SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Genetic and clinical investigations of Hungarian Maturity-Onset Diabetes of the Young patients

by Dr. Zsolt Gaál

Supevisor: Prof. Dr. István Balogh, DSc

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF MOLECULAR CELLULAR AND IMMUNE BIOLOGY

DEBRECEN, 2024

Genetic and clinical investigations of Hungarian Maturity-Onset Diabetes of the Young patients

By Dr. Zsolt Gaál, MD

Supervisor: Prof. Dr. István Balogh, DSc

Doctoral School of Molecular Cellular and Immune Biology,
University of Debrecen

Head of the **Defense Committee**:	Prof. Dr. Éva Csősz, DSc
Reviewers:	Dr. István Szatmári, PhD
	Dr. Tamás Várkonyi, PhD
Members of the Defense Committee:	Prof. Dr. Mariann Harangi, DSc
	Dr. Gábor Firneisz, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, at 13.00 p.m. on 17th of December 2024

1. INTRODUCTION

Epidemiology of diabetes mellitus

Diabetes mellitus is a group of symptoms associated caused by elevated blood glucose level, which, in the case of inadequate treatment, is characterized by a dramatically reduced quality of life and early death. Diabetes can be considered as epidemic. According to the 10th atlas of the International Diabetes Federation (IDF), published in 2021, the number of diabetic patients in the age group 20-79 years in the world is 536.6 million (https://diabetesatlas.org), which corresponds to be 10.5% prevalence. If this trend continues, this number will rise to 783.2 million by 2045, and the prevalence to 11.3%. According to the IDF, 6.7 million patients die each year due to diabetes mellitus. Currently, half of the diabetic patients does not even know about the disease, the number of undiagnosed cases is very high.

There are considerable differences between the diabetes data of different geographical areas and countries, especially regarding the most common type of diabetes, type 2 diabetes (T2DM). In the absence of a diabetes register in Hungary, we do not know the number of diabetic individuals. According to the IDF's estimate, the prevalence of diabetes in the age group 20-79 years in Hungary is 9.1% and the number of patients who do not know about their diabetes is 110,000. According to the results of the domestic database analysis, the annual number of adults (>18 years) with type 2 diabetes taking antidiabetic drugs increased from 422,700 to 743,800 between 2001 and 2016. The duration prevalence gradually increased until 2011, followed by a three-year peak period, then it started to decline modestly.

Monogenic diabetes

Monogenic diabetes is caused by one or more inherited mutations of a gene. There are two main groups of monogenic diabetes, the transient or permanent neonatal diabetes mellitus (NDM) and the Maturity-Onset Diabetes of the Young (MODY). The latter is a heterogeneous group of autosomal dominantly inherited monogenic diabetes. There are several data on the proportion of monogenic forms of diabetes. They might be responsible for 1-2% of diabetics under the age of 30, and in the pediatric diabetes population, monogenic forms of diabetes are present in 1-6%. In the case of all diabetes, thr proportion of MODY can be 2-4%. The vast majority, 80-90% of monogenic diabetes are not recognized: Frequently under- or misdiagnosed, and it is quite typical that it takes 10 years to reach the correct diagnosis. There

are more than a dozen known MODY genes. Molecular genetic identification of MODY subtypes affects many areas of clinical genetics and has very important consequences:
- confirms the clinical diagnosis,
- enables personalized treatment, including halting the treatment at the time of diagnosis or developing a treatment strategy appropriate to the affected causative gene,
- the exact prognosis can become known,
- enables the early, presymptomatic diagnosis of at-risk family members through the targeted genetic analysis of asymptomatic blood relatives, therefore delaying or avoiding the complications of diabetes can be possible.
There are 5 main criteria for MODY:
- development of diabetes before the age of 25,
- autosomal dominant inheritance,
- lack of need for insulin treatment or detectable C-peptide,
- β-cell dysfunction with normal insulin levels,
- lack of obesity.

These criteria may not be seen in all MODY patients. Recognition in older age does not exclude the possibility of MODY. This is also true for the absence of family accumulation, as it is known that *de novo* mutations might also occur, and in the case of *de novo* mutations, MODY is not present in relatives (ancestors) of the index patient. MODY patients do not have the type 2 diabetes type obesity, but MODY patients can also become obese as they age due to an unhealthy lifestyle.

Most common MODY types

Most MODY types are caused by heterozygous loss-of-function variants in transcription factor genes. The most common transcription factors that cause MODY belong to the hepatocyte nuclear factor (HNF) family responsible for MODY1 (*HNF4A*), MODY3 (*HNF1A*) and MODY5 (*HNF1B*). Other transcription factor genes causing MODY have already been described. *HNF* genes play an important role in the development and function of the liver, but in the case of MODY, their pancreatic activity is primarily affected. *HNF1A* and *HNF4A* mutations cause progressive β-cell dysfunction as they play an essential role in β-cell development. As a result, they appear with quite similar clinical picture, and late-onset diabetic complications are typical in the case of inadequate treatment. *HNF1A* mutations affect the

expression of glucose transport proteins and enzymes. The resulting hyperglycemia can progressively worsen, which can lead to the development of diabetic complications. Accordingly, strict treatment is required to achieve the target glucose ranges. HNF1A also plays a role in renal glucose reabsorption, which means that in *HNF1A* mutation carriers renal glucosuria can be seen due to the low renal threshold, which can precede the insulin secretion anomaly of β-cells by years. HNF1A-MODY has a high and age-related penetrance. Symptoms appear in 63% of these patients before the age of 25, in 93.6% before the age of 50 and in almost all patients by the age of 75.

HNF4A is involved in the regulation of glucose transport and metabolism as well and also in the expression of several proteins important in lipid metabolism. In the case of HNF4A-MODY, some patients have macrosomia at birth. The first-line treatment for HNF1A-MODY and HNF4A-MODY is a low-dose sulphonylurea (SU), which delays or even makes unnecessary the insulin treatment. According to international data, SU is sufficient for 80% of patients even seven years after the initiation of the treatment. Some patients may need additional insulin treatment, and in the case of late diagnosis of MODY, it is also possible that SU is no longer effective, however, after establishing the correct genetic diagnosis, switching to SU treatment is known to be effective even after long-term insulin therapy.

In the case of HNF1B-MODY, more extrapancreatic symptoms are to be expected, HNF1B plays a role in regulating the expression of kidney, liver and genitourinary genes. *HNF1B* gene defects are responsible for the development of RCAD (renal cysts and diabetes) syndrome. Since the average age of onset of diabetes in HNF1B-MODY is 24 years, the diagnosis is often made in a prediabetic state due to kidney abnormalities. Another special feature of HNF1B-MODY is the large number of *de novo* variations, i.e. the absence of multigenerational family accumulation, as well as a large deletion affecting 12 genes in some patients.

The enzyme glucokinase can be considered the glucose sensor of the pancreas. Changes in its activity influence the glucose secretion of the pancreatic β-cells. In GCK-MODY, heterozygous inactivating mutations of the *GCK* gene are associated with a small rise in fasting blood sugar levels detectable from birth. Paradoxically, neither micro- nor macrovascular complications are present in the case of GCK-MODY. One explanation for this may be that, although insulin secretion is triggered only at higher glucose levels, insulin exocytosis is still under strict control, and there is no significant postprandial increase. Pharmacological treatment is usually not necessary in the case of this type of diabetes, except for pregnancy and severe inflammatory diseases.

It is not surprising that pathogenic variations in the genes of key proteins on the glucose-insulin release axis of the β-cell can cause MODY. This is also the case with the insulin-encoding *INS* gene and the two genes encoding the ATP-dependent potassium channel, *KNCJ11* and *ABCC8*. The dynamics of the field is well illustrated by the fact that *BLK*, *KLF11* and *PAX4*, previously acknowledged as MODY genes, do not cause MODY according to the latest data. Very rare types - often affecting one or a few families - are PDX1-MODY, NEUROD1-MODY, CEL-MODY, APPL1-MODY and RFX6-MODY.

MODY prevalence

The estimated prevalence of MODY can be between 1-5% of all diabetes cases, but this depends largely on the studied population. The age of the studied cohort does not matter, since the majority of childhood autoantibody-negative cases are due to GCK-MODY, in which hyperglycemia is seen elevated since birth. A mutation of the *GCK* gene may be responsible for 20% of MODY cases, while mutations of the abovementioned transcription factor genes are responsible for 67%. Mutations of the *GCK* and *HNF1A* genes are seen in more than 70% of MODY cases, while *HNF4A* mutations are responsible for approximately 5%. The proportion of the two most common responsible genes varies widely in different countries. In the UK, GCK-MODY represents 32% and HNF1A-MODY 63% of the MODY background. According to data from the Norwegian MODY registry, HNF1A-MODY is present in 53% and GCK-MODY in 7.5% of all MODY cases. According to a Polish study, the most common form of MODY in Poland is GCK-MODY (more than 80%). Up to 5% of gestational diabetes can be MODY.

Genetic testing possibilities

Previously, sequential Sanger sequening of the most probable genes was utilized. Nowadays, clinical genetic testing methodology is gene panel sequencing. Using this method, all the genes in the panel can be tested simultaneously, significantly shortening the path to molecular diagnosis.

2. GOALS OF THE STUDIES

In this work, we focused on examining Hungarian MODY patients as follows:

I. Determination of the mutation spectrum and phenotypic characteristics of Hungarian MODY patients, definition of MODY subtypes.

II. Determination of diagnostic sensitivity for the clinically prescreened MODY-suspected cohort.

III. Analysis of current treatment in genetically confirmed MODY patients.

IV. Analysis of at-risk family members with cascade tests.

3. PATIENTS AND METHODS

Patients

This work included performing appropriate genetic tests and data processing in the case of 450 unrelated index patients. These patients with a clinical suspicion of MODY came to the Department of Laboratory Medicine, Division of Clinical Genetics of the University of Debrecen for diagnostic purposes. The processing of the clinical data and the evaluation of the clinical indication was carried out by Dr. Zsolt Gaál, the author of this dissertation. This included the followings:

- the creation of a special request form for the diagnosis of monogenic diabetes,
- the analysis of submitted requests before genetic analysis,
- determination of the order of the genetic analysis steps in the case of previous studies carried out using the Sanger sequencing method.

Cascade tests could be carried out for two reasons:

- if the index patient carried a genetic variation classified as pathogenic/likely pathogenic: in this case, the aim was to identify at-risk presymptomatic family members,
- if the classification of the variant detected in the index patient can be supported by the co-segregation of the given mutation with the disease.

Based on the above criteria, clinicians sent samples from 202 family members from almost all areas of Hungary.

Before the tests were performed, the patients were informed and signed the written informed consent. In each case, a request form was filled out, which contained the patient's most important historical data, relevant clinical and laboratory parameters.

Methods

Sanger sequencing

In the case of 102 patients, *GCK*, *HNF1A* and *HNF4A* genes were tested using Sanger sequencing. The genes were selected based on clinical and laboratory data. In many cases, a sequential examination was performed, if no causal difference could be detected in the previously analyzed gene(s). Sanger sequencing was performed using the Big DyeTerminator

v3.1 Cycle Sequencing kit according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Samples were separated on an ABI Prism 3100 Genetic Analyzer. Data analysis was performed using Sequencing Analysis Software (Applied Biosystems).

Next Generation DNA Sequencing (NGS)

First pyrosequencing technique was used. The raw data obtained during pyrosequencing were evaluated using the Amplicon Variant Analyzer software (Roche 454 Life Sciences). Following the development of technology, the analysis pipeline switched to Illumina-based sequencing. The tests were performed on two Illumina (Illumina, San Diego, CA) devices, the MiSeq with lower, and the NextSeq 500 with higher throughput.

DNA libraries were prepared using several different DNA library preparation kits. Each gene panel was designed to cover the coding regions and exon-intron boundaries of the studied genes. In all cases, the detected pathogenic/likely pathogenic variations were confirmed by Sanger sequencing. In the case of a total of 76 patients, the MODY MASTR (Multiplicom, Niel, Belgium) kit was used, which was based on the multiplex PCR principle. It simultaneously tested 7 different genes underlying the disease (*ABCC8, GCK, HNF1A, HNF1B, HNF4A, INS, KCNJ11*). Of the 76 patients, 33 patients were examined by pyrosequencing, while 43 patients were tested using Illumina sequencing. Illumina sequencing data were analyzed using NextGene evaluation software (SoftGenetics, State College, PA). In the case of 164 patients, we used a self-designed hybridization kit (Qiagen GmbH, Hilden, Germany) to examine the samples. The gene panel we designed examined 17 genes simultaneously, which were the following: *PAX4, NEUROD1, HNF4A, SLC16A1, KLF11, ABCC8, APPL1, KCNJ11, INS, HNF1B, GLUD1, PDX1, GCK, BLK, HNF1A, HADH, INSR*. Here, we used sequence-specific probes to separate the DNA regions of interest from the rest of the genome. The prepared samples were run on either Illumina Miseq or NextSeq instrumetnts. The third type of gene panel was self-designed and manufactured by Twist (Twist Bioscience, South San Francisco, CA). Two different versions of this with different numbers of genes were used. First version included 18 genes in 69 patient samples, which were the following: *ABCC8, GCK, HNF1A, HNF1B, HNF4A, INS, KCNJ11, SLC16A1, GLUD1, PDX1, INSR, KLF11, NEUROD1, APPL1, HADH, PAX4, BLK, RFX6*. The second panel design contained two more genes (*CEL, WFS1*), with which 6 more patients were tested.

MLPA analyses

A total of 32 patients required an MLPA (multiplex ligation-dependent probe amplification) test suitable for detecting large genetic variations (deletions, duplications). In the case of 4 patients as a single test was used, and in the case of 28 patients it was used as a confirmatory analysis to the copy number variations detected by one of the above-mentioned new generation methods . SALSA MLPA Probemix P241 MODY Mix 1 and/or SALSA MLPA Probemix P357 MODY Mix 2 were used for the tests according to the manufacturer's instructions (MRC Holland, Amsterdam, The Netherlands).

Bioinformatic analysis

During the filtering, all detected variations with a MAF (minor allele frequency) value exceeding 1% (GnomAD population database) were filtered out as non-pathogenic variations. The classification of the remaining variants was done according to the ACMG (American College of Medical Genetics and Genomics) recommendation, which uses five categories: pathogenic (the probability of pathogenicity is greater than 99%), likely pathogenic (the probability of pathogenicity is greater than 90%), of uncertain clinical significance, probably benign (the probability of the variant being benign is greater than 90%), benign (the probability of the variant being benign is greater than 99%).

4. RESULTS

Detection of pathogenic variants in the 450 patients

Of the 450 examined index patients, 132 patients were confirmed to have a pathogenic or likely pathogenic MODY-causing mutation. 89 mutations were detected, which sets the diagnostic sensitivity to be 29.3%. An additional 95 positive cases were described during targeted cascade tests of family members.

Mutations affecting the *GCK* and *HNF1A* genes accounted for 92.1% of the total number of variants. 73% (65/89) of the mutations affected the *GCK* gene, this figure was 19.1% for *HNF1A*. Altogether MODY was genetically confirmed in 132 patients (mutation-positive patients and their family members). The cascade tests shed light on another 95 cases - including 22 with presymptomatic diagnoses -, i.e. in a total of 227 patients, MODY was confirmed by genetic methods. In addition to the two most common causative genes, five other MODY genes (*ABCC8, HNF1B, HNF4A*, *INS* and *KCNJ11*) harbored responsible variantions, being in 7.9% of the variants, 8.3% of the index patients for, while for 7% of the combined patient + family member cohort.

Disease-causing genetic variations in the *HNF1A* gene

In the *HNF1A* gene, a total of 17 disease-causing variations (pathogenic, likely pathogenic classification) were detected, most of them being missense alterations. Two new variants were identified, c.2T>G (p.Met1Arg) and c.346G>C (p.Ala116Pro), not previously described in the literature. Among the disease-causing variations, 12 resulted in amino acid substitutions, 3 caused shift of the open reading frame (2 deletions, one insertion), and one caused premature termination. In one case, based on the predictions, the consequence can be either early termination or a splicing defect. Two more frequent variations were detected (p.Arg171* and p.Gln176*), in a total of eight families, six other variants were detectable in more than one case. The detected pathological variants showed intra-gene enrichment, mostly in exons 1-6.

Disease-causing genetic variations in *ABCC8, HNF1B, HNF4A, INS* and *KCNJ11* genes

7 disease-causing variants in the minor MODY genes were detected, of which 5 were probably pathogenic and 2 were classified as pathogenic. Two new, previously unknown mutations, in the *ABCC8* (c.3988+1G>A) and *INS* (c.128G>A) genes were described. The former presumably affects the maturation (splicing) of the resulting mRNA, while the *INS*: c.128G>A mutation is results in an amino acid change (p.Cys43Tyr).

Disease-causing genetic variations in the *GCK* gene

65 different pathogenic (18) and likely pathogenic (47) mutations were identified in the *GCK* gene. All mutations were present in heterozygous form. 85% (55/65) of the identified *GCK* mutations were missense, of which - based on the predictions - in 4 cases it could not be decided whether they really involve an amino acid exchange or disrupt mRNA splicing. Of the 65 mutations identified, 40% (28/65) were previously undescribed, novel mutations. 60% (37/65) of the disease-causing mutations have already been described in the literature. The p.Gly261Arg, p.Arg36Trp and p.Ser340Asn variants were quite common, and were detected in six, five and five cases/families, respectively. No large CNVs were detected. The proportion of mutations with different consequences was similar in the undescribed and known groups. 4 small deletions were detected, 2 of which cause a reading frame shift and early termination, 2 splicing defects affecting the invariable -1 and +1 positions of the introns, and 4 nonsense mutations that cause early chain termination.

Analysis of clinical data in genetically confirmed MODY

HNF1A-MODY

Of the 48 mutation-positive individuals, the number patients diagnosed with diabetes was 32, including the index patients and their family members. On average, the diagnosis of diabetes was made at age of 20 years (4-45 years), the average age was 32 years (10-79 years) when their genetic analysis was done, meaning that the genetic diagnosic delay was an average of 12 years (0-37 years). The average BMI was 24.3 kg/m^2 (12.9-36.1 kg/m^2), data was not available in 5 cases. 5/32 patients were obese, 22/32 were not obese, and data were not available in 5 cases. Complications developed in 4/32 cases (2 cases with retinopathy, proteinuria or acanthosis nigricans in one and one case). In 20/32 cases there were no complications, while in 8 cases no data were available. Fasting serum glucose in 22/32 cases averaged 8.6 mmol/L

(4.2-20 mmol/L), the 120-minute OGTT in 11/32 cases was 12.5 mmol/L on average (8.9-15.9 mmol/L). In 27/32 cases, the average HbA1c was 7.3% and 56.2 mmol/mol (5.2-12% and 33.3-107.7 mmol/mol). The MODY calculator showed an average of 43.4% in 20/32 cases (1.9-75.5%). In 8 of these cases, it indicated a probability of 75.5%. In 18 cases, several generations were involved during the family examination, while in 14 cases no family members were available for testing.

In the case of the index patients (23/48), diabetes was diagnosed at an average age of 18 years (10-36 years). They received the genetic result at an average age of 27 years (10-50 years), which is an average of 9 years (0-29 years) diagnostic delay in genetic diagnosis. In 21/23 cases, the average BMI was 23.4 kg/m^2 (12.9-36.1 kg/m^2), 2/23 patients were obese, while 19/23 were not. In 2 cases, data on BMI and obesity were not available. In one case, retinopathy developed as a complication, while in the other patient, in addition to retinopathy, proteinuria was also described. In 16/23 cases there were no complications, while in 5 cases no data were available. Average fasting serum glucose in 17/23 cases was 8.6 mmol/L (4.2-20 mmol/L), the 120-minute OGTT in 9/23 cases was 13.2 mmol/L (8.9-15.9 mmol/L). In 20/23 cases, the average HbA1c was 7.1% and 54.6 mmol/mol (5.2-10.6% and 33.3-92.4 mmol/mol). The MODY calculator averaged 46.5% (1.9-75.5%) in 16/23 cases, which showed 75.5% in 7 patients. In 9 cases, several generations were involved during the family examination, while in 14 cases the family members were not examined.

Regarding family members with diabetes (9/48), diabetes was diagnosed at an average age of 26 years (4-45 years), their genetic testing was done at an average age of 47 years (16-79 years), so the genetic diagnosis was made at an average age of 21 years (2-37 year) later. In 6/9 cases of the family members average BMI was 7.5 kg/m^2 (22.5-32.4 kg/m^2). 3 family members were obese, two of whom developed complications (retinopathy or acanthosis nigricans), 4 cases had no complications. 3 individuals were not obese, while in 3 cases data on BMI, obesity and complications were not available. The average fasting serum glucose for 5 family members was 8.4 mmol/L (6.0-11.3 mmol/L). OGTT was performed only in 2 family members (9.1 mmol/L and 9.8 mmol/L). In 7/9 cases, the average HbA1c was 7.7% and 61.0 mmol/mol (6.2-12% and 43.3-107.7 mmol/mol). The MODY probability calculator was completed in 4 cases with an average of 31% (4.6-75.5%) and only one patient had 75.5%.

Number of presymptomatic family members was 9/48, where the genetic test was performed at an average age of 18 years (1-37 years). BMI were available in 2 family members (17 kg/m^2 and 24.5 kg/m^2), no obesity or complications were observed. In 7 cases, there were no information about the clinical data.

In the case of 7 diabetic index patients, no data was available on the age at which diabetes was diagnosed. In their case, the genetic diagnosis was established at an average age of 24 years (13-42 years). More detailed clinical data were available in 3 patients: one of them received the genetic result at the age of 35, BMI value 25 kg/m^2, not obese, complications included retinopathy and ischemic heart disease, fasting glucose was 8.6 mmol/L. OGTT resulted in the 120-minute blood sugar to be 12 mmol/L, and the HbA1c result was 13.4% (123 mmol/mol). The other patient, who was genetically tested for MODY at the age of 15, was obese based on BMI, and his fasting glucose was 7.5 mmol/L. In both patients, several generations were affected by diabetes. The genetic analysis of the third patient was performed at the age of 18, and only HbA1c (8%, 63.9 mmol/mol) result was available.

GCK-MODY

GCK-MODY was confirmed in 163 individuals, of whom 115 patients were diagnosed with diabetes. Average age of 15 years (1-57 years) was at the time of the clinical diagnosis. Their genetic examination (113/115) was performed at an average age of 22 years (1-69 years), giving a 7 years (0-39 years) genetic diagnostic delay. In the remaining 2 patients, the exact date of the genetic diagnosis is not known. In 101/115 cases, the average BMI was 20.9 kg/m^2 (13.4-38 kg/m^2), 11/115 individuals were obese and 91/115 non-obese individuals were examined. A total of 10/115 cases had comorbidities, 6 patients had one, while another 4 patients had more than one. There were no complications in 83 cases. The average fasting serum glucose in 96/115 cases was 6.7 mmol/L (4.7-10.1 mmol/L), the result of the 120-minute OGTT was 9.6 mmol/L (5.2-22.9 mmol/L). In 97/115 cases, the average HbA1c was 6.4% and 46.2 mmol/mol (4.8-8.2% and 29.0-66.1 mmol/mol). The MODY probability calculator showed an average of 63.9% (1.9-75.5%) in 80/115 cases, of which 61 cases indicated a 75.5% probability. In 63/115 cases several generations were affected, in 33 cases the family members and in 3 cases the parents were not tested, in 12 cases the siblings were positive, but the parents were not tested. In 2 cases, the mother did not prove to be a carrier, while the father was not examined, and *de novo* GCK-MODY was also described in 2 cases.

In the index patients (76/115), diabetes was diagnosed at an average age of 12 years (1-35 years), and they received the genetic result at an average age of 18 years (2-59 years), which is 6.5 years (0-34 years) meant a delay in establishing a genetic diagnosis. In 70/76 cases, the average BMI was 20.2 kg/m^2 (13.4-32.7 kg/m^2), one patient was described with a normal BMI, in 5 cases no data were available. 6/76 patients were obese, 65/76 cases did not have obesity.

There were 6 people who reported complications. One complication was described in four patients (PCOS, acanthosis nigricans, recurrent acute laryngitis, possible celiac disease), while 2 people reported several complications (headache and increased respiratory rate, as well as hypertension and obesity). No complications developed in 58/76 patients. The average fasting serum glucose in 67/76 cases was 6.7 mmol/L (4.9-10.1 mmol/L), the 120-minute OGTT in 54/76 cases resulted in 9.8 mmol/L (5.4-22.9 mmol/L). In 71/76 cases, the average HbA1c was 6.4% and 46.1 mmol/mol (5.5-7.4% and 36.6-57.4 mmol/mol). In 66/76 cases, MODY probability calculator showed an average of 64.3% (1.9-75.5%). In the case of 52 patients, the calculator showed a 75.5% probability. In 30/76 cases several generations were affected, in 33 cases the family members were not tested, in 3 cases the parents were not tested, and in 6 cases the siblings were positive, but the parents were not tested. *De novo* descriptions fell into this group.

Family members (39/115) were diagnosed with diabetes at an average age of 22 years (1-57 years), the genetic diagnosis was received with an average delay of 8.6 years (0-39 years) at an average age of 30 years (1-69 years). In the case of one family member, no data were available on the date of his genetic diagnosis. The average BMI in 31/39 cases was 22.5 kg/m^2 (14.1-38.0 kg/m^2). 5 individuals were obese, while 26 were not. No complications occurred in 25/39 cases, and some complications were reported in 4 family members. Fasting serum glucose was known in 29 cases, their average was 6.9 mmol/L (4.7-9.2 mmol/L). OGTT was performed for 22 family members, with 9.2 mmol/L (5.2 -14.5 mmol/L) average. In 26/39 cases HbA1c were known with an average of 6.4% (4.8-8.2%) and 46.7 mmol/mol (29-66.1 mmol/mol). The MODY calculator was only determined for 14 family members, but in more than half of them (9/14) it indicated a probability of 75.5%. In 33 cases, it can be stated that several generations were affected with regard to GCK-MODY, 6 persons were examined where the siblings were positive, but the parents were not tested.

Due to a positive family history, genetic testing was performed in 12/163 cases of a non-diabetic family member, they (10/12) received the results of the genetic analysis at an average age of 22 years (1-48 years). In more than half of the cases, no clinical data on the family members were available. In 2 cases, BMI (normal, 26 kg/m^2) was determined. In 3 individuals there were data on fasting serum glucose, which showed an average of 6.5 mmol/L (5.4-7.1 mmol/L) and the 120-minute result of the OGTT, which was 7.1 mmol/L (5.9-9.1 mmol/L). In the case of one family member, only the result of fasting serum glucose (7.5 mmol/L) was given. HbA1c resulted in 6.2% and 44.3 mmol/mol and 7.0% and 53 mmol/mol (2/12 cases). MODY calculator did not give results in their case. In case of one family member, the mother

did not prove to be a carrier during the family screening, while the father was not tested, and in the other 11 cases it was shown that several generations were affected by GCK-MODY.
15 index patients and 21 family members (36/163 cases) who were previously diagnosed with diabetes, but the time of diagnosis was unknown were tested. In case of one patient, diabetes was diagnosed in childhood (the exact age was not available). Their genetic examination (35/36) was performed at an average age of 25 years (1-64 years). Average BMI in 11/36 cases was 19.7 kg/m^2 (15.1-29.5 kg/m^2), based on which none of them were obese. In 25 cases data was not available. Fasting serum glucose in 18/36 cases averaged 6.4 mmol/L (5.6-7.1 mmol/L), OGTT was determined in 9/36 cases, which averaged 7.6 mmol/L (5.9-9.4 mmol/L). HbA1c was available in 13 cases with an average of 6.3% (6.0-6.7%) and 45.4 mmol/mol (42.1-49.7 mmol/mol). MODY calculator was not determined for this group either. During the family examination, in the case of 25 individuals, several generations were involved, while in 11 cases we did not examine the family members.
In case of a 15/36 index patient, the average age when the genetic analysis was performed can be set at 20 years (8-36 years). In 5/15 cases, the average BMI was 19.4 kg/m^2 (16.2-25.0 kg/m^2), showing that none of them were obese. Fasting serum glucose in 9/15 cases averaged 6.3 mmol/L (5.6-7.1 mmol/L), OGTT was determined in 6 cases, which averaged 7.8 mmol/L (5.9-9.4 mmol/L). There were data on HbA1c in 6 cases, which showed an average of 6.3% (6.0-6.6%) and 45.4 mmol/mol (42.1-48.6 mmol/mol). We have no information about the MODY calculator, family members were examined in only 4 cases where several generations were involved.
In case of the 20 family members, the average age when their genetic analysis was performed was 29 years (1-64 years). In 6 cases, the average BMI was 19.1 kg/m^2 (15.1-29.5 kg/m^2), based on which none of them were obese. Fasting serum glucose in 9/21 cases averaged 6.4 mmol/L (5.7-7.1 mmol/L), OGTT was determined in 3/14 cases, which averaged 7.3 mmol/L (6.7-8.1 mmol/L). HbA1c was available in 7 cases with an average of 6.3% (6.0-6.7%) and 45.5 mmol/mol (42.1-49.7 mmol/mol). We have no information about the MODY calculator, in each case several generations were involved as determined by family screening.

Other MODY genes

In case of 16 individuals, during the genetic testing, mutation was identified in another MODY-causing gene, 14 of them were diagnosed with diabetes before the genetic result was available at an average age of 21 years (5-43 years). Their genetic examination (14/16) was performed

at an average age of 31 years (8-67 years), so the genetic diagnosis was received with a delay of 10 years (1-32 years). In 14/16 cases, the average BMI was 24.9 kg/m^2 (18.4-30.7 kg/m^2), 3/14 individuals were obese and 9/14 non-obese individuals were examined.
In case of 10/14 index patients, diabetes was diagnosed at an average age of 19 years (5-32 years), and the result of the genetic test was received at an average age of 27 years (8-45 years), which is an average of 8 years (1-30 years) genetic diagnostic delay. In 8/10 cases, the average BMI was 25.1 kg/m^2 (18.4-30.7 kg/m^2), in 2 cases no data was available. 3/14 patients were obese, obesity did not exist in 5/14 cases.
Family members (4/14) were diagnosed with diabetes at an average age of 26 years (10-43 years), and the genetic diagnosis was received with an average delay of 16 years (1-32 years) at an average age of 43 years (17-67 years). In their case, the average BMI was 24.6 kg/m^2 (19-28.7 kg/m^2), there was no obesity. We found a mutation in another MODY gene in 2 individuals for whom clinical data were not available (1 diabetic index patient - age at diagnosis of diabetes unknown, 1 family member - not (known) diabetic).

Treatment preceding genetic diagnosis of genetically confirmed MODY patients

In the two large groups (HNF1A- and GCK-MODY) the detailed analysis of the treatment before the genetic diagnosis was performed. The treatment showed a very varied picture. Therapeutic data were available for a total of 35 HNF1A-MODY and 125 GCK-MODY patients. 84 patients received appropriate treatment (4 HNF1A- and 80 GCK-MODY patients). 20 HNF1A-MODY and 19 GCK-MODY patients received insulin treatment. 4 HNF1A-MODY and 24 GCK-MODY patients received suboptimal oral antidiabetic treatment, while 2 patients each received combined treatment. In the case of five HNF1A-MODY patients, it was not possible to judge whether the treatment was adequate due to possible presymptomatic clinical suspicion (i.e. following an at-risk family member, in which case the mutation was later confirmed by genetic diagnosis, diet and no treatment groups).

5. DISCUSSION

In the course of this work, a workflow has been developed in the first time in Hungary which makes possible the rational completion of MODY analyses. This included the creation of a request form containing relevant data, appropriate clinical diabetological examinations and state-of-the-art clinical laboratory genetic testing, the latter of which has always followed technological development during the years of analysis.

In this 11-year study, the spectrum of MODY in Hungary has been established, and it was determined which MODY genes have pathogenic variations. In many cases, asymptomatic family members were also tested. These investigations covered the entire country, so the obtained data have not only of regional, but national significance.

In 132 cases of the 450 examined patients, the presence of a causative variant was confirmed. The diagnostic rate (29.3%) fits well with the international data (24-32%). We expanded our studies with cascade analyses, during which 95 additional mutation-positive cases were detected. In 22 of the latter cases, the diagnosis was presymptomatic, i.e. the patient's diabetes either had not yet manifested or had not yet been recognized.

It was found that - according to the current request practice - GCK-MODY is the most common in Hungary, followed by HNF1A-MODY, which together account for more than 90% of cases. The detailed analysis of the detected alterations has also been performed. A total of 17 causative variations were detected in the *HNF1A* gene, two of which, c.2T>G (p.Met1Arg) and c.346G>C (p.Ala116Pro), were new and not previously reported in the literature. A total of five rare MODY genes have been implicated as mutation-harboring. Among these, two new, previously unknown variations, in the *ABCC8* (c.3988+1G>A) and *INS* (c.128G>A) genes were described. 65 different causative variants in the *GCK* gene have been found. The rate of new, previously unknown mutations was very high (40%, 28/65).

A *de novo* mutation was detected in 2 families. This draws attention to the fact that the lack of family history does not rule out the possibility of MODY, so in some rare cases, one of the original MODY criteria (diabetes observed in several generations) is not always fulfilled. Clinical and genetic analysis of MODY is not a success story. In the case of MODY, several reasons for its relative ineffectiveness can be identified. First, the large number of the two main forms of diabetes inevitably diverts the attention of the clinic from the rarer, monogenic forms, which in many cases results in misdiagnosis and suboptimal treatment. Second, in clinical practice, the majority of patients do not receive genetic counseling, so pedigree analysis does not take place, thus multigenerational diabetes might escape attention. Third, the presence of

MODY in a family does not exclude the presence of T2DM or T1DM, even in the same patient. Fourth, the significant discoveries in this field in recent decades are difficult to translate into clinical practice - some clinician colleagues do not have up-to-date knowledge of the diagnostic and treatment options for MODY. Fifth, genetic tests are expensive and tied to centers, and the lack of clear patient pathways and financing protocols also makes integration into practice difficult. The specific nature of GCK-MODY - elevated blood sugar level from birth - would enable an early diagnosis, however, in the case of healthy patients, there is no inevitable blood sugar measurement, except during pregnancy. This is why many GCK-MODYs are diagnosed as gestational diabetes.

By the detailed analysis of the two large groups (GCK-MODY, HNF1A-MODY) several important conclusions can be drawn. In the case of mutation-positive HNF1A-MODY patients, diabetes was diagnosed at an average age of 20 years, while genetic confirmation was done with a 12-year diagnostic delay. These data were greatly improved when the genetic test confirmed HNF1A-MODY in the family, since in presymptomatic cases the genetic test was performed at an average age of 18 years, enabling correct and precise treatment. In the case of GCK-MODY, diabetes was diagnosed at an average age of 15 years, with a 7-year delay in genetic confirmation. In the case of rare MODY forms, the diagnostic delay of genetic confirmation was 10 years, but this data should be treated with caution due to the low number of cases.

Suboptimal treatment due to underdiagnosis is a known fact for MODY patients. Our results clearly prove that the genetic diagnosis of MODY greatly improves access to appropriate treatment. When examining the two largest MODY groups (information was available in 160 cases), it was revealed that only 52.5% of patients receive the appropriate treatment, be it sulphonylurea or diet/no treatment. It should be noted, that 24.4% of the patients received insulin treatment, which was completely unnecessary and in their case adversely affected the quality of life. In the future, we plan to follow up on these data, now together with health-economic calculations, in order to confirm the hypothesis that genetic diagnosis is a big step forward in three areas: establishing a genetic diagnosis of MODY and determining the subtype helps to choose the adequate therapy, stopping the wrong insulin treatment improves the quality of life, and finally, the above two directly and indirectly might have the potential to reduce healthcare expenditures in Hungary, similarly to positive international experiences. As one of the first steps of this, we used a simulation model to prove that MODY screening simultaneously reduces costs and improves quality of life.

6. SUMMARY

This is the first work to examine a large MODY cohort in Hungary, where the spectrum of MODY in Hungary was established, and determined which MODY genes have a detectable causative alteration. In many cases, asymptomatic family members were also tested. In 132 cases of the 450 examined patients, a causative genetic variation was confirmed. Cascade tests were also performed, during which 95 additional mutation-positive cases were found, in 22 of which the diagnosis was presymptomatic. It was found that GCK-MODY is the most common in Hungary, followed by HNF1A-MODY, accounting for more than 90% of cases. Causative variants were analyzed in details. 65 different causative variants in the *GCK* gene were described. The rate of new, previously unknown mutations was very high (40%, 28/65). A total of 17 pathological abnormalities were detected in the *HNF1A* gene, two of which had not been previously reported in the literature. In addition, causative variants in a total of five rare MODY genes were found. Among these, two new, previously unknown variants were described. A *de novo* mutation was detected in 2 families. In the case of mutation-positive HNF1A-MODY patients, diabetes was diagnosed at an average age of 20 years, while genetic confirmation occurred with a 12-year diagnostic delay. In the case of GCK-MODY, diabetes was diagnosed at an average age of 15 years, with a 7-year delay in genetic confirmation. These results prove that the genetic diagnosis of MODY greatly improves access to appropriate treatment. When examining the two largest MODY groups, it was revealed that 52.5% of the patients received the appropriate treatment, while 24.4% of the patients received unnecessary insulin treatment that adversely affected the quality of life.

7. NOVEL FINDINGS

1. We were the first to set up and examine a large MODY cohort in Hungary. In 132 of the 450 patients, we confirmed a causative genetic abnormality. During cascade tests, we found 95 additional mutation-positive cases, 22 of them in a presymptomatic state.

2. In Hungary, GCK-MODY is the most common, followed by HNF1A-MODY, accounting for more than 90% of all cases.

3. We described 65 different causative variants in the *GCK* gene, of which 28 were previously undescribed variations. We detected 17 pathological variations in the *HNF1A* gene, including two novel. We detected pathological variants in five rare MODY genes, with two new mutations. A *de novo* mutation was detected in 2 families.

4. In the case of HNF1A-MODY patients, the diagnosis of diabetes occurred at an average age of 20 years, while the genetic confirmation occurred with a 12-year diagnostic delay. These data were 15 and 7 years for GCK-MODY.

5. During the examination of the Hungarian cohort, we verified that the genetic diagnosis of MODY improves access to appropriate treatment. In the case of GCK-MODY and HNF1A-MODY, 52.5% of the patients received the appropriate treatment, while 24.4% of the patients received unnecessary insulin treatment that adversely affected the quality of life.

8. ACKNOWLEDGMENT

At the beginning of this work, I received a lot of guidance from Professor Andrew Hattersley, Professor Anikó Somogyi and I worked together with Doctor Irén Kántor. Professor István Balogh created the possibility of diagnosing monogenic diabetes in Hungary, he helped my clinical and scientific work in everything, thank you.

I would like to highlight Zsuzsanna Szűcs among the many colleagues who help me in my work. I got the most from my patients. I would like to thank my family for their support.

9. LIST OF PUBLICATIONS

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/481/2024.PL
Subject: PhD Publication List

Candidate: Zsolt Gaál
Doctoral School: Doctoral School of Molecular Cellular and Immune Biology

List of publications related to the dissertation

1. **Gaál, Z.**, Szűcs, Z., Kántor, I., Luczay, A., Tóth, H. P., Benn, O., Felszeghy, E. N., Karádi, Z., Madar, L., Balogh, I.: A Comprehensive Analysis of Hungarian MODY Patients-Part I: Gene Panel Sequencing Reveals Pathogenic Mutations in HNF1A, HNF1B, HNF4A, ABCC8 and INS Genes.
   Life (Basel). 11 (755), 1-13, 2021.
   DOI: http://dx.doi.org/10.3390/life11080755
   IF: 3.251
2. **Gaál, Z.**, Szűcs, Z., Kántor, I., Luczay, A., Tóth, H. P., Benn, O., Felszeghy, E. N., Karádi, Z., Madar, L., Balogh, I.: A Comprehensive Analysis of Hungarian MODY Patients-Part II: glucokinase MODY Is the Most Prevalent Subtype Responsible for about 70% of Confirmed Cases.
   Life (Basel). 11 (8), 1-20, 2021.
   DOI: http://dx.doi.org/10.3390/life11080771
   IF: 3.251

List of other publications

3. **Gaál, Z.**, Pálffy, Z., Varga, M., Mohamad, N. M., Pistai, M., Szegedi, J.: Tévutak, Betegutak és Lehetőségek: diabétesz, krónikus vesebetegség és társaik.
   Metabolizmus. 22 (2), 73-77, 2024.
4. Kovács, G., Nagy, D., Szilberhorn, L., Zelei, T., **Gaál, Z.**, Vellekoop, H., Huygens, S., Versteegh, M., Mölken, M. R. v., Koleva-Kolarova, R., Tsiachristas, A., Wordsworth, S., Nagy, B.: Cost-effectiveness of genetic-based screening strategies for maturity-onset diabetes of the young.
   Personalized Medicine. 20 (4), 375-385, 2023.
   DOI: http://dx.doi.org/10.2217/pme-2023-0023
   IF: 1.7

5. Jermendy, G., **Gaál, Z.**, Kempler, P., Lengyel, C., Rosta, L., Várkonyi, T., Wittmann, I.: DPP-4-gátlók a 2-es típusú diabetes kezelésében: megújult irányelvek, változó klinikai gyakorlat.
Diabetol. Hung. 28 (2), 69-75, 2020.
DOI: http://dx.doi.org/10.24121/dh.2020.6

6. Jermendy, G., **Gaál, Z.**, Gerő, L., Hídvégi, T., Kempler, P., Winkler, G., Wittmann, I.: Egészségügyi szakmai irányelv - A diabetes mellitus kórismézéséről, a cukorbetegek antihyperglykaemiás kezeléséről és gondozásáról felnőttkorban.
Diabetol. Hung. 28, 119-204, 2020.
DOI: http://dx.doi.org/10.24121/dh.2020.14.

7. Schernthaner, G., Karasik, A., Abraitienė, A., Ametov, A. S., **Gaál, Z.**, Gumprecht, J., Janež, A., Kaser, S., Lalic, K., Mankovsky, B. N., Moshkovich, E., Past, M., Prázný, M., Radulian, G., Smirčić Duvnjak, L., Tkáč, I., Trušinskis, K.: Evidence from routine clinical practice: EMPRISE provides a new perspective on CVOTs.
Cardiovasc Diabetol. 18 (1), 115, 2019.
DOI: http://dx.doi.org/10.1186/s12933-019-0920-3
IF: 7.332

8. **Gaál, Z.**, Balogh, I.: Monogenic Forms of Diabetes Mellitus.
In: Genetics of Endocrine Diseases and Syndromes. Eds.: Igaz Péter, Patócs Attila, Springer Nature Switzerland, Cham, 385-416, 2019.

9. Raccah, D., Chou, E., Colagiuri, S., **Gaál, Z.**, Lavalle, F., Mkrtumyan, A., Nikonova, E., Tentolouris, N., Vidal, J., Davies, M.: A global study of the unmet need for glycemic control and predictor factors among patients with type 2 diabetes mellitus who have achieved optimal fasting plasma glucose control on basal insulin.
Diabetes-Metab. Res. Rev. 33 (3), 1-39, 2017.
DOI: http://dx.doi.org/10.1002/dmrr.2858
IF: 3.904

10. Iván, G., Balogh, I., **Gaál, Z.**, Mosonyi, J., Szajbert, T.: Három évtizedes inzulinkezelés diagnosztizált HNF1A-MODY (MODY3) esete - hogyan tovább?
Diabetol. Hung. 24 (1), 48-52, 2016.

11. Jermendy, G., Balogh, I., **Gaál, Z.**: HNF-4-[alfa]-mutáció okozta monogénes diabetes mellitus (MODY-1) első hazai esete.
Orv. Hetil. 157 (12), 469-473, 2016.
DOI: http://dx.doi.org/10.1556/650.2016.30399
IF: 0.349

12. Kicsák, M., **Gaál, Z.**: Cukorbetegek iskolája-Csoportos betegoktatás tapasztalatai eredményei.
Diabetol. Hung. 22 (2), 83-88, 2014.

13. **Gaál, Z.**, Papp, Z., Bakó, B.: DIABOBHU obszervációs vizsgálat értékelése = Evaluation of the DIABOBHU Observational Study.
Orv. hetil. 155 (23), 903-910, 2014.
DOI: http://dx.doi.org/10.1556/OH.2014.29927

14. **Gaál, Z.**, Bakó, B., Gárdus, D., Gaál, B., Spisák, N., Balogh, I.: Neonatalis diabetes hátterének felnőttkori identifikálása.
Diabetol. Hung. 21 (1), 7-14, 2013.

15. **Gaál, Z.**: A glukózmonitorozás lehetőségei és kérdései.
Diabetol. Hung. 17 (1), 5-14, 2009.

16. Nádas, J., Putz, Z., Fövenyi, J., **Gaál, Z.**, Gyimesi, A., Hídvégi, T., Hosszúfalusi, N., Neuwirth, G., Oroszlán, T., Pánczél, P., Vándorfi, G., Winkler, G., Wittmann, I., Jermendy, G.: Cardiometabolic risk and educational level in adult patients with type 1 diabetes.
Acta Diabetol. 46 (2), 159-162, 2009.
DOI: http://dx.doi.org/10.1007/s00592-008-0065-4
IF: 1.549

17. Nádas, J., Putz, Z., Fövenyi, J., **Gaál, Z.**, Gyimesi, A., Hídvégi, T., Hosszúfalusi, N., Neuwirth, G., Oroszlán, T., Pánczél, P., Széles, G., Vándorfi, G., Winkler, G., Wittmann, I., Jermendy, G.: Cardiovascular Risk Factors Characteristic for the Metabolic Syndrome in Adult Patients with Type 1 Diabetes.
Exp. Clin. Endocrinol. Diabet. 117 (3), 107-112, 2009.
DOI: http://dx.doi.org/10.1055/s-0028-1082068
IF: 1.685

18. Nádas, J., Putz, Z., Fövenyi, J., **Gaál, Z.**, Gyimesi, A., Hídvégi, T., Hosszúfalusi, N., Neuwirth, G., Oroszlán, T., Pánczél, P., Vándorfi, G., Winkler, G., Wittmann, I., Jermendy, G.: A kardiometabolikus kockázati tényezők kezelésének eredményessége 1-es típusú diabetesben szenvedők körében.
Orv. hetil. 149 (27), 1263-1269, 2008.
DOI: http://dx.doi.org/10.1556/oh.2008.28328

19. **Gaál, Z.**, Kántor, I., Albert, L., Pap, K., Dicső, F., Hattersley, A., Sian, E., Pocsai, Z., Ádány, R., Valenta, B., Somogyi, A.: Glukokináz-MODY és gestatiós diabetes.
Diabetol. Hung. 14 (3), 227-232, 2006.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

20. **Gaál, Z.**, Ruzicska, É., Valenta, B., Somogyi, A.: Adhaesios molekulák szerepe atherosclerosisban és diabetes mellitusban.
Orv. hetil. 141 (46), 2483-2486, 2000.

Total IF of journals (all publications): 23,021
Total IF of journals (publications related to the dissertation): 6,502

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

23 September, 2024

DEBRECENI EGYETEM
Egyetemi és Nemzeti Könyvtár