Evidence-based tailoring of bioinformatics approaches to optimize methods that predict the effects of nonsynonymous amino acid substitutions in glucokinase

Daniela Šimčíková¹, Lucie Kocková¹, Kateřina Vackářová¹, Miroslav Těšínský¹, Petr Heneberg¹,*
Legends to supplementary materials

Fig. S1. The outcomes of prediction methods are only weakly correlated with clinical parameters measured in disease-affected patients. (a-d) Number of algorithms demonstrating effect of the nonsynonymous substitution as plotted against (a) fasting plasma glucose measured in PHHI (white circles), MODY (black circles) and PNDM (black triangles) patients, (b) OGTT glycemia at t = 120 min as measured in MODY patients, (c) HbA1c measured in MODY (black circles) and PNDM (white circles) patients, (d) age at diagnosis of PHHI (white circles), MODY (black circles) and PNDM (black triangles). (e-g) EVmutation scores plotted against (e) fasting plasma glucose of MODY and PNDM patients, (f) HbA1c of MODY and PNDM patients and (g) age at diagnosis of diabetes of MODY patients. Linear (a-d, f-g) and Gaussian (e) regression curves are shown.

Fig. S2. The alignment of amino acid sequences of GCK of 13 vertebrate species, which was used to calculate the Grantham variation (GV) scores. The alignment was obtained using the ClustalW algorithm, and includes the following sequences: Homo sapiens NP_000153.1; Mus musculus NP_034422.2; Rattus norvegicus XP_006251241; Bos taurus NP_001095772.1; Danio rerio NP_001038850; Cyprinus carpio ACD37722; Meleagris gallopavo XP_010725006; Aquila chrysaetos XP_011573674; Ficedula albicollis XP_005057963; Xenopus laevis NP_001079298; Nanorana parkeri XP_018422966; Anolis carolinensis XP_003224263; and Lepisosteus oculatus XP_006625388.

Table S1. Raw data used for the formation of Fig. 2. The table contains the numbers of families affected by the GCK nonsynonymous substitutions identified globally until year 2017, and these frequencies are matched with the GV values for each amino acid within the GCK molecule.

Table S2. The detailed overview of the GCK nonsynonymous substitutions known from humans and analyzed in the present study. The data are based on a thorough systematic review of previously published data and includes also data generated newly in course of the present study. The table includes the outcomes of prediction methods, previously published and newly generated experimental data on enzyme kinetics, available clinical data and relevant references.

Table S3. Nonsynonymous substitutions analyzed in vitro in this study and the corresponding primers used in site-directed mutagenesis.
Supplementary methods

Experimental procedures

We expressed glucokinase (GCK) from the expression vector, pGEX-5X-2, with an insert that encoded the wild-type GCK isoform 1; the expression vector was provided as a kind gift from Dr. Navas (Universidad Complutense de Madrid) [1]. We introduced variations into the expression construct via site-directed mutagenesis (QuikChange site-directed mutagenesis kit, Agilent Technologies, Santa Clara, CA); the list of primers used is provided as Suppl. Tab. S3. We verified all the constructs via bidirectional Sanger sequencing. Then, we prepared GCK and its mutant forms as fusion proteins with N-terminal glutathione-S-transferase in the Escherichia coli BL21 Gold(DE3) strain (Agilent Technologies, Santa Clara, CA). We grew the cells at 37°C to OD 0.7, and the expression was induced by adding IPTG to a final concentration of 0.2 mM. We incubated the cultures at 22°C for 16 hours with orbital shaking (240 rpm). Afterwards, we harvested the cells by centrifuging, and resuspended the pellets in a breaking buffer (25-fold smaller volume than the culture volume; PBS, pH 7.4 containing 4 mM MgCl2, 1 mM PMSF, 5 mM DTT, 0.5% Triton X-100, lysozyme and DNase I) followed by 30 min of incubation at room temperature. We lysed the cells via mild sonication on ice. We centrifuged the lysates (4°C; 20,000× g) and immediately incubated the supernatants with Glutathione Sepharose (GE Healthcare Life Sciences, Chicago, IL). Subsequently, we washed the beads twice and eluted GST-GCK with 50 mM Tris, 200 mM KCl, pH 8.0, containing 5 mM DTT and 10 mM glutathione. We performed the entire purification procedure at 4°C. We determined the protein concentration using a Bradford assay (Serva, Heidelberg, DE) with bovine serum albumin used as a standard.

We measured the GCK activity spectrophotometrically using a coupled reaction with glucose-6-phosphate dehydrogenase (Sigma-Aldrich, St. Louis, MO); the increasing concentration of NADPH was determined at 340 nm, as described previously [2-3]. One unit (U) of GCK was defined as the amount of enzyme that phosphorylated 1 µmol of glucose per min at 30°C under assay conditions. In the case of glucose as the variable substrate (0–200 mM), we measured these assays using two concentrations of ATP – 0.5 mM and 5 mM; the GCK activity exhibited a sigmoidal dependency, which satisfied the Hill equation. However, the GCK activity with variable ATP concentrations (0–5 mM) followed hyperbolic Michaelis-Menten kinetics; we performed these assays at two glucose concentrations: at the corresponding S0.5 and 50 mM. We performed the competitive inhibition with N-acetylglucosamine (GlcNAc) at 5 mM glucose and 5 mM ATP under identical assay conditions.

We determined the temperature stability at 30°C in the time course of 100 min at 50 mM glucose and 5 mM ATP. Protein concentrations varied over separate preparations (30–300 µg/mL) without having an effect on the protein stability.

We calculated, based on the determined kinetic variables (S0.5, nH, kcat and ATP KM), the relative activity index (RAI) and the glucose threshold for glucose-stimulated insulin release (GSIR-T). The RAI values serve as a direct comparison of the GCK mutants with the wild-type enzyme. The equation has been previously published [4]. We employed a minimal mathematical model, which reflects the kinetic characteristics of the wild-type GCK and its mutant forms, as well as
the stability coefficient and adaptation through the expression coefficient to predict the β-cell threshold for GSIR. The previously published consensual assumptions were fulfilled [2,5].

**Prediction methods**

For the prediction analyses mentioned below, we used a protein identifier (GCK NCBI code: NP_000153.1; GCK Swiss-Prot code: P35557), or directly an amino acid sequence in FASTA format.

We retrieved data related to the nonsynonymous single nucleotide variations (nonsynonymous substitutions, abbreviated as nsSNVs) in the expressed region of the GCK gene from the Ensembl [6], dbSNP [7], UniProtKB [8] and HGMD [9] databases and from a systematic review of the literature published in 2009-2017 and listed in the Web of Science database [10]. We obtained the structure of the closed form of GCK (PDB ID: 1V4S [11]) from the Protein Data Bank (PDB) [12].

In this study, we employed methods that use evolution-based sequence information (SIFT, PhD-SNP) and methods that take into account the chemical and physical characteristics of amino acids (Align-GVGD) or protein structural attributes combined with multiple sequence alignment-derived information (PolyPhen-2, SNAP2, SNPs&GO) to predict the phenotypic effect of nonsynonymous substitutions. A single amino acid substitution can result in a notable change in the protein stability, which is represented by a change in its Gibbs free energy (ΔΔG) upon folding. Therefore, we employed two predictors that focus on the stability properties of the nonsynonymous substitutions, I-Mutant 3.0 and PoPMuSiC 2.1. We also used the recently published prediction method, EVmutation, to compare the existing approaches and its new epistatic approach for protein function and the stability prediction.

The Sorting Intolerant From Tolerant (SIFT) method [13] is based on the hypothesis that protein evolution is correlated with protein function. Functionally relevant amino acids should be conserved in the protein family, whereas less important positions should be diverse. The SIFT Human Protein predicts whether nonsynonymous substitutions affect the protein function for all Ensembl transcripts with an assigned ENSP number (GCK ENSP: ENSP00000384247). Based on their scores, the substitutions are considered to be damaging (≤0.05) or tolerated (>0.05), ideally with median sequence information (also referred as the median conservation value) between 2.75 and 3.25. The median sequence information provides an assessment of the confidence, and SIFT computes the conservation value at each position in the alignment. The conservation value ranges from 0, which means that all 20 amino acids are at that position, to 4.32, which means that the position is completely conserved. A sufficient diversity within the aligned sequences is maintained by median sequence information of ~3.0.

The PolyPhen-2 (Polymorphism Phenotyping v2) method [14] estimates the probability of the nonsynonymous substitution to adversely affect protein function based on sequence, phylogenetic and structural features. The nonsynonymous substitution is predicted as probably damaging (0.85–1.00), possibly damaging (0.15–0.84) or benign (<0.15). We identified the nonsynonymous substitution effect according to the HumDiv score. The model was trained on a
dataset that involved known effects of damaging alleles that cause human Mendelian diseases that are annotated in the UniProtKB database.

SNAP2 (Screening for non-acceptable polymorphisms) [15] is a neural network-based classifier that predicts the impact of nonsynonymous substitutions based on evolutionary information, structural features and solvent accessibility. The score ranges from -100 (strong neutral prediction) to +100 (strong effect prediction).

PhD-SNP (Predictor of human Deleterious Single Nucleotide Polymorphisms) [16] is a support vector machine (SVM)-based classifier that distinguishes disease-related nonsynonymous substitutions from neutral ones by reflecting the nature of the substitution and properties of the neighboring sequence environment. The method was optimized using a dataset of neutral and deleterious variations taken from the UniProtKB/Swiss-Prot.

The SNPs&GO method [17] is based on a principle very similar to PhD-SNP. In contrast to PhD-SNP, the SNPs&GO also takes into account protein function information that is defined by Gene Ontology (GO) [18-19] terms. GO terms are directly retrieved only if a Swiss-Prot code is used. If GO terms are not included (only protein sequence input), the accuracy of the method is thought to be lower and comparable with PhD-SNP [17].

Align-GVGD [20-21] classifies the amino acid substitutions and their functional effect according to the “C-score” (from C0 – neutral to C65 – deleterious), which is based on the cross-species protein multiple sequence alignment with a comparison of the physical and chemical characteristics of amino acids. The Align-GVGD combines the GV (Grantham variation) and GD (Grantham deviation) score. We expressed the evolutionary conservation of the amino acid sequence of the pancreatic isoform of GCK in the form of a GV score, which was based on the alignment of the human GCK protein sequence with the GCK sequences of 12 other vertebrate species. Because the GCK sequence is highly conserved, the alignment included not only mammals (three species) but also birds, amphibians, reptiles and fish (Suppl. Fig. S2). To calculate the GV score, we used the multiple sequence alignment, which was formed using ClustalW in MEGA6, and built on the following sequences: Homo sapiens NP_000153.1; Mus musculus NP_034422.2; Rattus norvegicus XP_006251241; Bos taurus NP_001095772.1; Danio rerio NP_001038850; Cyprinus carpio ACD37722; Meleagris gallopavo XP_010725006; Aquila chrysaetos XP_011573674; Ficedula albicollis XP_005057963; Xenopus laevis NP_001079298; Nanorana parkeri XP_018422966; Anolis carolinensis XP_003224263; Lepisosteus oculatus XP_006625388. Positions with zero GV score have the same amino acids across all species and are thus invariant, whereas the GV increases when the alignment demonstrates evidence for variation in the particular residue. The GCK gene does not have any insertions or deletions of amino acids in the studied species, except for the N- and C-terminal parts of the molecule; thus, nearly all the variability was assigned to nonsynonymous substitutions.

I-Mutant 3.0 [22] was designed to estimate the protein stability change caused by nonsynonymous substitutions. The tool was trained on a dataset built on the information from ProTherm [23], which is a comprehensive thermodynamic database of experimental data for wild-type and mutant proteins. Based on the protein structure or the sequence, the difference (ΔΔG value) between the unfolding Gibbs free energies of the mutated and wild-type protein is calculated. In the present study, we based the ΔΔG values on the protein structure of GCK (PDB ID: 1V4S [11]). Nonsynonymous substitutions with ΔΔG>0.5 kcal/mol are considered to be
largely stabilizing, and those with $\Delta\Delta G<-0.5$ kcal/mol are predicted as largely destabilizing. Other nonsynonymous substitutions with $\Delta\Delta G$ in the range from -0.5 to 0.5 kcal/mol have a weak effect.

Another web server that allows predicting the thermodynamic stability changes upon the nonsynonymous substitution is PoPMuSiC-2.1 [24]. This method reflects the solvent accessibility of the mutated residue. The predictions are derived from the structure of the target protein (GCK PDB ID:1V4S). The $\Delta\Delta G$ values lower than 0 kcal/mol are assigned to stabilizing nonsynonymous substitutions, and those that are higher than 0 kcal/mol are assigned to destabilizing nonsynonymous substitutions.

The recently reported prediction method, EVmutation [25], exploits the epistatic approach. Thus, it takes into account explicitly modelling of interactions between all the pairs of residues in the proteins and bases in RNAs to predict nonsynonymous substitution effects. Within validation, EVmutation predictions were compared with outcomes from 34 high-throughput mutagenesis experiments. The EVmutation scores ($\Delta E$) below 0 are assigned to deleterious nonsynonymous substitutions, values above 0 correspond to beneficial nonsynonymous substitutions, and values equal to 0 correspond to neutral nonsynonymous substitutions.

The developers of all prediction methods suggested interpreting the resulting predictions using arbitrary scores as threshold values. We presented the calculations using these arbitrarily suggested interpretations and thresholds in Table 2. However, arbitrary thresholds were associated with extreme uncertainty and overestimated the effects of neutral nonsynonymous substitutions. Nevertheless, we found that three prediction methods, PolyPhen-2, SNAP2 and EVmutation, allowed differentiating at least in part between the neutral and MODY-associated nonsynonymous substitutions when considering their numerical outcomes. Thus, for these three methods, we computed (PolyPhen-2 and SNAP2) or retrieved (EVmutation) predictions for all possible amino acid exchanges within the GCK molecule, irrespectively on whether they are already known from humans or not. For SNAP2, we retrieved 8,837 predictions with mean value 4.54±0.63 (min -99, max 96, median 13, 25th percentile -52, 75th percentile 58). For PoPMuSiC 2.1, we retrieved 8,856 predictions with mean value 1.10±0.01 (min -1.88, max 5.77, median 0.85, 25th percentile 0.32, 75th percentile 1.70). For EVmutation, we retrieved 8,191 predictions with mean value -5.35±0.03 (min -10.15, max 4.10, median -5.36, 25th percentile -7.06, 75th percentile -3.79). We applied two types of adjusted thresholds in order to be able to predict nonsynonymous substitutions, which are likely to serve as causative MODY nonsynonymous substitutions, and which are likely to be benign or activating. We calculated the thresholds by computing the medians and SDs of scores for nonsynonymous substitutions, which do not cause any monogenically inherited disease. We calculated the threshold for predicting the MODY-associated nonsynonymous substitutions as median of scores for nonsynonymous substitutions, which do not cause any monogenically inherited disease, with the addition of 2 SDs. We calculated the threshold for predicting the benign (or activating) nonsynonymous substitutions as the median value of scores for nonsynonymous substitutions, which do not cause any monogenically inherited disease. We used these evidence-based thresholds for a further validation of these methods.

We analyzed the data by one-way ANOVA with Tukey’s post-tests and computed the $S_{0.5}$, Hill coefficient $n_H$, $k_{cat}$ and ATP $K_M$ via non-linear regression analyses. We obtained $IC_{50}$ using four parameters logistic curve fitting. Multiparametric analyses included the detrended
correspondence analyses. We calculated Pearson product moment correlation coefficients and Spearman rank order correlation coefficients in order to correlate the numerical outputs of EVmutation, PoPMuSiC 2.1 and SNAP2 prediction methods applied to total hypothetic GCK nonsynonymous substitutions for which the outcomes of all the three prediction methods were available (n_PoPMuSiC 2.1 / EVmutation = 8,493 nonsynonymous substitutions, n_SNAP2 / PoPMuSiC 2.1 and SNAP2 / EVmutation = 8,189 nonsynonymous substitutions). We also calculated the two correlation coefficients in order to compare GV with the frequency of families (n = 465 residues, of that 279 residues were disease-associated (1596 disease-associated families) and 164 residues were not evolutionarily conserved). Data were shown as means ± SE, unless stated otherwise. We performed the calculations and plotted the figures in PAST 2.14 and SigmaPlot 12.0.

Supplementary references for Supplementary Methods

1 García-Herrero CM, Galán M, Vincent O, et al. (2007) Functional analysis of human glucokinase gene mutations causing MODY2: exploring the regulatory mechanisms of glucokinase activity. Diabetologia 50:325–333

2 Davis EA, Cuesta-Muñoz A, Raoul M, et al. (1999) Mutants of glucokinase cause hypoglycaemia- and hyperglycaemia syndromes and their analysis illuminates fundamental quantitative concepts of glucose homeostasis. Diabetologia 42:1175–1186

3 Liang Y, Kesavan P, Wang L, et al. (1995) Variable effects of maturity-onset-diabetes-of-youth (MODY)-associated glucokinase mutations on substrate interactions and stability of the enzyme. Biochem J 309:167–173

4 Matschinsky FM (2009) Assessing the potential of glucokinase activators in diabetes therapy. Nat Rev Drug Discov 8:399–416

5 Matschinsky FM, Davis EA, Cuesta-Muñoz A, et al. (2000) The glucokinase system and the regulation of blood sugar. In: Matschinsky DM, Magnuson MA (eds) Molecular pathogenesis of MODYs. Karger, Basel, pp 99–108

6 Ensembl (2017) Ensembl genome browser 88; http://www.ensembl.org/; accessed 11 April 2017

7 NCBI (2017) NCBI dbSNP Build 150; https://www.ncbi.nlm.nih.gov/projects/SNP/index.html; accessed 11 April 2017

8 EMBL-EBI, SIB, PIR (2017) UniProteorlease 2017_04; http://www.uniprot.org; accessed 11 April 2017

9 Stenson PD, Mort M, Ball EV, et al. (2014) The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Hum Genet 133:1–9

10 Thomson Reuters (2017) Web of Science. http://apps.webofknowledge.com; accessed 11 April 2017

11 Kamata K, Mitsuya M, Nishimura T, Eiki J, Nagata Y (2004) Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase. Structure 12:429–438
12 RSCB (2017) PDB-101. http://www.rcsb.org/pdb/home/home.do; accessed 11 April 2017
13 Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4:1073–1081
14 Adzhubei IA, Schmidt S, Peshkin L, et al. (2010) A method and server for predicting damaging missense mutations. Nat Methods 7:248–249
15 Hecht M, Bromberg Y, Rost B (2015) Better prediction of functional effects for sequence variants. BMC Genomics 16(Suppl 8):S1–S12
16 Capriotti E, Calabrese R, Casadio R (2006) Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. Bioinformatics 22:2729–2734
17 Calabrese R, Capriotti E, Fariselli P, Martelli PL, Casadio R (2009) Functional annotations improve the predictive score of human disease-related mutations in proteins. Hum Mutat 30:1237–1244
18 Ashburner M, Ball CA, Blake JA, et al. (2000) Gene Ontology: tool for the unification of biology. Nat Genet 25:25–29
19 The Gene Ontology Consortium (2015) Gene Ontology Consortium: going forward. Nucleic Acids Res 43:D1049–D1056
20 Tavtigian SV, Deffenbaugh AM, Yin L, et al. (2005) Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J Med Genet 43:295–305
21 Mathe E, Olivier M, Kato S, Ishioka C, Hainaut P, Tavtigian SV (2006) Computational approaches for predicting the biological effect of p53 missense mutations: a comparison of three sequence analysis based methods. Nucleic Acids Res 34:1317–1325
22 Capriotti E, Fariselli P, Rossi I, Casadio R (2008) A three-state prediction of single point mutations on protein stability changes. BMC Bioinformatics 9(Suppl 2):S6–S14
23 Kumar MD, Bava KA, Gromiha MM, et al. (2006) ProTherm and ProNIT: thermodynamic databases for proteins and protein-nucleic acid interactions. Nucleic Acids Res 34:D204–D206
24 Dehouck Y, Kwasigroch JM, Gilis D, Rooman M (2011) PoPMuSiC 2.1: a web server for the estimation of protein stability changes upon mutation and sequence optimality. BMC Bioinformatics 12:151–162
25 Hopf TA, Ingraham JB, Poelwijk FJ, et al. (2017) Mutation effects predicted from sequence co-variation. Nat Biotechnol 33:128–135
Suppl. Table S1. Raw data used for the formation of Fig. 2. The table contains the numbers of families affected by disease-causing GCK nonsynonymous substitutions identified globally until year 2017, and these frequencies are matched with the GV values for each amino acid within the GCK molecule.

| Position | Amino acid | GV score | Frequency of mutations [number of families] |
|----------|------------|----------|--------------------------------------------|
| 1        | M          | 353.86   | 1                                          |
| 2        | L          | 353.86   | 0                                          |
| 3        | D          | 353.86   | 0                                          |
| 4        | D          | 353.86   | 1                                          |
| 5        | R          | 353.86   | 0                                          |
| 6        | A          | 353.86   | 0                                          |
| 7        | R          | 243.26   | 0                                          |
| 8        | M          | 91.64    | 1                                          |
| 9        | E          | 168.08   | 0                                          |
| 10       | A          | 152.19   | 0                                          |
| 11       | A          | 218.83   | 0                                          |
| 12       | K          | 173.05   | 0                                          |
| 13       | K          | 125.10   | 0                                          |
| 14       | E          | 107.83   | 0                                          |
| 15       | K          | 108.93   | 1                                          |
| 16       | V          | 64.43    | 2                                          |
| 17       | E          | 44.60    | 0                                          |
| 18       | Q          | 42.81    | 0                                          |
| 19       | I          | 10.12    | 1                                          |
| 20       | L          | 0        | 3                                          |
| 21       | A          | 99.13    | 0                                          |
| 22       | E          | 0        | 0                                          |
| 23       | F          | 0        | 1                                          |
| 24       | Q          | 116.58   | 0                                          |
| 25       | L          | 0        | 0                                          |
| 26       | Q          | 124.29   | 0                                          |
| 27       | E          | 56.87    | 0                                          |
| 28       | E          | 0        | 1                                          |
| 29       | D          | 44.60    | 0                                          |
| 30       | L          | 0        | 2                                          |
| 31       | K          | 128.24   | 0                                          |
| 32       | K          | 126.69   | 0                                          |
| 33       | V          | 31.78    | 6                                          |
| 34       | M          | 14.30    | 1                                          |
| 35       | R          | 237.70   | 0                                          |
| 36       | R          | 0        | 30                                         |
| 37       | M          | 14.30    | 1                                          |
| 38       | Q          | 42.81    | 2                                          |
| 39       | K          | 116.42   | 0                                          |
| 40       | E          | 0        | 25                                         |
| 41       | M          | 0        | 3                                          |
| 42       | D          | 44.60    | 0                                          |
| 43       | R          | 0        | 22                                         |
| 44       | G          | 0        | 34                                         |
| 45       | L          | 0        | 0                                          |
| 46       | R          | 38.73    | 1                                          |
| 47       | L          | 31.78    | 0                                          |
| 48       | E          | 0        | 0                                          |
| 49       | T          | 0        | 1                                          |
| 50       | H          | 68.35    | 8                                          |
| 51       | E          | 105.70   | 0                                          |
| 52       | E          | 0        | 0                                          |
| 53       | A          | 0        | 3                                          |
| 54       | S          | 0        | 0                                          |
| 55       | V          | 0        | 2                                          |
| 56       | K          | 0        | 0                                          |
| 57       | M          | 0        | 3                                          |
| 58       | L          | 0        | 1                                          |
| 59       | P          | 0        | 10                                         |
|   |   |   |   |
|---|---|---|---|
| 60 | T | 0 | 4 |
| 61 | Y | 0 | 3 |
| 62 | V | 0 | 7 |
| 63 | R | 0 | 0 |
| 64 | S | 0 | 1 |
| 65 | T | 0 | 3 |
| 66 | P | 0 | 0 |
| 67 | E | 44.60 | 0 |
| 68 | G | 0 | 4 |
| 69 | S | 0 | 0 |
| 70 | E | 0 | 7 |
| 71 | V | 0 | 2 |
| 72 | G | 0 | 25 |
| 73 | D | 0 | 2 |
| 74 | F | 0 | 0 |
| 75 | L | 0 | 0 |
| 76 | S | 99.13 | 4 |
| 77 | L | 0 | 3 |
| 78 | D | 0 | 5 |
| 79 | L | 0 | 0 |
| 80 | G | 0 | 5 |
| 81 | G | 0 | 8 |
| 82 | T | 0 | 3 |
| 83 | N | 0 | 0 |
| 84 | F | 0 | 0 |
| 85 | R | 0 | 0 |
| 86 | V | 0 | 0 |
| 87 | M | 0 | 0 |
| 88 | L | 0 | 0 |
| 89 | V | 0 | 0 |
| 90 | K | 0 | 0 |
| 91 | V | 0 | 1 |
| 92 | G | 0 | 0 |
| 93 | E | 0 | 0 |
| 94 | G | 93.77 | 0 |
| 95 | E | 171.76 | 0 |
| 96 | E | 134.33 | 0 |
| 97 | G | 125.13 | 0 |
| 98 | Q | 154.67 | 0 |
| 99 | W | 0 | 7 |
|100 | S | 123.62 | 0 |
|101 | V | 0 | 2 |
|102 | K | 101.46 | 0 |
|103 | T | 0 | 4 |
|104 | K | 77.74 | 0 |
|105 | H | 83.33 | 0 |
|106 | Q | 53.64 | 1 |
|107 | M | 0 | 0 |
|108 | Y | 0 | 10 |
|109 | S | 0 | 0 |
|110 | I | 0 | 2 |
|111 | P | 0 | 1 |
|112 | E | 121.33 | 0 |
|113 | D | 0 | 0 |
|114 | A | 0 | 1 |
|115 | M | 0 | 1 |
|116 | T | 0 | 1 |
|117 | G | 0 | 2 |
|118 | T | 0 | 1 |
|119 | A | 0 | 1 |
|120 | E | 56.87 | 1 |
|121 | M | 0 | 0 |
|122 | L | 0 | 5 |
|123 | F | 0 | 2 |
|124 | D | 0 | 7 |
|125 | Y | 0 | 1 |
|126 | I | 0 | 0 |
|   |   |   |   |
|---|---|---|---|
|  127 | S | 99.13 | 1 |
|  128 | E | 107.72 | 0 |
|  129 | C | 0 | 28 |
|  130 | I | 0 | 7 |
|  131 | S | 0 | 3 |
|  132 | D | 0 | 1 |
|  133 | F | 21.61 | 1 |
|  134 | L | 0 | 6 |
|  135 | D | 0 | 0 |
|  136 | K | 53.23 | 0 |
|  137 | H | 24.08 | 2 |
|  138 | Q | 45.75 | 3 |
|  139 | M | 14.30 | 0 |
|  140 | K | 0 | 0 |
|  141 | H | 0 | 0 |
|  142 | K | 0 | 0 |
|  143 | K | 0 | 0 |
|  144 | L | 0 | 1 |
|  145 | P | 0 | 0 |
|  146 | L | 0 | 7 |
|  147 | G | 0 | 0 |
|  148 | F | 0 | 1 |
|  149 | T | 0 | 5 |
|  150 | F | 0 | 15 |
|  151 | S | 0 | 4 |
|  152 | F | 0 | 3 |
|  153 | P | 0 | 0 |
|  154 | V | 0 | 2 |
|  155 | R | 0 | 1 |
|  156 | H | 0 | 2 |
|  157 | E | 0 | 4 |
|  158 | D | 0 | 0 |
|  159 | I | 4.86 | 1 |
|  160 | D | 0 | 9 |
|  161 | K | 0 | 1 |
|  162 | G | 0 | 3 |
|  163 | I | 0 | 0 |
|  164 | L | 0 | 7 |
|  165 | L | 0 | 2 |
|  166 | N | 0 | 0 |
|  167 | W | 0 | 1 |
|  168 | T | 0 | 7 |
|  169 | K | 0 | 3 |
|  170 | G | 0 | 4 |
|  171 | F | 0 | 4 |
|  172 | K | 0 | 0 |
|  173 | A | 0 | 5 |
|  174 | S | 0 | 2 |
|  175 | G | 0 | 13 |
|  176 | A | 0 | 1 |
|  177 | E | 0 | 1 |
|  178 | G | 0 | 10 |
|  179 | N | 0 | 0 |
|  180 | N | 0 | 14 |
|  181 | V | 29.61 | 1 |
|  182 | V | 0 | 27 |
|  183 | G | 0 | 0 |
|  184 | L | 0 | 0 |
|  185 | L | 0 | 2 |
|  186 | R | 0 | 2 |
|  187 | D | 0 | 3 |
|  188 | A | 0 | 32 |
|  189 | I | 0 | 1 |
|  190 | K | 0 | 0 |
|  191 | R | 0 | 78 |
|  192 | R | 0 | 2 |
|  193 | G | 0 | 3 |
| 194 | D | 0 | 0 |
| 195 | F | 0 | 0 |
| 196 | E | 0 | 0 |
| 197 | M | 0 | 3 |
| 198 | D | 0 | 1 |
| 199 | V | 0 | 1 |
| 200 | V | 0 | 4 |
| 201 | A | 0 | 3 |
| 202 | M | 0 | 7 |
| 203 | V | 0 | 18 |
| 204 | N | 0 | 0 |
| 205 | D | 0 | 6 |
| 206 | T | 0 | 35 |
| 207 | V | 0 | 2 |
| 208 | A | 0 | 8 |
| 209 | T | 0 | 16 |
| 210 | M | 0 | 10 |
| 211 | I | 0 | 1 |
| 212 | S | 0 | 2 |
| 213 | C | 0 | 9 |
| 214 | Y | 0 | 3 |
| 215 | Y | 0 | 0 |
| 216 | E | 0 | 0 |
| 217 | D | 0 | 2 |
| 218 | H | 28.82 | 0 |
| 219 | Q | 111.62 | 0 |
| 220 | C | 0 | 3 |
| 221 | E | 0 | 8 |
| 222 | V | 0 | 0 |
| 223 | G | 0 | 40 |
| 224 | M | 14.30 | 4 |
| 225 | I | 0 | 6 |
| 226 | V | 0 | 42 |
| 227 | G | 0 | 4 |
| 228 | T | 0 | 26 |
| 229 | G | 0 | 3 |
| 230 | C | 0 | 1 |
| 231 | N | 0 | 4 |
| 232 | A | 0 | 3 |
| 233 | C | 0 | 4 |
| 234 | Y | 0 | 1 |
| 235 | M | 0 | 9 |
| 236 | E | 0 | 5 |
| 237 | E | 0 | 1 |
| 238 | M | 0 | 0 |
| 239 | Q | 193.50 | 7 |
| 240 | N | 105.58 | 3 |
| 241 | V | 0 | 3 |
| 242 | E | 0 | 0 |
| 243 | L | 0 | 1 |
| 244 | V | 0 | 5 |
| 245 | E | 0 | 0 |
| 246 | G | 0 | 4 |
| 247 | D | 48.73 | 0 |
| 248 | E | 0 | 2 |
| 249 | G | 0 | 5 |
| 250 | R | 0 | 6 |
| 251 | M | 0 | 13 |
| 252 | C | 0 | 8 |
| 253 | V | 0 | 3 |
| 254 | N | 0 | 4 |
| 255 | T | 0 | 6 |
| 256 | E | 0 | 20 |
| 257 | W | 0 | 2 |
| 258 | G | 0 | 11 |
| 259 | A | 0 | 16 |
| 260 | F | 0 | 1 |
| 261 | G  | 0   | 49  |
|-----|----|-----|-----|
| 262 | D  | 126.14 | 0 |
| 263 | S  | 99.45 | 9  |
| 264 | G  | 55.27 | 5  |
| 265 | E  | 0   | 15  |
| 266 | L  | 0   | 2   |
| 267 | D  | 44.60 | 0 |
| 268 | E  | 44.60 | 3  |
| 269 | F  | 0   | 1   |
| 270 | L  | 101.88 | 0 |
| 271 | L  | 0   | 0   |
| 272 | E  | 0   | 0   |
| 273 | Y  | 0   | 2   |
| 274 | D  | 0   | 4   |
| 275 | R  | 28.82 | 8  |
| 276 | L  | 31.78 | 1  |
| 277 | V  | 29.61 | 2  |
| 278 | D  | 0   | 10  |
| 279 | E  | 0   | 4   |
| 280 | S  | 127.17 | 0 |
| 281 | S  | 0   | 5   |
| 282 | A  | 96.19 | 0  |
| 283 | N  | 0   | 0   |
| 284 | P  | 0   | 0   |
| 285 | G  | 0   | 0   |
| 286 | Q  | 24.08 | 0  |
| 287 | Q  | 0   | 4   |
| 288 | L  | 0   | 0   |
| 289 | Y  | 0   | 8   |
| 290 | E  | 0   | 1   |
| 291 | K  | 0   | 3   |
| 292 | L  | 14.30 | 1  |
| 293 | I  | 0   | 1   |
| 294 | G  | 55.27 | 6  |
| 295 | G  | 0   | 2   |
| 296 | K  | 0   | 0   |
| 297 | Y  | 0   | 1   |
| 298 | M  | 0   | 6   |
| 299 | G  | 0   | 10  |
| 300 | E  | 0   | 6   |
| 301 | L  | 4.86 | 0   |
| 302 | V  | 64.43 | 4  |
| 303 | R  | 0   | 7   |
| 304 | L  | 0   | 3   |
| 305 | V  | 0   | 0   |
| 306 | L  | 0   | 2   |
| 307 | L  | 14.30 | 1  |
| 308 | R  | 26.00 | 4  |
| 309 | L  | 14.30 | 2  |
| 310 | V  | 0   | 0   |
| 311 | D  | 48.73 | 0  |
| 312 | E  | 0   | 1   |
| 313 | N  | 23.01 | 0  |
| 314 | L  | 0   | 1   |
| 315 | L  | 31.78 | 13 |
| 316 | F  | 0   | 1   |
| 317 | H  | 118.22 | 0 |
| 318 | G  | 0   | 23  |
| 319 | E  | 61.28 | 1  |
| 320 | A  | 103.68 | 0 |
| 321 | S  | 111.67 | 0 |
| 322 | E  | 44.60 | 0  |
| 323 | Q  | 125.10 | 0 |
| 324 | L  | 0   | 3   |
| 325 | R  | 26  | 0   |
| 326 | T  | 0   | 1   |
| 327 | R  | 26  | 1   |
| 328 | G | 0 | 0 |
| 329 | A | 103.68 | 0 |
| 330 | F | 0 | 0 |
| 331 | E | 0 | 1 |
| 332 | T | 57.75 | 1 |
| 333 | R | 42.81 | 0 |
| 334 | F | 21.82 | 0 |
| 335 | V | 29.61 | 0 |
| 336 | S | 0 | 3 |
| 337 | Q | 0 | 1 |
| 338 | V | 29.61 | 0 |
| 339 | E | 0 | 10 |
| 340 | S | 103.39 | 5 |
| 341 | D | 0 | 0 |
| 342 | T | 79.02 | 2 |
| 343 | G | 95.09 | 0 |
| 344 | D | 0 | 1 |
| 345 | R | 97.03 | 0 |
| 346 | K | 26.00 | 0 |
| 347 | Q | 0 | 0 |
| 348 | I | 89.28 | 2 |
| 349 | Y | 224.33 | 0 |
| 350 | N | 0 | 0 |
| 351 | I | 0 | 0 |
| 352 | L | 0 | 1 |
| 353 | S | 111.49 | 0 |
| 354 | T | 200.43 | 0 |
| 355 | L | 21.82 | 0 |
| 356 | G | 97.85 | 1 |
| 357 | L | 31.78 | 0 |
| 358 | R | 116.23 | 0 |
| 359 | P | 26.87 | 1 |
| 360 | S | 57.75 | 0 |
| 361 | T | 182.66 | 0 |
| 362 | T | 162.25 | 0 |
| 363 | D | 0 | 2 |
| 364 | C | 0 | 0 |
| 365 | D | 81.24 | 1 |
| 366 | I | 93.66 | 0 |
| 367 | V | 0 | 2 |
| 368 | R | 28.82 | 0 |
| 369 | R | 102.84 | 2 |
| 370 | A | 64.43 | 0 |
| 371 | C | 0 | 10 |
| 372 | E | 0 | 0 |
| 373 | S | 135.70 | 1 |
| 374 | V | 0 | 5 |
| 375 | S | 0 | 1 |
| 376 | T | 0 | 0 |
| 377 | R | 0 | 10 |
| 378 | A | 0 | 17 |
| 379 | A | 0 | 5 |
| 380 | H | 108.82 | 6 |
| 381 | M | 14.30 | 6 |
| 382 | C | 0 | 6 |
| 383 | S | 103.39 | 30 |
| 384 | A | 0 | 5 |
| 385 | G | 0 | 5 |
| 386 | L | 0 | 6 |
| 387 | A | 0 | 10 |
| 388 | G | 0 | 1 |
| 389 | V | 29.61 | 7 |
| 390 | I | 4.86 | 0 |
| 391 | N | 93.88 | 0 |
| 392 | R | 104.93 | 1 |
| 393 | M | 0 | 5 |
| 394 | R | 28.82 | 0 |
Supplementary references for Suppl. Table S1:

Agladioglu SY, Aycan Z, Cetinkaya S, et al. (2016) Maturity onset diabetes of youth (MODY) in Turkish children: sequence analysis of 11 causative genes by next generation sequencing. J Pediatr Endocrinol Metab 29:487–496

Ajala ON, Huffman DM, Ghibrial II (2016) Glucokinase mutation – a rare cause of recurrent hypoglycemia in adults: a case report and literature review. J Community Hosp Intern Med Perspect 26:32983

Almeida C, Silva SR, Garcia E, Leite AL, Teles A, Campos RA (2014) A novel genetic mutation in a Portuguese family with GCK-MODY. J Pediatr Endocrinol Metab 27:129–133

Ang SF, Lim SC, Tan CSH, et al. (2016) A preliminary study to evaluate the strategy of combining clinical criteria and next generation sequencing (NGS) for the identification of monogenic diabetes among multi-ethnic Asians. Diabetes Res Clin Pract 119:13–22

Antosik K, Gnys P, De Franco E, et al. (2016) Single patient in GCK-MODY family successfully re-diagnosed into GCK-PNDM through targeted next-generation sequencing technology. Acta Diabetol 53:337–338

Barbetti F, Cobo-Vuilleumier N, Dionisi-Vici C, et al. (2009) Opposite clinical phenotypes of glucokinase disease: description of a novel activating mutation and contiguous inactivating mutations in human glucokinase (GCK) gene. Mol Endocrinol 23:1983–1989

Bazalová Z, Ryápáková B, Brož J, et al. (2010) Three novel mutations in MODY and its phenotype in three different Czech families. Diabetes Res Clin Pract 88:132–138

Beer NL, van de Bunt M, Colclough K, et al. (2011) Discovery of a novel site regulating glucokinase activity following characterization of a new mutation causing hyperinsulinemic hypoglycemia in humans. J Biol Chem 286:19118–19126

Beer NL, Osbak KK, van de Bunt, et al. (2012) Insights into the pathogenicity of rare missense GCK variants from the identification and functional characterization of compound heterozygous and double mutations inherited in cis. Diabetes Care 35:1482–1484

Bennett JT, Vasta V, Zhang M, NarayananJ, Gerrits P, Hahn SH (2015) Molecular genetic testing of patients with monogenic diabetes and hyperinsulinism. Mol Genet Metab 114:451–458

Borowiec M, Antosik K, Fendler W, et al. (2012) Novel glucokinase mutations in patients with monogenic diabetes – clinical outline of GCK-MD and potential for founder effect in Slavic population. Clin Genet 81:278–283

Borowiec M, Fendler W, Antosik K, et al. (2012) Doubling the referral rate of monogenic diabetes through a nationwide information campaign – update on glucokinase gene mutations in a Polish cohort. Clin Genet 82:587–590

Brah N, De Franco E, Dawes A, et al. (2016) Permanent neonatal diabetes mellitus due to a novel homozygous GCK mutation in a premature baby with IUGR and its management. Horm Res Paediatr 86(Suppl 1):208

Caetano LA, Jorge AAL, Malaquias AC, et al. (2012) Incidental mild hyperglycemia in children: two MODY 2 families identified in Brazilian subjects. Arq Bras Endocrinol Metab 56:519–524

Calcaterra V, Martinetti M, Salina A, Aloì C, Larizza D (2011) The coexistence of type 1 diabetes, MODY2 and metabolic syndrome in a young girl. Acta Diabetol 49:401–404

Capuano M, Garcia-Herrero CM, Tinto N, et al. (2012) Glucokinase (GCK) mutations and their characterization in MODY2 children of Southern Italy. PLoS ONE 7:e38906

Carmody D, Naylor RN, Bell CD, et al. (2016) GCK-MODY in the US National Monogenic Diabetes Registry: frequently misdiagnosed and unnecessarily treated. Acta Diabetol 53:703–708

Challis BG, Harris J, Sleigh A, et al. (2014) Familial adult onset hyperinsulinism due to an activating glucokinase mutation: implications for pharmacological glucokinase activation. Clin Endocrinol 81:855–861
Constantini S, Malerba G, Contreas G, et al. (2015) Genetic and bioinformatics analysis of four novel GCK missense variants detected in Caucasian families with GCK-MODY phenotype. Clin Genet 87:440–447.

Cuesta-Muñoz AL, Tuomi T, Cobø-Vuilleumier N, et al. (2010) Clinical heterogeneity in monogenic diabetes caused by mutations in the glucokinase gene (GCK-MODY). Diabetes Care 33:290–292.

d’Annunzio G, Marchi M, Aloi C, Salina A, Lugani F, Lorini R (2013) Hyperglycaemia and β-cell antibodies: is it always pre-type 1 diabetes? Diabetes Res Clin Pract 100:e20–e22.

DellaManna T, da Silva MR, Chacca AR, et al. (2012) Clinical follow-up of two Brazilian subjects with glucokinase-MODY (MODY2) with description of a novel mutation. Arq Bras Endocrinol Metab 56:490–495.

Delvecchio M, Ludovico O, Bellacchio E, et al. (2013) MODY type 2 P59S GCK mutant: founder effect in South of Italy. Clin Genet 83:83–87.

Demirbilek H, Arya VB, Ozbek MN, et al. (2015) Clinical characteristics and molecular genetic analysis of 22 patients with neonatal diabetes from the South-Eastern region of Turkey: predominance of non-K\textsubscript{ATP} channel mutations. Eur J Endocrinol 172:697–705.

Doddabela-vangala Mruthyunjaya M, Chapla A, Hasarghatta Shyamasunder A, et al. (2017) Comprehensive maturity onset diabetes of the young (MODY) gene screening in pregnant women with diabetes in India. PLoS ONE 12:e0168656.

Emelyanov AO, Sechkio E, Koksharova E, et al. (2017) A glucokinase gene mutation in a young boy with diabetes mellitus, hyperinsulinemia, and insulin resistance. Int Med Case Rep J 10:77–80.

Esquiaveto-Aun AM, De Mello MP, Paulino MF, Minicucci WJ, Guerra-Júnior, De Lemos-Marini (2015) A new compound heterozygosis for inactivating mutations in the glucokinase gene as cause of permanent neonatal diabetes mellitus (PNDM) in double-first cousins. Diabetol Metab Syndr 7:101.

Fahr K, Böckmann A, Wildhardt G, Gessler P (2012) MODY 2 diabetes in a mature newborn due to a new mutation in the GCK-gene. Klin Padiatr 224:316–317.

Garcia-Herrero CM, Rubio-Cabezas O, Azriel S, et al. (2012) Functional characterization of MODY2 mutations highlights the importance of the fine-tuning of glucokinase and its role in glucose sensing. PLoS ONE 7:e30518.

Giuffrida FMA, Callieri LE, DellaManna T, et al. (2013) A novel glucokinase deletion (p.Lys32del) and five previously described mutations co-segregate with the phenotype of mild familial hyperglycaemia (MODY2) in Brazilian families. Diabetes Res Clin Pract 100:e42–e45.

Giuffrida FMA, Moises RS, Weinert LS, et al. (2017) Maturity-onset diabetes of the young (MODY) in Brazil: establishment of a national registry and appraisal of available genetic and clinical data. Diabetes Res Clin Pract 123:134–142.

Gozlan Y, Tenenbaum A, Shalitin S, et al. (2012) The glucokinase mutation p.T206P is common among MODY patients of Jewish Ashkenazi descent. Pediatr Diabetes 13:e14–e21.

Haliloglu B, Hysenaj G, Atay Z, et al. (2016) GCK gene mutations are a common cause of childhood-onset MODY (maturity-onset diabetes of the young) in Turkey. Clin Endocrinol 85:393–399.

Heggie AJ, Walker M (2016) A patient with a glucokinase mutation: a cautionary tale. Diabet Med 33(Suppl 1):102.

Henquin JC, Sempoux C, Marchandise J, et al. (2013) Congenital hyperinsulinism caused by hexokinase I expression or glucokinase-activating mutation in a subset of β-cells. Diabetes 62:1689–1696.

Jha S, Siddiqui S, Waghdhare S, et al. (2016) Identification of a novel glucokinase mutation in an Indian woman with GCK-MODY. Lancet Diabetes Endocrinol 4:302.

Juszczak A, Bartlett K, Mackillop L, Owen K (2016) Pregnancy outcome in hypoglycaemia caused by an activating glucokinase mutation. Diabet Med 33(Suppl 1):100.

Kanthimathi S, Jahnani S, Balamurugan K, et al. (2014) Glucokinase gene mutations (MODY 2) in Asian Indians. Diabetes Technol Ther 16:180–185.

Kassem S, Bhandari S, Rodriguez-Bada P, et al. (2010) Large islets, beta-cells proliferation, and a glucokinase mutation. N Engl J Med 362:1348–1350.

Kawakita R, Hosokawa Y, Fujimaru R, et al. (2014) Molecular and clinical characterization of glucokinase maturity-onset diabetes of the young (GCK-MODY) in Japanese patients. Diabet Med 31:1357–1362.
Kuraeva TL, Sechko EA, Zilberman LI, et al. (2015) Molecular genetics and clinical variants MODY2 and MODY3 in children in Russia. Probl Endocrinol 61:14–25

Li Q, Cao X, Qiu HY, Lu J, et al. (2016) A three-step programmed method for the identification of causative gene mutations of maturity onset diabetes of the young (MODY). Gene 588:141–148

Lopez AP, de Dios A, Chiesa I, Perez MS, Frechtel GD (2016) Analysis of mutations in the glucokinase gene in people clinically characterized as MODY2 without a family history of diabetes. Diabetes Res Clin Pract 118:38–43

Marks SD, Couch RM (2010) Identification of two new mutations in the glucokinase gene that result in maturity-onset diabetes of the young. Diabetes Care 33:e94

Martinez R, Gutierrez-Nogues A, Fernández-Ramos C, et al. (2017) Heterogeneity in phenotype of hyperinsulinism caused by activating glucokinase mutations: a novel mutation and its characterization. Clin Endocrinol, doi: 10.1111/cen.13318

Morishita K, Kyo C, Yonemoto T, Kosugi R, Ogawa T, Inoue T (2017) Asymptomatic congenital hyperinsulinism due to a glucokinase-activating mutation, treated as adrenal insufficiency for twelve years. Case Rep Endocrinol 2017:4709262

Negahdar M, Aukrust I, Molnes J, et al. (2014) GCK-MODY diabetes as a protein misfolding disease: the mutation R275C promotes protein misfolding, self-association and cellular degradation. Mol Cell Endocrinol 382:55–65

Osbak KK, Colclough K, Saint-Martin C, et al. (2009) Update on mutations in glucokinase (GCK), which cause maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia. Hum Mutat 30:1512–1526

Papadimitriou DT, Willems PJ, Bothou C, Karpathios T, Papadimitriou A (2015) A novel heterozygous mutation in the glucokinase gene is responsible for an early-onset mild form of maturity-onset diabetes of the young, type 2. Diabetes Metab 41:342–345

Pihoker C, Gilliam LK, Ellard S, et al. (2013) Prevalence, characteristics and clinical diagnosis of maturity-onset diabetes of the young due to mutations in HNF1A, HNF4A, and glucokinase: results from the SEARCH for diabetes in youth. J Clin Endocrinol Metab 98:4055–4062

Ping Xiao Y, Hua Xu X, Lan Fang Y, et al. (2016) GCK mutations in Chinese MODY2 patients: a family pedigree report and review of Chinese literature. J Pediatr Endocrinol Metab 29:959–964

Pruhova S, Dusatkova P, Sumnik Z, et al. (2010) Glucokinase diabetes in 103 families from a country-based study in the Czech Republic: geographically restricted distribution of two prevalent GCK mutations. Pediatr Diabetes 11:529–535

Pull K, Arbo T, Kahre T, Peet A, Tillmann V (2012) MODY2 caused by a novel mutation of GCK gene. J Pediatr Endocrinol Metab 25:801–803

Raimondo A, Chakera AJ, Thomsen SK, et al. (2014) Phenotypic severity of homozygous GCK mutations causing neonatal or childhood-onset diabetes is primarily mediated through effects on protein stability. Hum Mol Genet 23:6432–6440

Shammas C, Neocleous V, Phelan MM, Lian LY, Skordis N, Phylactou LA (2013) A report of 2 new cases of MODY2 and review of the literature: implications in the search for type 2 diabetes drugs. Metabolism 62:1535–1542

Shen Y, Cai M, Liang H, Wang H, Weng J (2011) Insight into the biochemical characteristics of a novel glucokinase gene mutation. Hum Genet 129:231–238

Shoemaker AH, Zienkiewicz J, Moore DJ (2012) Clinical assessment of HNF1A and GCK variants and identification of a novel mutation causing MODY2. Diabetes Res Clin Pract 96:e36–e39

Stanik J, Dusatkova P, Cinek O, et al. (2014) De novo mutations of GCK, HNF1A and HNF4A may be more frequent in MODY than previously assumed. Diabetologia 57:480–484

Steele AM, Tribble ND, Caswell R, et al. (2011) The previously reported T342P GCK missense variant is not a pathogenic mutation causing MODY. Diabetologia 54:2202–2205
Szopa M, Ludwig-Galezowska A, Radkowski P, et al. (2015) Genetic testing for monogenic diabetes using targeted next-generation sequencing in patients with maturity-onset diabetes of the young. Pol Arch Med Wewn 125:845–851

Valentinová L, Beer NL, Staník J, et al. (2012) Identification and functional characterisation of novel glucokinase mutations causing maturity-onset diabetes of the young in Slovakia. PLoS ONE 7:e34541

Wang Z, Ping F, Zhang Q, et al. (2017) Preliminary screening of mutations in the glucokinase gene of Chinese patients with gestational diabetes. J Diabetes Investig, doi: 10.1111/jdi.12664

Weinert LS, Silveiro SP, Giuffrida FMA, et al. (2014) Three unreported glucokinase (GCK) missense mutations detected in the screening of thirty-two Brazilian kindreds for GCK and HNF1A-MODY. Diabetes Res Clin Pract 106:e44–e48

Yellapu NK, Valasani KR, Pasupuleti SK, Gopal S, Potukuchi Venkata Gurunadha Krishna S, Matcha B (2014) Identification and analysis of novel R308K mutation in glucokinase of type 2 diabetic patient and its kinetic correlation. Biotechnol Appl Biochem 61:572–581

Yilmaz AA, Elmaogullari S, Demirel F, et al. (2016) A novel glucokinase gen mutation: MODY type-2 case. Horm Res Paediatr 86(Suppl 1):247

Yokota I, Moritani M, Nishisho K, Miyoshi Y, Kotani Y, Kagami S (2011) Detection of glucokinase gene defects in non-obese Japanese children diagnosed with diabetes by school medical examinations. Endocr J 58:741–746

Yorifuji T, Fujimaru R, Hosokawa Y, et al. (2012) Comprehensive molecular analysis of Japanese patients with pediatric-onset MODY-type diabetes mellitus. Pediat Diabetes 13:26–32

Ziemssen F, Bellanné-Chantelot C, Osterhoff M, Schatz H, Pfeiffer AFH (2002) To: Lindner T, Cockburn BN, Bell GI (1999). Molecular genetics of MODY in Germany. Diabetologia 42:121-123. Diabetologia 45:286–287
| Exon 1 | Exon 2 | Exon 3 | Exon 4 | Exon 5 | Exon 6 | Exon 7 | Exon 8 | Exon 9 | Exon 10 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1Q106P | 2Y108H | 3T103N | 4T116P | 5V16Q  | 6S151T | 7S151P | 8L146R | 9P111L | 10G881D |

**Variation**

| Phenotype | Type | Age at diagnosis (days) | PG or FPG [mM] | C-peptide [%] |
|-----------|------|-------------------------|----------------|--------------|
|          |      |                         |                |              |

**References**

| References | Enzyme kinetics | Clinical phenotype |
|------------|-----------------|--------------------|
|            |                 |                    |

**Diagnosis**

| Diagnosis | Type | Age at diagnosis (days) | PG or FPG [mM] | C-peptide [%] |
|-----------|------|-------------------------|----------------|--------------|
|           |      |                         |                |              |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 118; 130 | 2.2; 1.9 | 3.83 | 1.04; 1.51 | 0.00041 | 113.43 | 1.33 | 10.18 | < 0.01 |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|----------|----------|-------|-------------|----------|---------|------|-------|--------|
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 15.5 | 1.43 | 0.63 | 36.8 | 0.4 | 0.35 | 0 N | 0.6 | -97 | C0 | 31.78 | 0 | 2.11 |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 7.81 | 0.85 | 9.5 | 0.98 | 0.06 | 6.9 | 0.01 | 0.838 | N | 1.75 | -45 | D | 0 | -0.96 |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 1 | D | 0.63 | 57 | D | D | -0.79 | C65 | 0 | 176.58 | -5.5 | This study | [2] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 0.006 | D | 1.75 | 30 | N | D | -0.7 | C55 | 0 | 60 | -4.18 | [2] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 0.848 | D | 2.22 | 44 | D | D | -1.59 | C45 | 0 | 49.94 | -7.29 | [2] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 0.809 | D | 1.08 | -19 | D | D | -1.36 | C15 | 0 | 21.52 | -4.65 | [2] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 0.999 | D | 3.43 | 71 | D | D | -1.74 | C45 | 14.3 | 86.59 | -5.86 | [20] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 12 | 6.6 | 8.1 | 0 | 0.999 | D | 1.1 | -19 | D | D | -0.9 | C45 | 28.82 | 169.74 | -6.45 | [13] | [58] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 1 | D | 3.47 | 84 | D | D | -2.03 | C65 | 0 | 159.94 | -8.04 | [6] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 1 | D | 2.55 | 0 | D | D | -2.21 | C65 | 0 | 112.44 | -7.59 | [2] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 1 | D | 0.97 | 50 | D | D | -1.36 | C45 | 0 | 49.94 | -7.07 | [74] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0 | 1 | D | 2.55 | 85 | D | D | -1.5 | C65 | 0 | 94.49 | -6.56 | [2] |
|                  |                  |                  |                  |                  |                  |                  |                  |                  | 0,39 | 0,738 | N | -0.09 | -61 | N | N | -0.23 | C55 | 0 | 56.87 | -3.99 | [2] |

This study [2]
Reference: Grantham, K. and P. (2019). "Permanent neonatal diabetes mellitus." Journal of Pediatrics, 102(2), 10-16.

N/D: not clear, whether the effect is disease-related or neutral

NA: no activity

GV: Grantham Variation

PNDM: permanent neonatal diabetes mellitus

| Amino Acid | Reference | Effect | p-value | Beta-coefficient | 95% CI | Additional Notes |
|------------|-----------|--------|----------|------------------|--------|-----------------|
| A460R      | 1,94      | 1,1    | 0,55     |                  |        |                 |
| G464D      | 5,9       | 1,15   | 0,65     |                  |        |                 |
| R447Q      | 10,85     | 1,52   | 0,74     | 51,55            | 0,41   |                 |
| H438Q      | 24,49     | 1,39   | 1,36     | 6,7              | 0,04   |                 |
| V452L      | 16        | 1,39   | 0,69     | 8,17             | 0,22   |                 |

| Amino Acid | Reference | Effect | p-value | Beta-coefficient | 95% CI | Additional Notes |
|------------|-----------|--------|----------|------------------|--------|-----------------|
| A460R      | 1,94      | 1,1    | 0,55     |                  |        |                 |
| G464D      | 5,9       | 1,15   | 0,65     |                  |        |                 |
| R447Q      | 10,85     | 1,52   | 0,74     | 51,55            | 0,41   |                 |
| H438Q      | 24,49     | 1,39   | 1,36     | 6,7              | 0,04   |                 |
| V452L      | 16        | 1,39   | 0,69     | 8,17             | 0,22   |                 |
Njolstad PR, Sovik O, Cuesta-Olarte AL, Huopio H, Otonkoski T, et al. (2004) Severe persistent hyperinsulinemic hypoglycaemia due to a de novo glucokinase mutation. Diabetes 53:2164–2168.
### Suppl. Table S3. Nonsynonymous substitutions analyzed in vitro in this study and the corresponding primers used in site-directed mutagenesis.

| Nonsynonymous substitution | Primers |
|---------------------------|---------|
|                          | **Fw** |                         |
|                          | **Rv** |                         |
| V33A c.98T>C              | GGAGGACCTGAAGAGGCGATGACGGATG | CTCTCATGCCCTTCTTCAGGTCTCC |
| R63S c.187C>T             | CACCTACGTGTGCTCCACCCAGAACGGTCAGAAGTC | AGCCCTTGCGGAGGACACAGTGGGGCCAGC |
| G81D c.242G>A             | GACCTGGTGACACTAATCCAGGGTG | GAAGTTATGTCACCCAGTGCCAGGAAGATGCC |
| F150L c.450C>A            | GGCGCTTCACCTATCCCTCTTGAGGAG | CTCCTACAGGAGGAAGGCTGAAAGG |
| T209K c.626C>A            | CACGGCTGGGCAAGGATGCTCTGCTACTAC | CAGGAGATCATGGCCACCAGTGCATTC |
| R250C c.748C>T            | GGCGACAGGGGCTGATGCTGACAATACCGAGTGGG | GAGCGACATGCACGGCCTCTGCCCTTCG |
| M251C c.751_753 delinsTGT | GACGGAGGCGCTGTTGCTGCAATACCGAGTGGG | CGGTATTGACGCATATGCGGCCCTCGTCCC |
| M251I c.753G>A            | AGGCCGCAGAGGGGCGATGCTGCAATACCGAGTGGG | GAGCGACATGCACGGCCTCTGCCCTTCG |
| M251V c.751A>G            | CGAGGGCCGGGCGTGCAGTCAGAATACCGAGTGGG | CGGTATTGACGCATATGCGGCCCTCGTCCC |
| C252R c.754T>C            | GAGGGCCCGATGCGAGGCGCTCTGCCCTTCGCCC | CAGGAGATCATGGCCACCAGTGCATTC |
| F260L c.778T>C            | GAGTGGGGGCGCCCTCGGAGAATACGGAGTGGG | CCTCGCGGAGGCGCACGACCTTCCTGCCC |
| G295D c.884G>A            | GAAGCTCATAGGAGACAAGTACATGGGCAGCTGG | CGGTATTGACGCATATGCGGCCCTCGTCCC |
| L314P c.941T>C            | GAGCGAAACCCGCTTCTGCCAGGAGGAGGAGGAG | CACTGCGCTGAGAGTCAGGAGGAGGAG |
| F316V c.946T>G            | GAAAAACCTCTGCTGCTAGGAGGAGGAGGAGGAG | CGACCCGACTCGGAGGAGGAGGAGGAG |
| G318R c.952G>A            | CTCTCTCAGGAGGAGGCGCTCCAGGACGACGAC | CGGTATTGACGCATATGCGGCCCTCGTCCC |
| G385W c.1153G>T           | GTGCCTGGCCGTGCTGCGGAGGAGGAGGAG | CAGGAGATCATGGCCACCAGTGCATTC |
| F419L c.1255T>C           | CAAGCTGACACCCAGGAGGAGGAGGAGGAG | CGGTATTGACGCATATGCGGCCCTCGTCCC |
| C434Y c.1301G>A           | GGCTGACCGCCAGCTACGAGATCCTCCTCATCG | CGGTATTGACGCATATGCGGCCCTCGTCCC |
| A454E c.1361C>A           | GGGCGCCTGGCTCAGGAGGAGGAGGAG | CACTGCGCTGAGAGTCAGGAGGAGGAG |

| Primers | **Fw** |                         |
|---------|--------|-------------------------|
|         |        |                         |

**G385W c.1153G>T**  
**F419L c.1255T>C**  
**C434Y c.1301G>A**  
**A454E c.1361C>A**