A Comprehensive Analysis of Hungarian MODY Patients—Part II: Glucokinase MODY Is the Most Prevalent Subtype Responsible for about 70% of Confirmed Cases

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Abstract: MODY2 is caused by heterozygous inactivating mutations in the glucokinase (GCK) gene that result in persistent, stable and mild fasting hyperglycaemia (5.6–8.0 mmol/L, glycosylated haemoglobin range of 5.6–7.3%). Patients with GCK mutations usually do not require any drug treatment, except during pregnancy. The GCK gene is considered to be responsible for about 20% of all MODY cases, transcription factors for 67% and other genes for 13% of the cases. Based on our findings, GCK and HNF1A mutations together are responsible for about 90% of the cases in Hungary, this ratio being higher than the 70% reported in the literature. More than 70% of these patients have a mutation in the GCK gene, this means that GCK-MODY is the most prevalent form of MODY in Hungary. In the 91 index patients and their 72 family members examined, we have identified a total of 65 different pathogenic (18) and likely pathogenic (47) GCK mutations of which 28 were novel. In two families, de novo GCK mutations were detected. About 30% of the GCK-MODY patients examined were receiving unnecessary OAD or insulin therapy at the time of requesting their genetic testing, therefore the importance of having a molecular genetic diagnosis can lead to a major improvement in their quality of life.

Keywords: MODY2; GCK-MODY; GCK mutations; Hungary

1. Introduction
1.1. GCK-MODY (MODY2)

MODY2 is caused by heterozygous inactivating mutations in the glucokinase (GCK) gene encoding a key regulator glycolytic enzyme of the hexokinase family [1]. It has two tissue-specific promoters and a different exon 1, the upstream promoter being functional in the pancreas (exon 1a) and brain, while the downstream one only in the liver (exons 1b and 1c), resulting in different isoforms of the GCK gene [2,3].

GCK has an important role in carbohydrate metabolism. It is responsible for the catalysis of the first reaction of the glycolytic pathway, the glucose phosphorylation [1]. GCK acts as a glucose sensor of the pancreatic beta-cells [1], therefore it is critical in the process of the regulation of insulin secretion and release.
In the case of GCK-MODY, a mildly elevated glucose level is caused by heterozygous loss-of-function mutations in the GCK gene. Any of the 10 exons and promoter of the pancreatic isoform of the GCK gene might be affected as no mutational hotspots have been identified. The mutations might affect enzyme kinetics or protein folding [4,5]. To date, almost 800 disease-causing small scale GCK mutations have been reported in the professional version of the HGMD (Human Gene Mutation Database, version 2021.1) associated with the MODY phenotype, the majority of them being missense alterations resulting in abnormal structure and/or function of the mutant protein, often affecting its kinetic parameters.

GCK gene mutations result in abnormal glucose sensing, raising the threshold of glucose-mediated insulin secretion. As a consequence, stable and mild fasting hyperglycaemia (5.6–8.0 mmol/L, glycosylated haemoglobin range of 5.6–7.3%) persists that does not deteriorate with age and is not associated with an increased risk of complications [6,7]. The clinical manifestation of GCK-MODY is generally nonprogressive, usually asymptomatic in childhood. The elevated glucose level is present from birth, therefore it is mostly detected incidentally [8,9]. Performing an oral glucose tolerance test (OGTT) can help to distinguish GCK-MODY patients from other types of MODY as in the case of GCK-MODY, patients generally have a small (<3.5 mmol/L) 2 h glucose increment [10].

Patients with GCK mutations usually do not require any drug treatment (except during pregnancy or in critical clinical situations), however, they often receive unnecessary insulin therapy or oral antidiabetic drug treatment [9]. Good glycaemic control can usually be achieved with only diet and exercise [11].

1.2. MODY Prevalence

The estimated MODY prevalence is around 1–5% of all diabetes mellitus cases, but it varies depending on the population studied [12,13]. The GCK gene is considered to be responsible for about 20% of all MODY cases, transcription factors for 67% and other genes for 13% of the cases [14]. GCK and HNF1A genes together are responsible for about 70% of all known MODY cases, the ratio of the two genes widely varying between countries [15]. For example in the United Kingdom, the prevalence of GCK-MODY is reported to be 32% [6,16], and 63% in the case of HNF1A-MODY [17]. The Norwegian MODY Registry reports a distribution of 53% HNF1A-MODY, 30% GCK-MODY, 7.5% HNF4A-MODY and 5.6% HNF1B-MODY [18]. A Polish study reports GCK-MODY to be the most prevalent with 83% [19] while the American SEARCH study reports HNF1A-MODY as the most prevalent form with roughly 60%, GCK-MODY being in the second position with 30% [20].

2. Materials and Methods

As this paper is Part II of two accompanying publications in the Journal, the patients and methods presented in this section are the same as the ones described in Part I of this article. The genes tested and genetic methods used during the study are presented in the Supplementary file (Part I of these articles).

2.1. Patients

A total of 450 unrelated index patients with suspected MODY diagnosis and their 202 family members have been referred to our laboratory for genetic testing from all around Hungary. All participants or their guardians have given informed consent to genetic testing according to national regulations.

2.2. Methods

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp Blood Mini kit (Qiagen GmbH, Hilden, Germany).

In the case of 102 index patients, Sanger sequencing of the GCK, HNF1A or HNF4A genes was performed using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol.
Bidirectional pyrosequencing with a minimum coverage of $40 \times$ was performed on Roche GS Junior 454 pyrosequencing system (Roche 454 Life Sciences, Branford, CT, USA) in the case of 33 index patients.

The 311 index patient samples were sequenced on Illumina Miseq or NextSeq 550 (Illumina, San Diego, CA, USA) sequencer systems in $2 \times 150$ cycle (or $2 \times 250$ cycle in the case of the MODY MASTR kit) paired-end mode. Three different library preparation methods were used before sequencing. The MODY MASTR kit (Multiplicom, Niel, Belgium) was used to examine 7 genes in the case of 76 index patients. A custom-made and enrichment-based DNA library preparation kit (Qiagen, GmbH, Hilden, Germany) containing 17 genes was used in the case of 164 index patients, and another custom-designed gene panel (Twist Bioscience, South San Francisco, CA, USA) was used, examining 18 genes in the case of 69, and 20 genes in the case of 6 index patients. (Supplementary Table S1, see Part I) In the case of Illumina sequenced data, data analysis was performed using the NextGene software (SoftGenetics, State College, PA, USA).

MLPA (multiplex ligation-dependent probe amplification) was performed in the case of 32 index patients (as a single test in the case of 4 index patients and in addition to one of the above-mentioned methods in the case of 28 index patients) using SALSA MLPA Probemix P241 MODY Mix 1 and/or SALSA MLPA Probemix P357 MODY Mix 2 (MRC Holland, Amsterdam, Netherlands) according to the manufacturer’s protocol.

The testing method(s) used in the case of every index patient is described in the Supplementary Table S2 (Part I of these articles).

Cascade testing was performed in 202 family members usually by targeted Sanger sequencing of the respective exon of the MODY-causing gene in which their relative had a possibly pathogenic mutation.

2.3. Variant Confirmation

All variants obtained with next-generation sequencing that were suspected to be disease-causing were validated by Sanger sequencing. Furthermore, when the amplicon’s minimum coverage was $<40 \times$ in the NGS data, the respective exons were also sequenced using the Sanger method.

2.4. Variant Filtering and Interpretation

All detected variants having a MAF > 0.01 (minor allele frequency) in the gnomAD population database were filtered. The remaining variants were classified according to the ACMG standards and guidelines [21,22]. A web-based interpretation tool, Franklin (Genoox) [23] was used to assist the classification. HGMD Professional and ClinVar databases were also used in variant interpretation.

2.5. Clinical Data Collection

Clinical data of patients and family members having a ‘pathogenic’ (‘P’) or ‘likely pathogenic’ (‘LP’) mutation in one of the MODY-causing genes was collected from their application form sent and filled out by their clinician at the time of requesting the genetic testing. The MODY probability calculator (https://www.diabetesgenes.org/, accessed on 20 March 2021) was used to calculate the probability of the patient having MODY when all the information required was available and the patient was under the age of 35, as the calculator cannot be used in case of patients older than that.

3. Results

GCK Mutations

From the 450 index patients examined, 132 tested positive for a pathogenic or likely pathogenic classified variant in one of the MODY-causing genes with a total of 89 mutations. GCK and HNF1A mutations together were responsible for about 90% of the cases, this ratio being higher in Hungary than the 70% reported in the literature [15]. More than 70% (65/89) of the mutations among the index patients were found in the GCK gene (Table 1).
With targeted cascade testing of family members, we identified an additional 95 positive cases, resulting in a total of 227 patients with a molecular genetic diagnosis of MODY. More than 70% of these patients have a mutation in the GCK gene, which means that GCK-MODY is the most prevalent form of MODY in Hungary.

Table 1. Number of patients harbouring a pathogenic/likely pathogenic mutation in one of the MODY-causing genes.

| Gene           | No. of Index Patients with ‘P’/‘LP’ Mutations | No. of Index Patients and Their Family Members with ‘P’/‘LP’ Mutations |
|----------------|---------------------------------------------|-------------------------------------------------|
| GCK            | 91 (68.9%)                                  | 163 (71.8%)                                    |
| HNF1A          | 30 (22.7%)                                  | 48 (21.1%)                                     |
| other MODY-causing gene ¹ | 11 (8.3%)                                  | 16 (7.0%)                                     |
| **Total**      | **132 (100.0%)**                            | **227 (100.0%)**                               |

¹ ABCC8, HNF1B, HNF4A, INS, KCNJ11. P: pathogenic; LP: likely pathogenic.

In the 91 index patients and their 72 family members, we have identified a total of 65 different pathogenic (18) and likely pathogenic (47) GCK mutations, summarized in Table 2 and Figure 1. Every mutation detected was in heterozygous form. Eighteen mutations were found in more than one apparently unrelated families, the most frequent ones being p.Arg36Trp (5 families), p.Gly261Arg (G > A 5 families and G > C 1 family) and p.Ser340Asn (5 families). Of the detected mutations, 40% (28/65) are novel, while 60% (37/65) have been previously described in the literature. Almost 85% (55/65) of the detected GCK mutations were missense mutations resulting in an amino acid change. In addition, four such mutations were found at exon/intron boundaries of the coding sequence, possibly disrupting exon splicing as well (Table 3).

In the case of two families (F101, F173), the p.Ala188Thr and p.Val226Glu mutations were both detected and co-segregated in the proband and her parent, suggesting a cis position.

Table 4 presents the clinical data of the index patients and their family members. Obesity is not characteristic of these patients, only about 10% of them have their BMI out of the range considered healthy. The age of diagnosis differs widely among the patients, and they have generally received their molecular genetic diagnosis of MODY several years after their diagnosis of diabetes. We had information regarding their treatment in 125 cases. Almost half of the patients examined do not receive any treatment or control their blood sugar levels only by maintaining a healthy diet, which is in accordance with the literature. However, around 10% of these patients receive unnecessary insulin treatment and another 16% are on some oral antidiabetic drug, also unnecessary (Table 5). Their HbA1c levels are generally around 7.0% or lower.

The detected GCK mutation was shown to be de novo in two cases (Table 4, F041 and F375).
| Nucleotide Change | Protein Change | Exon/Intron | Function | ACMG | ACMG Evidence | ClinVar | gnomAD Alleles (MAF) | Pr/FM | Family ID | Novel/Known | Reference |
|------------------|----------------|-------------|----------|------|---------------|---------|----------------------|-------|-----------|-------------|-----------|
| c.98T > C        | p.Val33Ala     | exon 2      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP2 (1); PP3 (1); PP5 (3) | Pathogenic (1) | N/A | 2/0 | F306, F454 | known |
| c.106C > T       | p.Arg36Trp     | exon 2      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (2); PP2 (1); PP3 (1); PP5 (3) | Pathogenic/likely pathogenic (4) | 4 (0.0001414) | 5/4 | F028, F015, F310, F433, F434 | known |
| c.115_117delAAG  | p.Lys39del     | exon 2      | In-Frame | Pathogenic | PM1 (2); PM2 (2); PM4 (2); PP1 (3) | N/A | N/A | 1/2 | F044 | known |
| c.130G > A       | p.Gly44Ser     | exon 2      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (3); PP2 (1); PP3 (1); PP5 (3) | Pathogenic (1) | N/A | 1/3 | F133 | known |
| c.171G > T       | p.Met57Ile     | exon 2      | Missense | Pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1); PP5 (3); PS1 (1) | Pathogenic (1) | N/A | 1/0 | F463 | novel |
| c.208G > A       | p.Glu70Lys     | exon 2      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (1); PP3 (1); PP5 (1) | N/A | N/A | 1/0 | F389 | known |
| c.234A > C       | p.Gly72Ala     | exon 3      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (2); PP1 (2); PP2 (1); PP3 (1) | N/A | N/A | 2/3 | F069, F274 | novel |
| c.244A > C       | p.Thr82Pro     | exon 3      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1) | N/A | N/A | 1/3 | F006 | known |
| c.266T > G       | p.Val89Gly     | exon 3      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1) | N/A | N/A | 1/1 | F472 | known |
| c.351A > T       | p.Asparagine   | exon 3      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (2); PP1 (1); PP2 (1); PP3 (1); PP5 (2) | Likely pathogenic (1) | N/A | 1/1 | F083 | novel |
| c.425A > C       | p.Lys142Thr    | exon 4      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1) | N/A | N/A | 1/0 | F250 | novel |
| c.437T > C       | p.Leu146Arg    | exon 4      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (1); PP3 (1) | N/A | N/A | 1/0 | F018 | known |
| c.457C > T       | p.Pro153Ser    | exon 4      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (1); PP2 (1); PP3 (1); PP5 (1) | Uncertain significance (2) | N/A | 1/1 | F103 | known |
| c.460G > A       | p.Val154Met    | exon 4      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (2); PP2 (1); PP3 (1) | N/A | N/A | 1/1 | F381 | novel |
| c.494T > C       | p.Leu165Pro    | exon 5      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1) | N/A | N/A | 2/0 | F092, F291 | known |
| c.501G > C       | p.Trp167Cys    | exon 5      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1) | N/A | N/A | 1/0 | F349 | novel |
| c.518C > A       | p.Ala173Asp    | exon 5      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (1); PP3 (1) | N/A | N/A | 1/0 | F002 | novel |
| Nucleotide Change | Protein Change | Exon/Intron | Function | ACMG | ACMG Evidence | ClinVar | gnomAD Alleles (MAF) | Pr/FM | Family ID | Novel/Known | Reference |
|------------------|----------------|-------------|----------|------|---------------|---------|---------------------|-------|-----------|-------------|-----------|
| c.562G > A       | p.Ala188Thr    | exon 5      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (2); PP3 (1); PP5 (3) | Pathogenic (2) | 1 (0.000003982) | 2/2 | F101, F173 | known       | [34]      |
| c.572G > C       | p.Arg191Pro    | exon 5      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP2 (1); PP3 (1) | N/A | N/A | 1/0 | F374 | novel       |           |
| c.599T > C       | p.Val200Ala    | exon 6      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (2); PP3 (1) | N/A | N/A | 1/0 | F094 | known       | [10]      |
| c.617C > T       | p.Thr206Met    | exon 6      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP2 (1); PP3 (1); PP5 (1) | N/A | 1 (0.000003977) | 2/2 | F031, F116 | known       | [35]      |
| c.620T > C       | p.Val207Ala    | exon 6      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (2); PP3 (1) | N/A | N/A | 1/0 | F316 | novel       |           |
| c.622G > T       | p.Ala208Ser    | exon 6      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP2 (1); PP3 (1) | N/A | N/A | 1/0 | F205 | novel       |           |
| c.623C > T       | p.Ala208Val    | exon 6      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (3); PP2 (1); PP3 (1) | Uncertain significance (1) | 1 (0.000003977) | 1/2 | F227 | known       | [36]      |
| c.626C > T       | p.Thr209Met    | exon 6      | Missense | Pathogenic | PM1 (2); PM2 (2); PP1 (2); PP2 (1); PP3 (1); PP5 (1) | Pathogenic (1) | N/A | 3/1 | F041, F042, F153 | known     | [25]      |
| c.649G > A       | p.Asp217Asn    | exon 6      | Missense | Likely pathogenic | BS4 (1); PM1 (2); PM2 (2); PP2 (1); PP3 (1) | Uncertain significance (4) | 1 | 1/0 | F150 | known       |           |
| c.660C > A       | p.Cys220*      | exon 6      | Nonsense | Likely pathogenic | PM2 (2); PV51 (4) | N/A | N/A | 1/0 | F165 | known       | [38]      |
| c.668G > A       | p.Gly223Asp    | exon 6      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP2 (1); PP3 (1) | N/A | N/A | 1/2 | F411 | novel       |           |
| c.677T > A       | p.Val226Glu    | exon 6      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (2); PP2 (1); PP3 (1) | N/A | N/A | 2/2 | F101, F173 | known       | [39]      |
| c.683C > T       | p.Thr228Met    | exon 7      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (2); PP2 (1); PP3 (1); PP5 (3) | Pathogenic (5) | 1 (0.000003999) | 1/0 | F065 | known       | [40]      |
| c.702C > A       | p.Tyr234*      | exon 7      | Non sense | Pathogenic | PM2 (2); PP1 (2); PV51 (4) | N/A | N/A | 2/2 | F031, F244 | known       | [41]      |
| c.709_711delGAG  | p.Glu237del    | exon 7      | In-frame | Likely pathogenic | PM1 (2); PM2 (2); PM4 (2); PP1 (2) | N/A | N/A | 2/1 | F070, F246 | novel       |           |
| c.724G > T       | p.Glu242*      | exon 7      | Nonsense | Pathogenic | PM2 (2); PP1 (3); PV51 (4) | N/A | N/A | 1/3 | F382 | novel       |           |
| c.730G > A       | p.Val244Met    | exon 7      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1); PP5 (1) | Conflicting (1 LP, 1 VUS) | N/A | 1/0 | F400 | novel       |           |
| c.742G > A       | p.Glu248Lys    | exon 7      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1); PP5 (1) | N/A | 1 (0.000003982) | 1/0 | F219 | known       | [42]      |
| c.752T > G       | p.Met251Arg    | exon 7      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (3); PP2 (1); PP3 (1) | N/A | N/A | 1/4 | F145 | known       | [10]      |
| c.778T > G       | p.Phe260Val    | exon 7      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP2 (1); PP3 (1) | N/A | N/A | 2/2 | F408, F455 | known       | [43]      |
Table 2. Cont.

| Nucleotide Change | Protein Change | Exon/Intron | Function | ACMG | ACMG Evidence | ClinVar | gnomAD Alleles (MAF) | Pr/FM | Family ID | Novel/Known | Reference |
|-------------------|----------------|-------------|----------|------|----------------|---------|----------------------|-------|-----------|-------------|-----------|
| c.781G > A        | p.Gly261Arg    | exon 7      | Missense | Pathogenic | PM1 (2); PM2 (2); PP1 (1); PP2 (1); PP3 (1); PP5 (3); PS1 (3) | Pathogenic (4) | 1 (0.000003983) | 4/1  | F126, F191, F202, F222 | known       | [40]       |
| c.781G > C        | p.Gly261Arg    | exon 7      | Missense | Pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1); PP5 (3); PS1 (3) | Pathogenic (1) | N/A | 1/0       | F108        | known       | [44]       |
| c.790G > A        | p.Gly264Ser    | exon 7      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1); PP5 (1) | Pathogenic (1) | N/A | 2/0       | F080, F216   | known       | [55]       |
| c.790G > T        | p.Glu265*      | exon 7      | Missense | Pathogenic | PM2 (2); PP1 (1); PP5 (3); PVSI (4) | Pathogenic (2) | N/A | 1/1       | F035        | known       | [45]       |
| c.822C > A        | p.As274Glu     | exon 7      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1) | N/A | 1 (0.000003992) | 1/0  | F280        | novel       |           |
| c.824G > C        | p.Arg275Pro    | exon 7      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP2 (1); PP4 (1) | N/A | N/A | 1/0       | F107        | novel       |           |
| c.863T > C        | p.Leu288Pro    | exon 7      | Missense/splicing | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1) | N/A | N/A | 1/1       | F403        | novel       |           |
| c.869A > G        | p.Glu290Gly    | exon 8      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (1); PP2 (1); PP3 (1) | N/A | N/A | 2/6       | F043, F046   | known       |           |
| c.872A > T        | p.Lys291Met    | exon 8      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (3); PP2 (1); PP3 (1) | N/A | N/A | 1/1       | F038        | known       | [39]       |
| c.884G > A        | p.Gly295Asp    | exon 8      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (1); PP2 (1); PP3 (1) | N/A | N/A | 2/5       | F085, F162, F353 | known       | [47]       |
| c.886A > C        | p.Lys296Gln    | exon 8      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP2 (1); PP3 (1) | N/A | 2 (0.000008008) | 1/0  | F286        | novel       |           |
| c.908G > A        | p.Arg309Gln    | exon 8      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (3); PP3 (1); PP5 (2) | N/A | 1/1       | F167 | known       | [46]       |
| c.952G > A        | p.Gly318Arg    | exon 8      | Missense | Pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (3); PP3 (1); PP5 (2) | Pathogenic (1) | N/A | 3/5       | F016, F209, F335 | known       | [47]       |
| c.982delG         | p.Gly328Gluufs*25 | exon 8    | Frameshift | Pathogenic | PM2 (2); PP1 (3); PVSI (4) | N/A | N/A | 2/5 | F085, F162 | novel       |           |
| c.989T > C        | p.Phe330Ser    | exon 8      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (1); PP2 (1); PP3 (1) | Likely pathogenic (1) | N/A | 1/1       | F122        | known       | [48]       |
| c.1019G > A       | p.Ser340Asn    | exon 8      | Missense/splicing | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (3); PP2 (1); PP3 (1) | N/A | N/A | 5/4 | F027, F062, F163, F187, F197 | known       | [32]       |
| c.1019G > C       | p.Ser340Thr    | exon 8      | Missense/splicing | Likely pathogenic | PM1 (2); PM2 (2); PM5 (1); PP1 (1); PP2 (1); PP3 (1) | N/A | N/A | 1/1 | F435 | known       | [49]       |
| c.1019 + 1G > T   | Splice         | intron 8    | Splicing | Likely pathogenic | PM2 (2); PVSI (3) | N/A | N/A | 1/0 | F131        | known       | [50]       |
| c.1130G > C       | p.Arg377Pro    | exon 9      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PM5 (2); PP2 (1); PP3 (1) | N/A | N/A | 1/0 | F482 | novel       |           |
| c.1139A > C       | p.His380Pro    | exon 9      | Missense | Likely pathogenic | PM1 (2); PM2 (2); PP1 (1); PP2 (1); PP3 (1) | N/A | N/A | 1/1 | F200 | novel       |           |
| Nucleotide Change   | Protein Change | Exon/Intron | Function     | ACMG          | ACMG Evidence                  | ClinVar | gnomAD Alleles (MAF) | Pr/FM | Family ID | Novel/Known | Reference |
|---------------------|----------------|-------------|--------------|---------------|-------------------------------|---------|----------------------|-------|-----------|-------------|-----------|
| c.1186_1193delA     | p.Ser396Argfs*60 | exon 9      | Frameshift   | Likely        | PM2 (2); PVSI (4)             | N/A     | N/A                  | 1/0   | F314      | novel       |           |
| c.1225G > C         | p.Asp409His     | exon 9      | Missense     | Likely        | PM1 (2); PM2 (2); PP1 (1); PP2 (1); PP3 (1) | N/A     | N/A                  | 1/1   | F113      | novel       |           |
| c.1268T > C         | p.Phe423Ser     | exon 10     | Missense     | Likely        | PM1 (2); PM2 (2); PM5 (1); PP1 (2); PP2 (1); PP3 (1) | Uncertain significance (1) | N/A     | 1/0      | F275        | known     | [51]      |
| c.1340G > A         | p.Arg447Gln     | exon 10     | Missense     | Likely        | PM1 (2); PM2 (2); PM5 (1); PP1 (2); PP2 (1); PP3 (1); PP5 (2) | Likely pathogenic (1) | N/A     | 2/2      | F201, F373 | known     | [59,42]   |
| c.1340G > C         | p.Arg447Pro     | exon 10     | Missense     | Likely        | PM1 (2); PM2 (2); PM5 (1); PP1 (2); PP2 (1); PP3 (1) | Uncertain significance (1) | N/A     | 1/0      | F273        | known     | [52]      |
| c.1355T > G         | p.Val452Gly     | exon 10     | Missense     | Likely        | PM1 (2); PM2 (2); PP1 (2); PP3 (1) | N/A     | N/A                  | 1/0   | F263      | novel       |           |

GCK reference sequence: NM_000162.5, novel mutations are shown in bold. ACMG: shows the classification of the mutation based on the ACMG guidelines; ACMG evidence: the criteria and their strength used for the ACMG classification, as follows: (1)—supporting, (2)—moderate, (3)—strong, (4)—very strong, (5)—stand-alone; ClinVar: the classification of the mutation according to ClinVar, with the number of submissions in brackets; gnomAD MAF: minor allele frequency of the mutation in the gnomAD database; Pr/FM: number of probands/their family members the mutation was found in; family ID: identification of the families the mutation was found in.
Table 3. GCK mutations distributed by the amino acid consequence.

| Consequence               | No. of Mutations |
|---------------------------|------------------|
| Missense                  | 51 (78.5%)       |
| Missense and/or splicing  | 4 (6.2%)         |
| Splicing                  | 21 (3.1%)        |
| Nonsense                  | 4 (6.2%)         |
| Frameshift                | 2 (3.1%)         |
| In-frame                  | 2 (3.1%)         |

Figure 1. GCK mutations detected in the index patients. Novel mutations are shown in colour.
Table 4. Clinical data of patients with GCK mutation.

| Family ID | Sample ID | Age at Diagnosis of Diabetes | Age at Receiving Genetic Dg | BMI * | Obesity | Complications | Therapy BEFORE Genetic Diagnosis | FPG (0') (mmol/L) | PPG (120') (mmol/L) | HbA1c % (mmol/mol) | MODY Calculator (%) | Family Screening |
|-----------|-----------|-----------------------------|-----------------------------|-------|---------|--------------|---------------------------------|----------------|----------------|------------------|------------------|-------------------|
| F002      | P002      | 32                          | 47                          | 26.2  | no      | none          | OAD—metformin                  | 7.3            | N/A             | 6.3 (45.4)       | 15.1             | no family members tested |
| F006      | P015      | 31                          | N/A                         | 23    | no      | none          | insulin                        | 6.8            | 19.0            | 6.7 (49.7)       | 12.6             | multiple generations affected |
| F006      | P016      | 46                          | N/A                         | 33    | yes     | HLD, PAD      | insulin                        | 7.0            | 12.0            | 8.2 (66.1)       | N/A              | multiple generations affected |
| F006      | P017      | 3                           | 4                           | 15.6  | no      | none          | diet                           | 6.0            | 9.0             | N/A              | N/A              | multiple generations affected |
| F006      | P018      | no diabetes                 | 1                           | N/A   | N/A     | N/A           | N/A                            | N/A            | N/A             | N/A              | N/A              | multiple generations affected |
| F011      | P028      | 17                          | 27                          | 19.8  | no      | none          | OAD—acarbose                   | 7.0            | 6.0             | 6.2 (44.3)       | 75.5             | multiple generations affected |
| F011      | P029      | 42                          | 53                          | 24.7  | no      | none          | OAD—sulphonylurea              | 9.2            | 5.2             | 6.5 (47.5)       | N/A              | multiple generations affected |
| F011      | P030      | 2                           | 3                           | 15.4  | no      | none          | diet                           | 6.7            | N/A             | N/A              | N/A              | multiple generations affected |
| F016      | P037      | childhood                   | 30                          | 25    | no      | none          | OAD—metformin                  | 5.8            | 6.2             | 6.6 (48.6)       | N/A              | multiple generations affected |
| F016      | P038      | no diabetes                 | 3                           | N/A   | N/A     | none          | N/A                            | N/A            | N/A             | N/A              | N/A              | multiple generations affected |
| F016      | P040      | 15                          | 33                          | N/A   | N/A     | none          | diet                           | 7.6            | N/A             | N/A              | N/A              | multiple generations affected |
| F016      | P041      | N/A                         | 5                           | N/A   | N/A     | none          | diet                           | 5.8            | 8.1             | 6.1 (43.2)       | N/A              | multiple generations affected |
| F016      | P042      | N/A                         | 55                          | N/A   | N/A     | N/A           | N/A                            | N/A            | N/A             | N/A              | N/A              | multiple generations affected |
| F016      | P044      | N/A                         | 14                          | N/A   | N/A     | N/A           | N/A                            | N/A            | N/A             | N/A              | N/A              | multiple generations affected |
| F018      | P047      | 10                          | 14                          | N/A   | N/A     | none          | diet                           | 6.5            | N/A             | 6.3 (45.4)       | N/A              | no family members tested |
| F027      | P063      | N/A                         | 25                          | N/A   | N/A     | none          | diet                           | 6.1            | 8.8             | 6.2 (44.3)       | N/A              | no family members tested |
| F028      | P064      | 10                          | 12                          | 19.4  | no      | none          | N/A                            | 7.1            | 8.5             | N/A              | N/A              | multiple generations affected |
| F028      | P065      | N/A                         | 11                          | 23.2  | no      | none          | OAD—metformin                  | 6.2            | 7.1             | 6.5 (47.5)       | N/A              | multiple generations affected |
| F028      | P066      | N/A                         | 41                          | N/A   | N/A     | none          | N/A                            | 6.7            | 6.7             | N/A              | N/A              | multiple generations affected |
| F031      | P069      | 8                           | 11                          | 16.2  | no      | none          | OAD—metformin                  | 7.6            | 7.9             | 6.7 (49.7)       | 75.5             | multiple generations affected |
| F031      | P070      | 27                          | 40                          | N/A   | N/A     | N/A           | diet                           | N/A            | N/A             | N/A              | N/A              | multiple generations affected |
| F035      | P074      | 8                           | 11                          | 19.1  | no      | none          | diet                           | 6.0            | 15.2            | 6.2 (43.2)       | 75.5             | multiple generations affected |
| F035      | P075      | 26                          | 40                          | N/A   | N/A     | N/A           | OAD—metformin                  | N/A            | N/A             | N/A              | N/A              | multiple generations affected |
| F038      | P078      | N/A                         | 36                          | N/A   | N/A     | N/A           | N/A                            | N/A            | N/A             | N/A              | N/A              | multiple generations affected |
| F038      | P079      | 7                           | 12                          | 15.2  | no      | none          | diet                           | 6.5            | 8.6             | N/A              | N/A              | multiple generations affected |
| F041      | P082      | 8                           | 10                          | 14.31 | no     | none          | diet                           | 8.1            | 11.6            | 6.2 (44.3)       | 75.5             | de novo            |
### Table 4. Cont.

| Family ID | Sample ID | Age at Diagnosis of Diabetes | Age at Receiving Genetic Dg | BMI | Obesity | Complications | Therapy BEFORE Genetic Diagnosis | FPG (0′) (mmol/L) | PPG (120′) (mmol/L) | HbA1c % (mmol/mol) | MODY Calculator (%) | Family Screening |
|-----------|-----------|-----------------------------|-----------------------------|-----|---------|---------------|----------------------------------|-----------------|-----------------|------------------|-----------------|-----------------|
| F042      | P085      | 4                           | 7                           | 15.7| no      | none          | diet                            | 6.0             | 8.5             | 6.4 (46.4)       | 75.5            | siblings positive, parents not tested |
| F042      | P086      | 1                           | 1                           | N/A | N/A     | N/A           | diet                            | N/A             | N/A             | 4.8 (29.0)       | N/A             | siblings positive, parents not tested |
| F043      | P088      | 20                          | 34                          | 21.5| no      | none          | diet                            | 7.9             | N/A             | 6.4 (46.4)       | 75.5            | no family members tested |
| F044      | P089      | 14                          | 17                          | 24  | no      | none          | insulin                         | 5.1             | N/A             | 6.8 (50.8)       | 49.4            | multiple generations affected |
| F044      | P090      | N/A                         | 48                          | 29.5| no      | renal cysts   | N/A                             | 6.9             | N/A             | 6.7 (49.7)       | N/A             | multiple generations affected |
| F044      | P091      | 46                          | 69                          | 21  | no      | TIA, glaucoma, osteoporosis | OAD—metformin    | 5.1             | N/A             | 6.6 (48.6)       | N/A             | multiple generations affected |
| F046      | P104      | 18                          | 45                          | 22.0| no      | none          | none                            | N/A             | N/A             | 5.5 (36.6)       | 75.5            | multiple generations affected |
| F046      | P105      | 13                          | 18                          | 23.7| no      | none          | insulin                         | 7.3             | 11.2            | 6.7 (49.7)       | 49.4            | multiple generations affected |
| F046      | P106      | 14                          | 24                          | 18.5| no      | none          | insulin                         | 6.0             | 9.5             | 6.2 (44.3)       | 75.5            | multiple generations affected |
| F046      | P108      | 15                          | 20                          | 19.4| no      | none          | insulin                         | 7.7             | 8.7             | 7.0 (53.0)       | 8.2             | multiple generations affected |
| F046      | P113      | N/A                         | 1                           | N/A | N/A     | N/A           | N/A                             | N/A             | N/A             | N/A             | N/A             | multiple generations affected |
| F046      | P738      | N/A                         | 3                           | 15.5| no      | none          | none                            | 5.7             | N/A             | 6.0 (42.1)       | N/A             | multiple generations affected |
| F046      | P739      | N/A                         | 6                           | 15.7| no      | none          | none                            | 5.8             | N/A             | 6.1 (43.2)       | N/A             | multiple generations affected |
| F062      | P128      | 3                           | 3                           | N/A | N/A     | none          | none                            | 6.0             | 5.4             | N/A             | N/A             | multiple generations affected |
| F062      | P129      | no diabetes                 | N/A                         | N/A | N/A     | N/A           | N/A                             | N/A             | N/A             | N/A             | N/A             | multiple generations affected |
| F062      | P130      | no diabetes                 | N/A                         | N/A | N/A     | N/A           | N/A                             | N/A             | N/A             | N/A             | N/A             | multiple generations affected |
| F065      | P133      | 11                          | 23                          | 30  | yes     | N/A           | insulin                         | N/A             | N/A             | 6.6 (48.6)       | 12.6            | no family members tested |
| F069      | P141      | 16                          | 18                          | 18.7| no      | none          | OAD—metformin                   | 6.8             | N/A             | 5.7 (38.8)       | 75.5            | multiple generations affected |
| F069      | P142      | N/A                         | 51                          | N/A | N/A     | N/A           | N/A                             | 6.9             | N/A             | 6.4 (46.4)       | N/A             | multiple generations affected |
| F069      | P143      | N/A                         | 64                          | N/A | N/A     | N/A           | N/A                             | 7.1             | N/A             | 6.4 (46.4)       | N/A             | multiple generations affected |
| F070      | P144      | N/A                         | 23                          | 18.7| no      | ligament tear | OAD—metformin                   | 7.1             | N/A             | 6.0 (42.1)       | N/A             | no family members tested |
| F080      | P138      | N/A                         | 8                           | N/A | N/A     | N/A           | N/A                             | N/A             | N/A             | N/A             | N/A             | no family members tested |
| F083      | P157      | N/A                         | 24                          | N/A | N/A     | PCOS          | none                            | 6.9             | 7.9             | N/A             | N/A             | multiple generations affected |
| F083      | P158      | 15                          | 50                          | N/A | N/A     | N/A           | OAD—metformin                   | 7.78            | N/A             | 6.3 (45.4)       | N/A             | multiple generations affected |
| F085      | P160      | 16                          | 18                          | 20.1| no      | none          | diet                            | 7.1             | 7.9             | 6.8 (50.8)       | 75.5            | multiple generations affected |
| F085      | P161      | 15                          | 32                          | 17.8| no      | none          | diet                            | 6.0             | N/A             | 6.1 (43.2)       | 75.5            | multiple generations affected |
| F085      | P162      | 2                           | 2                           | N/A | N/A     | granuloma annulare | diet                        | 6.1             | N/A             | 6.1 (43.2)       | N/A             | multiple generations affected |
| Family ID | Sample ID | Age at Diagnosis of Diabetes | Age at Receiving Genetic Dg | BMI * | Obesity | Complications | Therapy BEFORE Genetic Diagnosis | FPG (0’) (mmol/L) | PPG (120’) (mmol/L) | HbA1c % (mmol/mol) | MODY Calculator (%) | Family Screening |
|-----------|-----------|-----------------------------|-----------------------------|-------|---------|---------------|----------------------------------|-----------------|-----------------|-------------------|-------------------|------------------|
| F092      | P170      | N/A                         | 15                          | N/A   | N/A     | none          | diet                            | 6.6             | 9.4             | 6.5 (47.5)        | N/A               | no family members tested |
| F094      | P172      | 10                          | 15                          | 19.4  | no      | none          | diet                            | 7.2             | 9.6             | 6.4 (46.4)        | 75.5              | parents not tested |
| F101      | P192      | 6                           | 7                           | 13.4  | no      | none          | none                            | 6.0             | 10.0            | 6.0 (42.1)        | 75.5              | multiple generations affected |
| F101      | P193      | no diabetes                 | 46                          | normal| no      | N/A           | N/A                             | N/A             | N/A             | N/A               | N/A               | multiple generations affected |
| F103      | P196      | 5                           | 6                           | 17    | no      | none          | diet                            | 6.2             | 8.8             | 6.3 (45.4)        | 75.5              | multiple generations affected |
| F103      | P197      | 36                          | 42                          | 24    | no      | none          | insulin                         | 6.9             | 13.6            | 6.0 (42.1)        | N/A               | multiple generations affected |
| F105      | P199      | 9                           | 14                          | 20.9  | no      | N/A           | diet                            | 7.3             | N/A             | N/A               | N/A               | multiple generations affected |
| F105      | P200      | 25                          | 37                          | 30.1  | yes     | none          | none                            | N/A             | N/A             | N/A               | N/A               | multiple generations affected |
| F105      | P202      | N/A                         | 6                           | 15.1  | no      | none          | none                            | N/A             | N/A             | N/A               | N/A               | multiple generations affected |
| F107      | P204      | 16                          | 17                          | 19    | no      | none          | diet                            | 7.9             | 8.8             | 7.0 (53.0)        | 75.5              | no family members tested |
| F108      | P205      | 21                          | 30                          | 15.1  | no      | insulin      | N/A                             | 6.2             | 10.4            | 6.0 (42.1)        | 75.5              | no family members tested |
| F113      | P210      | 12                          | 17                          | 17    | no      | insulin      | N/A                             | 6.3             | 45.4            | 12.6              | multiple generations affected |
| F113      | P211      | 43                          | 45                          | 22.2  | no      | none          | diet                            | 6.9             | 9.2             | 6.3 (45.4)        | N/A               | multiple generations affected |
| F116      | P214      | 7                           | 19                          | 19.8  | no      | headache, elevated RR | OAD—metformin | 5.8             | 14              | 6.8 (50.8)        | 75.5              | cousin positive, parents not tested |
| F116      | P215      | 9                           | 15                          | 21.1  | no      | none          | diet                            | 5.8             | 6.4             | 6.5 (47.5)        | 75.5              | cousin positive, parents not tested |
| F122      | P222      | 14                          | 20                          | 25    | no      | N/A           | diet                            | 6.5             | N/A             | 6.2 (44.3)        | 75.5              | siblings positive, parents not tested |
| F122      | P223      | 14                          | 24                          | 24    | no      | none          | diet                            | 6.3             | N/A             | N/A               | N/A               | siblings positive, parents not tested |
| F126      | P227      | 14                          | 36                          | 22.5  | no      | PCOS          | OAD—metformin | 5.9             | >11             | 5.8 (39.9)        | 75.5              | no family members tested |
| F131      | P232      | 19                          | 28                          | 19.4  | no      | none          | none                            | 8.4             | N/A             | 6.8 (50.8)        | 75.5              | no family members tested |
| F133      | P236      | 13                          | 14                          | 19    | no      | none          | diet                            | 6.4             | 9.4             | 6.4 (46.4)        | 75.5              | multiple generations affected |
| F133      | P237      | 11                          | 18                          | 19    | no      | insulin      | N/A                             | N/A             | 6.4 (46.4)       | 8.2               | multiple generations affected |
| F133      | P240      | 31                          | 42                          | 21    | no      | none          | OAD                             | 7.8             | 10.00           | 6.4 (46.4)        | 58                | multiple generations affected |
| F133      | P241      | 57                          | 63                          | 38    | yes     | IHD           | OAD                             | 8.7             | 10              | N/A               | N/A               | multiple generations affected |
| F145      | P257      | 6                           | 12                          | 15.2  | no      | insulin      | N/A                             | 7.1             | 54.1            | 12.6              | multiple generations affected |
| F145      | P258      | 37                          | 38                          | N/A   | N/A     | N/A           | N/A                             | 7.3             | 7.8             | 5.8 (39.9)        | N/A               | multiple generations affected |
| F145      | P259      | no diabetes                 | 2016                        | N/A   | none    | N/A           | N/A                             | 7.0             | 5.9             | N/A               | N/A               | multiple generations affected |
| F145      | P260      | 4                           | 5                           | N/A   | N/A     | none          | N/A                             | 9.7             | 6.8             | 6.8 (50.8)        | N/A               | multiple generations affected |
| F145      | P261      | 39                          | 40                          | 24.3  | no      | N/A           | N/A                             | 7.2             | 5.9             | 6.3 (45.4)        | N/A               | multiple generations affected |
Table 4. Cont.

| Family ID | Sample ID | Age at Diagnosis of Diabetes | Age at Receiving Genetic Dg | BMI ¹ | Obesity | Complications | Therapy BEFORE Genetic Diagnosis | FPG (0′) (mmol/L) | PPG (120′) (mmol/L) | HbA1c % (mmol/mol) | MODY Calculator (%) | Family Screening |
|-----------|-----------|-----------------------------|----------------------------|-------|---------|---------------|---------------------------------|----------------|----------------|------------------|-----------------|-----------------|
| F150      | P266      | 35                          | 59                         | 21.6  | no      | none          | OAD—metformin                   | 10.1           | N/A             | 7.4 (57.4)       | 15.1            | parents not tested |
| F153      | P271      | 9                           | 16                         | 23.6  | no      | none          | diet                           | 6.9            | 12.0            | 6.2 (44.3)       |                | no family members tested |
| F162      | P280      | 9                           | 12                         | 32.4  | yes     | acanthosis nigricans | OAD—metformin                 | N/A            | N/A             | 6.5 (47.5)       | 75.5            | multiple generations affected |
| F162      | P281      | 3                           | 4                          | 14.1  | no      | N/A           | diet                           | 4.7            | 5.7             | 6.4 (46.4)       | 75.5            | multiple generations affected |
| F162      | P282      | N/A                         | 2                          | N/A   | N/A     | N/A           | diet                           | 5.4            | 6.2             | N/A             | N/A            | multiple generations affected |
| F162      | P283      | no diabetes                 | 21                         | N/A   | N/A     | N/A           | none                           | 10.0           | 6.3             | 6.2 (44.3)       | 75.5            | multiple generations affected |
| F163      | P284      | 7                           | 11                         | 29.0  | yes     | none          | none                           | 6.6            | 10.9            | 5.9 (41.0)       | 75.5            | multiple generations affected |
| F163      | P285      | 9                           | 13                         | 23.5  | no      | none          | none                           | 10.0           | 6.3             | 6.2 (44.3)       | 75.5            | multiple generations affected |
| F165      | P288      | 10                          | 11                         | 18.0  | no      | N/A           | diet                           | 7              | 10.8            | 5.9 (41.0)       | 75.5            | no family members tested |
| F167      | P290      | 13                          | 13                         | 19.2  | no      | none          | diet                           | 5.2            | 10.7            | 5.7 (38.8)       | 75.5            | multiple generations affected |
| F167      | P292      | no diabetes                 | 48                         | N/A   | N/A     | N/A           | N/A                            | N/A            | N/A             | N/A             |                | multiple generations affected |
| F173      | P298      | 1                           | 2                          | 15    | no      | N/A           | diet                           | 5.6            | 9.9             | 6.4 (46.4)       | 75.5            | multiple generations affected |
| F173      | P299      | 16                          | 33                         | N/A   | N/A     | N/A           | N/A                            | N/A            | N/A             | N/A             |                | multiple generations affected |
| F187      | P319      | 1                           | 4                          | 16.5  | no      | none          | none                           | 6.3—7          | 5.8—7           | 6.5 (47.5)       | 75.5            | no family members tested |
| F191      | P323      | 1                           | 12                         | 16.8  | no      | none          | diet                           | 6.6            | 7.3             | 6.6 (48.6)       | 75.5            | no family members tested |
| F197      | P335      | 13                          | 27                         | 21.9  | no      | none          | diet                           | 6.9            | 8.5             | 6.5 (47.5)       | 75.5            | no family members tested |
| F200      | P338      | 5                           | 18                         | 17.7  | no      | none          | diet                           | 6.17           | 8.3             | 6.8 (50.8)       | 75.5            | multiple generations affected |
| F200      | P339      | 47                          | 51                         | 23.9  | no      | none          | OAD—metformin + sulphonylurea   | N/A            | N/A             | N/A             |                | multiple generations affected |
| F201      | P340      | 23                          | 31                         | 19.7  | no      | none          | OAD—sulphonylurea               | 6.6            | 11.2            | 6.1 (43.2)       | 75.5            | multiple generations affected |
| F201      | P341      | 57                          | 60                         | 30.8  | yes     | N/A           | diet                           | 6.9            | 14.5            | 6.3 (45.4)       | N/A            | multiple generations affected |
| F201      | P342      | 22                          | 35                         | 23.1  | no      | none          | diet                           | 7.3            | 9.8             | 5.8 (39.9)       | 75.5            | multiple generations affected |
| F202      | P343      | 5                           | 6                          | 17.0  | no      | none          | none                           | 6.2            | 8.2             | 6.4 (46.4)       | 75.5            | siblings positive, parents not tested |
| F202      | P344      | 9                           | 10                         | 15.4  | no      | none          | none                           | 6.5            | 6.9             | 6.9 (51.9)       | 75.5            | siblings positive, parents not tested |
| F205      | P349      | 4                           | 10                         | 16.5  | no      | none          | diet                           | 5.8            | 8.5             | 5.7 (38.8)       | 75.5            | no family members tested |
| F209      | P353      | 9                           | 43                         | 29    | no      | none          | OAD—metformin                  | 7.2            | 9.5             | 7.0 (53.0)       | 75.5            | no family members tested |
Table 4. Cont.

| Family ID | Sample ID | Age at Diagnosis of Diabetes | Age at Receiving Genetic Dg | BMI * | Obesity | Complications | Therapy BEFORE Genetic Diagnosis | FPG (0’) (mmol/L) | PPG (120’) (mmol/L) | HbA1c % (mmol/mol) | MODY Calculator (%) | Family Screening |
|-----------|-----------|-----------------------------|-----------------------------|-------|---------|---------------|----------------------------------|-----------------|-------------------|-------------------|------------------|------------------|
| F213      | P357      | N/A                         | 12                          | 17.8  | no      | none           | diet                             | 7.1             | 8.6               | 6.2 (44.3)        | N/A              | multiple generations affected |
| F213      | P358      | N/A                         | 34                          | N/A   | N/A     | N/A           | diet                             | N/A             | N/A               | N/A               | N/A              | multiple generations affected |
| F216      | P361      | N/A                         | 14                          | 16.2  | no      | none           | diet                             | 5.6             | 5.9               | 6.3 (45.4)        | N/A              | no family members tested |
| F219      | P364      | 32                          | 39                          | 25.2  | no      | none           | diet                             | 6.9             | 8.7               | 5.5 (36.6)        | 62.4             | parents not tested |
| F227      | P373      | 7                           | 21                          | 25.7  | no      | none           | insulin                          | 7.0             | 14.7              | 6.1 (43.2)        | 75.5             | multiple generations affected |
| F227      | P374      | N/A                         | 15                          | 15.5  | no      | N/A           | N/A                              | 6.8             | N/A               | N/A               | N/A              | multiple generations affected |
| F227      | P377      | 45                          | 51                          | 33    | yes     | none           | OAD—metformin                    | 7.8             | 8.9               | N/A               | N/A              | multiple generations affected |
| F244      | P394      | 15                          | 16                          | 17.6  | no      | none           | diet                             | 6.5             | 8.8               | 6.2 (44.3)        | 75.5             | no family members tested |
| F246      | P396      | 6                           | 7                           | 19.9  | yes     | none           | none                             | 6.7             | 9.4               | 6.3 (45.4)        | 75.5             | multiple generations affected |
| F246      | P398      | no diabetes                 | 43                          | N/A   | N/A     | N/A           | N/A                              | 7.5             | N/A               | 7.0 (53.0)        | N/A              | multiple generations affected |
| F250      | P403      | N/A                         | 35                          | N/A   | N/A     | cardiac       | N/A                              | N/A             | N/A               | N/A               | N/A              | no family members tested |
| F263      | P426      | 18                          | 31                          | 21.5  | no      | N/A           | OAD—metformin                    | 6.9             | 8.5               | 6.4 (46.4)        | 75.5             | no family members tested |
| F272      | P435      | 20                          | 22                          | 17.4  | no      | none           | none                             | 6.7             | 22.9              | 6.0 (42.1)        | 75.5             | no family members tested |
| F273      | P436      | N/A                         | 18                          | 19.5  | no      | N/A           | N/A                              | 5.8             | N/A               | N/A               | N/A              | no family members tested |
| F274      | P437      | 5                           | 16                          | 32.7  | yes     | hypertension, obesity | OAD—metformin | 6.5             | 9.4               | 6.5 (47.5)        | 75.5             | siblings positive, parents not tested |
| F274      | P438      | 2                           | 7                           | 16.7  | no      | none           | diet                             | 6.7             | N/A               | 6.4 (46.4)        | 75.5             | siblings positive, parents not tested |
| F275      | P439      | 8                           | 10                          | 16.3  | no      | none           | diet                             | 6.1             | 8.3               | 6.1 (43.2)        | 75.5             | no family members tested |
| F280      | P444      | 10                          | N/A                         | N/A   | N/A     | N/A           | N/A                              | N/A             | N/A               | N/A               | N/A              | no family members tested |
| F291      | P455      | 10                          | 16                          | 19.2  | no      | none           | insulin                          | 6.5             | 11.7              | 6.8 (50.8)        | 4                | no family members tested |
| F296      | P460      | 13                          | 18                          | N/A   | N/A     | none           | insulin                          | N/A             | N/A               | N/A               | N/A              | no family members tested |
| F306      | P470      | 7                           | 8                           | 14.6  | no      | repeated acute laryngitis | none | 6.10            | N/A               | 6.0 (42.0)        | 75.5             | no family members tested |
| F310      | P477      | 14                          | 15                          | 22.2  | no      | N/A           | N/A                              | N/A             | N/A               | 6.4 (46.4)        | 75.5             | mother no carrier, father not tested |
| F314      | P482      | 4                           | 6                           | 14.9  | no      | none           | diet                             | N/A             | N/A               | 6.0 (42.1)        | 75.5             | no family members tested |
| F316      | P488      | 30                          | 37                          | 20.6  | no      | none           | OAD—metformin                    | 6.3             | 6.9               | 6.3 (45.4)        | 35.8             | no family members tested |
| F349      | P532      | 17                          | 39                          | 20.6  | no      | none           | OAD—metformin                    | 5.7             | N/A               | 6.4 (46.4)        | 75.5             | no family members tested |
| F353      | P536      | 14                          | 23                          | 26.7  | no      | none           | diet                             | 6.9             | 9.6               | 6.5 (47.5)        | 75.5             | no family members tested |
| F373      | P557      | 8                           | 18                          | 19.2  | no      | N/A           | diet                             | 5.7             | 9                 | 5.6 (37.7)        | 75.5             | no family members tested |
| Family ID | Sample ID | Age at Diagnosis of Diabetes | Age at Receiving Genetic Dg | BMI * | Obesity | Complications | Therapy BEFORE Genetic Diagnosis | FPG (0′) (mmol/L) | PPG (120′) (mmol/L) | HbA1c % (mmol/mol) | MODY Calculator (%) | Family Screening |
|-----------|-----------|-----------------------------|----------------------------|-------|---------|---------------|----------------------------------|------------------|---------------------|-------------------|------------------|------------------|
| F374      | P558      | 2                           | 19                         | 20    | no      | N/A           | diet                             | 6.0              | 9.4                 | 6.7 (49.7)        | 75.5             | no family members tested |
| F375      | P559      | 8                           | 10                         | normal | no      | none          | diet                             | 7.9              | 9.5                 | 6.7 (49.7)        | N/A              | de novo           |
| F381      | P570      | 12                          | 12                         | 21.2  | no      | none          | diet                             | 5.3              | 8.8                 | 6.8 (50.8)        | 75.5             | multiple generations affected |
| F381      | P572      | 2                           | 41                         | 24    | no      | N/A           | diet                             | N/A              | N/A                 | N/A               | N/A              | multiple generations affected |
| F382      | P574      | 13                          | 13                         | 22    | no      | none          | diet                             | 7.7              | 9.8                 | 6.3 (45.4)        | 75.5             | multiple generations affected |
| F382      | P575      | N/A                         | 41                         | N/A   | N/A     | N/A           | diet                             | N/A              | N/A                 | N/A               | N/A              | multiple generations affected |
| F382      | P577      | N/A                         | 61                         | N/A   | N/A     | N/A           | insulin                          | 6.0              | 11.0                | 6.3 (45.4)        | 12.6             | mother no carrier, father not tested |
| F382      | P578      | N/A                         | 29                         | N/A   | N/A     | N/A           | insulin                          | N/A              | N/A                 | N/A               | N/A              | mother no carrier, father not tested |
| F389      | P586      | 28                          | 31                         | 20    | no      | none          | insulin                          | 7.2              | 9.5                 | 6.3 (45.4)        | 62.4             | no family members tested |
| F400      | P598      | 14                          | 18                         | 21    | no      | none          | insulin                          | 6.7              | 8.9                 | 5.7 (38.8)        | 75.5             | no family members tested |
| F403      | P601      | 10                          | 11                         | 15.1  | no      | none          | insulin                          | 6.0              | 11.0                | 6.3 (45.4)        | 12.6             | mother no carrier, father not tested |
| F403      | P604      | no diabetes                 | 7                          | N/A   | N/A     | N/A           | insulin                          | N/A              | N/A                 | N/A               | N/A              | mother no carrier, father not tested |
| F408      | P610      | 5                           | 18                         | 18.7  | no      | none          | insulin                          | 7.3              | N/A                 | 6.4 (46.4)        | 1.9              | siblings positive, parents not tested |
| F408      | P611      | 14                          | 14                         | 17.9  | no      | none          | insulation                       | 7.2              | 8.8                 | 7.1 (54.1)        | 75.5             | siblings positive, parents not tested |
| F411      | P614      | 12                          | 12                         | 14.7  | no      | none          | diet                             | 5.9              | 9.3                 | 7.0 (53.0)        | 75.5             | multiple generations affected |
| F411      | P616      | no diabetes                 | 11                         | N/A   | N/A     | N/A           | insulin                          | N/A              | N/A                 | N/A               | N/A              | multiple generations affected |
| F411      | P617      | no diabetes                 | 42                         | 26    | no      | none          | diet                             | 7.1              | 9.1                 | 6.2 (44.3)        | N/A              | multiple generations affected |
| F433      | P639      | N/A                         | 13                         | N/A   | N/A     | N/A           | insulin                          | N/A              | N/A                 | N/A               | N/A              | no family members tested |
| F434      | P640      | N/A                         | 10                         | N/A   | N/A     | N/A           | insulin                          | N/A              | N/A                 | N/A               | N/A              | no family members tested |
| F435      | P641      | 7                           | 13                         | 18    | no      | none          | insulin                          | N/A              | N/A                 | 7.1 (54.1)        | 8.2              | multiple generations affected |
| F435      | P642      | 24                          | 37                         | 24    | no      | none          | insulin                          | N/A              | N/A                 | 7.2 (55.2)        | 62.4             | multiple generations affected |
| F454      | P665      | 10                          | 11                         | 17.4  | no      | possible coeliac disease | none                         | 6.2              | 5.9                 | N/A               | N/A              | no family members tested |
Table 4. Cont.

| Family ID | Sample ID | Age at Diagnosis of Diabetes | Age at Receiving Genetic Dg | BMI * | Obesity | Complications | Therapy BEFORE Genetic Diagnosis | FPG (0′) (mmol/L) | PPG (120′) (mmol/L) | HbA1c % (mmol/mol) | MODY Calculator (%) | Family Screening |
|-----------|-----------|-------------------------------|----------------------------|-------|---------|---------------|---------------------------------|------------------|------------------|------------------|------------------|------------------|
| F455      | P666      | 3                             | 4                          | N/A   | N/A     | N/A           | diet                            | 4.9              | 12.7             | 6.1 (43.2)       | N/A              | multiple generations affected |
| F455      | P667      | N/A                           | N/A                        | N/A   | N/A     | N/A           | N/A                            | N/A              | N/A              | N/A              | N/A              | multiple generations affected |
| F463      | P675      | 20                            | 27                         | 18.9  | no      | none          | OAD—metformin + sulphonylurea   | 7.2              | N/A              | 6.6 (48.6)       | 75.5             | no family members tested |
| F472      | P684      | 1                             | 2                          | N/A   | N/A     | N/A           | N/A                            | 6.4              | 8.6              | 6.9 (51.9)       | N/A              | multiple generations affected |
| F472      | P733      | N/A                           | 50                         | N/A   | N/A     | N/A           | N/A                            | N/A              | N/A              | N/A              | N/A              | multiple generations affected |
| F482      | P696      | 6                             | 6                          | 28    | yes     | N/A           | diet                            | 6.4              | 11.0             | 6.2 (44.3)       | 75.5             | no family members tested |
| F499      | P713      | N/A                           | 21                         | N/A   | N/A     | N/A           | N/A                            | N/A              | N/A              | N/A              | N/A              | no family members tested |

Index patients are shown in bold. Age at diabetes: N/A—the patient has diabetes, but no information was received regarding the age of diagnosis; no diabetes—no information was given in the application form that the patient shows any signs of diabetes. dg—diagnosis; OAD—oral antidiabetic drug; IHD—Ischemic Heart Disease; PAD—Peripheral Arterial Disease; TIA—transient ischemic attack; PCOS—Polycystic Ovary Syndrome; RR—respiratory rate. * BMI data refers to the time of referral for genetic testing.
Table 5. The distribution of the types of therapy received before proper genetic diagnosis.

| Therapy                                | Number of Patients |
|----------------------------------------|--------------------|
| insulin                                | 19 (11.7%)         |
| OAD—sulphonylurea                      | 2 (1.2%)           |
| OAD—metformin                          | 19 (11.7%)         |
| OAD—metformin and sulphonylurea        | 2 (1.2%)           |
| OAD—acarbose                           | 1 (0.6%)           |
| other OAD                              | 2 (1.2%)           |
| diet                                   | 56 (34.4%)         |
| no treatment                           | 24 (14.7%)         |
| N/A                                    | 38 (23.3%)         |

We had enough information to use the MODY calculator in about half of the patients. In the case of about three-quarters of these patients (62/81), the calculator showed a 75.5% probability of the patient having MODY, this was the highest probability we could get using the calculator.

4. Discussion

Two hundred and twenty-seven patients were diagnosed with MODY in our examined cohort from all over Hungary in about 10 years with a 70% mutation rate in the GCK gene, meaning that the most prevalent form of monogenic diabetes in Hungary is the GCK-MODY.

Although GCK-MODY patients generally do not need any treatment, around 30% of the patients examined were receiving an unnecessary OAD or insulin therapy. We would like to emphasize once again the importance of having a proper molecular genetic diagnosis, as this can lead to a major improvement in the patients’ quality of life by stopping their drug treatment.

The majority of the examined patients had an HbA1c level of 7.0% or lower, this being in accordance with the mildly elevated level reported in the literature and in contrast with the HNF1A patients we examined, where about 60% of the patients had a value of 7.0% or above.

As two families had de novo GCK mutations, one criterion of MODY about the transgenerational occurrence of the disease should be treated with caution—the lack of apparent inheritance pattern does not exclude the possibility of having a MODY.

The effect of the pathogenic and likely pathogenic mutations on the kinetics of the glucokinase enzyme is still not precisely known in many cases, therefore we plan to further investigate this question in the future.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/life11080771/s1, Table S1. The list of genes examined with the different library preparation kits; Table S2. Methods used for testing the index patients; Table S3. Clinical data of patients with HNF1A mutation; Table S4. Clinical data of patients having a mutation in other MODY-causing genes.

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Institutional Review Board Statement: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Patients gave informed written consent. The laboratory is approved by the National Public Health and Medical Officer Service (approval number: 094025024).
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