franco E, Saint-Martin C, Brusgaard K, et al. Update of variants identified in the pancreatic β-cell KATP channel genes KCNJ11 and ABCC8 in individuals with congenital hyperinsulinism and diabetes. Hum Mutat. 2020; 41: 884-905.

42 Urrutia I, Martínez R, Rica I, et al. Negative autoimmunity in a Spanish pediatric cohort suspected of type 1 diabetes, could it be monogenic diabetes? PloS One. 2019; 14: e0220634.

43 Weber S, Moriniere V, Knüppel T, et al. Prevalence of mutations in renal developmental genes in children with renal hypodysplasia: results of the ESCAPE study. J Am Soc Nephrol. 2006; 17: 2864-2870.

44 Urhammer SA, Rasmussen SK, Kaisaki PJ, et al. Genetic variation in the hepatocyte nuclear factor-1 alpha gene in Danish Caucasians with late-onset NIDDM. Diabetologia. 1997; 40: 473-475.

45 Owen KR, Stride A, Ellard S, Hattersley AT. Etiological investigation of diabetes in young adults presenting with apparent type 2 diabetes. Diabetes Care. 2003; 26: 2088-2093.

46 Ellard S, Colclough K. Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1 alpha (HNF1A) and 4 alpha (HNF4A) in maturity-onset diabetes of the young. Hum Mutat. 2006; 27: 854-869.

47 Najmi LA, Aukrust I, Flannick J, et al. Functional investigations of HNF1A identify rare variants as risk factors for type 2 diabetes in the general population. Diabetes. 2017; 66: 335-346.

48 Billings LK, Jablonski KA, Warner AS, et al. Variation in maturity-onset diabetes of the young genes influence response to interventions for diabetes prevention. J Clin Endocrinol Metab. 2017; 102: 2678-2689.

49 Hattersley AT, Patel KA. Precision diabetes: learning from monogenic diabetes. Diabetologia. 2017; 60: 769-777.

50 Shepherd MH, Shields BM, Hudson M, et al. A UK nationwide prospective study of treatment change in MODY: genetic subtype and clinical characteristics predict optimal glycaemic control after discontinuing insulin and metformin. Diabetologia. 2018; 61: 2520-2527.