Glucokinase Gene Mutations: Structural and Genotype-Phenotype Analyses in MODY Children from South Italy

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Abstract

Background: Maturity onset diabetes of the young type 2 (or GCK MODY) is a genetic form of diabetes mellitus provoked by mutations in the glucokinase gene (GCK).

Methodology/Principal Findings: We screened the GCK gene by direct sequencing in 30 patients from South Italy with suspected MODY. The mutation-induced structural alterations in the protein were analyzed by molecular modeling. The patients’ biochemical, clinical and anamnestic data were obtained. Mutations were detected in 16/30 patients (53%); 9 of the 12 mutations identified were novel (p.Glu707Asp, p.Phe123Lue, p.Asp132Asn, p.His137Asp, p.Gly162Asp, p.Thr168Ala, p.Arg192Ser, p.Glu290X, p.Gln106_Met107delinsLeu) and are in regions involved in structural rearrangements required for catalysis. The prevalence of mutation sites was higher in the small domain (7/12: ~59%) than in the large (4/12: 33%) domain or in the connection (1/12: 8%) region of the protein. Mild diabetic phenotypes were detected in almost all patients [mean (SD) OGTT = 7.8 mMol/L (1.8)] and mean triglyceride levels were lower in mutated than in unmutated GCK patients (p = 0.04).

Conclusions: The prevalence of GCK MODY is high in southern Italy, and the GCK small domain is a hot spot for MODY mutations. Both the severity of the GCK mutation and the genetic background seem to play a relevant role in the GCK MODY phenotype. Indeed, a partial genotype-phenotype correlation was identified in related patients (3 pairs of siblings) but not in two unrelated children bearing the same mutation. Thus, the molecular approach allows the physician to confirm the diagnosis and to predict severity of the mutation.

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Background

Glucokinase (also called hexokinase IV) catalyzes the ATP-dependent phosphorylation of glucose to glucose-6-phosphate. It is homologous to hexokinases I, II and III, but its lower affinity for glucose, restricted localization to a few cell types and peculiar kinetic properties, compared to those of the other hexokinases, confer it distinctive properties. Indeed, GCK acts as a glucose sensor in the pancreas and liver, and presents a peculiar sigmoidal glucose saturation curve, which indicates cooperative behaviour.

Elucidation of crystal structure of GCK yielded data that could help to establish structure-function correlations [7]. Indeed, the protein folds into two domains known as the small and the large domain with the glucose binding site in between. An unexpected “super-open” form and a “closed” form of the enzyme were identified [7]; the latter is similar to the form found in the hexokinase I structure [8]. The two forms differ in the relative spatial orientation of the two domains. The catalytic mechanism...
requires dramatic GCK conformational changes. In fact, when bound to glucose, the enzyme goes from the super-open inactive form to the closed form. Therefore, the inter-domain motions are crucial for the enzyme activity.

In this study, we report the identification of 9 novel and 3 known mutations of the GCK gene in children from south Italy. All mutations co-segregated with the diabetic phenotype in the respective families and resulted in perturbation of the 3D structure of the protein. Our data show that molecular screening is useful in diagnosis of MODY because it allows to confirm the diagnosis and to predict severity of the mutation.

Methods

Subjects

Thirty patients aged 1–14 years, 9 boys and 21 girls whose clinical presentation was suggestive of MODY were selected for GCK gene screening from among 240 diabetic children seen in the Paediatric Clinic of our Medical School between 2001 and 2006. Other MODY genes were not investigated. Most patients were unrelated; 6 were except (3 pairs of siblings, 1: M022–M023, 2: M024–M025, 3: M028–M029). Inclusion criteria were: early onset (<25 years) of diabetes, mild hyperglycaemia, no autoimmune markers of type 1 diabetes, without obesity [c.o. body mass index (BMI) z score (>2) and family history of diabetes for at least two consecutive generations [1,9]. No treatment was administered to the patients and no diabetes complications were evident up to diagnosis. A fasting blood sample was drawn from both parents of mutated patients and from 100 unrelated controls, who came from the same geographical area, and used for the GCK molecular characterization. The parents of all subjects gave their written informed consent to the study. The research was conducted according to Helsinki II declaration and approved by the ethics committee of our Faculty.

Clinical and anamnetic examination

We collected the following data for each patient upon diagnosis: age, birth weight, family history of diabetes and/or other diseases and BMI. The BMI was transformed into BMI-z-score (z-BMI) based on the Centre for Disease Control normative curves [10,11].

Biochemical analyses

The following biochemical parameters were measured on fasting blood samples: plasma glucose (FPG) by the enzymatic hexokinase method and triglycerides by the standard enzymatic method (Hitachi Modular, Tokyo, Japan); glycosylated haemoglobin (HbA1c) by HPLC (HLC-723 G7 TOSOH Bioscience Tokyo, Japan); serum insulin by the chemiluminescence method (Immuliight 2000; Medical System Genoa, Italy). After an oral glucose dose of 0.75g/kg body weight (maximum 75 g), oral glucose tolerance test (OGTT) was evaluated on blood samples collected every 30 min up to 2 h. The first-phase insulin response (FPIR) was calculated as the sum of T+1 and +3 min serum insulin concentrations, evaluated after an i.v. glucose dose of 0.5 g/kg body weight in 3 min [12].

DNA extraction

Genomic DNA from patients, parents, and controls was extracted from a blood sample plus EDTA using Nucleon BACC 2 kit (Amersham Biosciences Europe, Milan, Italy).

Sequence analysis

Exons and flanking intron regions of GCK, including tissue specific variants of exon 1, were amplified by PCR using previously reported primers (exons 1a, 2, 3, 4) [13] or chosen by the Primer 3 program (exons 1b, 1c, 5, 6, 7, 8, 9 and 10) [14]. The PCR mixture contained in a final volume of 50 µl: 20 µM each primer, 1× PCR buffer (Applied Biosystems, Foster City, CA, USA), 10 µM each deoxynucleotide triphosphate, 2.5 U of Taq DNA polymerase (Applied Biosystems) and 200 ng of genomic DNA. Each PCR was performed on Gene-Amp PCR system 9700 thermocycler (Applied Biosystems) and consisted of a initial denaturation step at 95°C for 5 min and a final extension at 72°C for 7 min.

The primers and PCR conditions are detailed in table 1. Product sizes were evaluated by agarose gel electrophoresis and amplicons were sequenced in both directions using the Big Dye terminator sequencing kit (Applied Biosystems) on ABI PRISM 3730 (Applied Biosystems). We preliminarily analyzed the GCK coding sequence of a healthy subject by sequence analysis and verified that it overlapped the wild-type reference sequence (GenBank NM_000162). All sequences of the

### Table 1. Primers used for PCR amplification of GCK exons and PCR conditions.

| Exons | Forward primer | Reverse primer | PCR annealing temperatures | PCR annealing times | PCR extension times (72°C) | Number of cycles |
|-------|----------------|----------------|----------------------------|---------------------|---------------------------|-----------------|
| 1a    | 5’-TCCACCTCAGAGGCTCTAGCT | 5’-TCAATTCTGAGGCCTCAAAC | 60°C | 30'' | 60'' | 35 |
| 1b    | 5’-GCCAAGGAGACCTCCTGCTTG | 5’-AGGAGAGCAGAGGAGTGAGG | 62°C | 20'' | 30'' | 30 |
| 1c    | 5’-GGCCTCACTCAGGCTGGAAC | 5’-AGCAGGAGGAGAGGAGGAGG | 54°C | 20'' | 30'' | 30 |
| 2     | 5’-GCAGGAGGAGGAGGAGGAGG | 5’-CACAGCTGCTTGGATGAGG | 60°C | 15'' | 30'' | 30 |
| 3     | 5’-CTGAGAGGAGGAGGAGGAGG | 5’-CAGAGGAGGAGGAGGAGGAGG | 60°C | 15'' | 30'' | 30 |
| 5     | 5’-CTGAGAGGAGGAGGAGGAGG | 5’-CTGAGAGGAGGAGGAGGAGG | 60°C | 15'' | 30'' | 30 |
| 7     | 5’-CCAGAAGGAGGAGGAGGAGG | 5’-CTGCTCTTGGAGGTGTTT | 54°C | 20'' | 45'' | 35 |
| 8     | 5’-GGGAGGAGGAGGAGGAGGAGG | 5’-CTGCTCTTGGAGGTGTTT | 54°C | 20'' | 45'' | 35 |
| 9     | 5’-CTGAGAGGAGGAGGAGGAGG | 5’-CTGCTCTTGGAGGTGTTT | 54°C | 20'' | 45'' | 35 |
| 10    | 5’-GAGTCTTCTGACCCCTTTTG | 5’-CACCGAAAACCTGAGGAAAGG | 55°C | 20'' | 45'' | 35 |

1 GenBank accession n° (AH005826)
2 Taken from Stoffel M. et al. 1992 Proc Natl Acad Sci USA 89:7698–7702.
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patients were analyzed and compared with the wild-type published reference sequence with the ABI Seqscape software v2.5 (Applied Biosystems).

All mutations were also validated on a second PCR product. Finally, the children’s parents and 100 unrelated healthy individuals were screened for these mutations.

Conservation of residues was evaluated from a multiple sequence alignment of 15 sequences in the PFAM349 and PFAM3724 protein families [15]. The result was validated by a multiple sequence alignment of 341 protein sequences homolog to GCK and extracted from the non redundant sequence database RefSeq, available online [16]. Furthermore, for the 1v4s structure, a multiple sequence alignment of 143 sequences in the CouSurf_HSSP database [17] was carried out. The outcomes from the three approaches were in accordance.

Statistical analysis
Continuous variables are reported as mean (SD) and comparisons among variables were made with the t-test. Inter-group differences were considered statistically significant at p<0.05.

We calculated the distribution in the large domain, in the small domain and in the connection region of the protein of our GCK mutations and of those described in literature.

Mutations nomenclature
All mutations are described according to the recommended nomenclature available online [18–20]. Nucleotide numbers are derived from cDNA GCK sequence (GenBank NM_000162) considering nucleotide +1 the A of the first ATG translation initiation codon in the reference sequence.

Molecular modelling
We used molecular modelling to investigate alterations of the GCK 3D structure and dynamics associated to the various mutations. The active form of GCK served as template because it has the highest resolution structure (2.3 Å, PDB code: 1v4s) [7]. Under the assumption that point mutations are likely to preserve the overall fold of the proteins, it follows the procedure for generating models. The first step of this procedure is the prompt substitution of the residues, which we did with the program MODELLER [21]. The program replaces the side chains by selecting the most abundant conformers and performs a simulated annealing procedure to optimize side chain conformations. This was followed by energy minimization in explicit solvent with the GROMACS program [22] using the GROMOS96 force field [23]. Finally, the system was optimized by means of a short molecular dynamics calculation of 1ns. The calculation was made by restraining the main chain conformations and allowing the side chain dynamics. The notation used for secondary structure was taken from Kamata et al. [7]. Accordingly, sequences 1–64 and 206–439 belong to the large domain, sequences 72–201 and 445–465 belong to the small domain, and sequences 65–71, 202–205 and 440–444 belong to the three loops connecting the domains.

Results
Table 2 shows the molecular characterization of the 16/30 our diabetic children who had mutations in the GCK gene. Ten patients were unrelated (two of these had the same mutation) and 6 were siblings from 3 families. Therefore, GCK MODY diagnosis was confirmed in these 16 patients. All mutations were detected at the heterozygous state, 9 mutations were novel; 8 of them caused variations in amino acid residues well conserved among species, and one produced a truncated protein of 289 amino acids (p.Glu70Asp, p.Phe123Leu, p.Asp132Asn, p.His137Asp, p.Gly162Asp, p.Ser160Ala, p.Arg329Ser, p.Glu290X, p.Gln106_Met107delinsLeu). Three other GCK mutations were already known: 2 of them caused truncated proteins (p.Lys396X and p.Ser453X) [24,25] and 1 was a missense mutation (p.Glu263Lys) [Bellante-Chantelot C et al. Abstract, Diabetologia 1998; 41:A109, 423]. The structural analysis of mutation sites indicated 3D clustering. In fact, among the GCK variants in our population, 7/12 (~59%), 4/12 (33%) and 1/12 (8%) were localized in the small domain, in the large domain and in the connecting loops, respectively (Fig. 1). The effects of GCK mutations on the enzyme’s 3D structure are reported in Table 2. Figures 2 and 3 show the structural features of some of the new mutations detected in our GCK MODY patients.

Table 3 shows the phenotypic characteristics of our mutated children at diagnosis. All mutations were also present in either the mother or the father of the GCK MODY patients in association with diabetes and mean birth weight did not differ between children who inherited GCK mutations from their father or mother (2.84 kg vs 2.98 kg). Almost but one patient had mild hyperglycemia [mean (range) TFG = 6.60 (5.7–7.9) mmol/L, M013 = 10.0 mmol /L], mildly altered OGTT [mean (SD) 2 h OGTT = 7.8 (1.8) mmol/L] and 2h insulin always <2.8 except in two patients: M024 = 3.3 and M005 = 4.9; EPIR values between 24.6 and 156.0 μU/mL and slightly elevated levels of HbA1c [mean (SD) HbA1c = 6.1 (0.4) %]. The mean triglyceride concentration (0.58 mmol/L) was lower in our mutated patients (p = 0.04) than in unmutated GCK MODY patients (0.80 mmol/L) and below 50th percentile of our laboratory age-related reference range (0.36–1.10 mmol/L).

Discussion
In our Italian population, GCK gene mutations account for 6% (16/240) diabetic children and for 53% (16/30) with suspected MODY. This GCK MODY prevalence is in line with those detected in southern European countries, particularly in France and in Italy, namely between 8% and 56% [25–27]. An analysis of the 3D structure of the protein mutants yielded evidence of structural perturbations, which supports the GCK MODY-causing nature of these mutations. In particular, the p.Glu70Asp mutation (detected in 1 patient) substitutes the highly conserved glutamate with aspartate. These amino acids are polar acidic residues but the binding to lysine 458, in the α3 helix, appears to be weakened compared with the wild-type enzyme. This substitution could slightly modify enzymatic activity and/or stability. Substitution of glutamate by lysine is reported, in association with diabetes, to reduce the enzyme’s glucose affinity but protein stability is preserved [28].

The p.Gln106_Met107delinsLeu mutation (detected in 1 patient) altered respectively, a poorly and moderately conserved residues but the binding to lysine 458, in the α3 helix, appears to be weakened compared with the wild-type enzyme. This substitution could slightly modify enzymatic activity and/or stability. Substitution of glutamate by lysine is reported, in association with diabetes, to reduce the enzyme’s glucose affinity but protein stability is preserved [28].

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Table 2. GCK mutations in children from south Italy affected by GCK MODY

| Patient code | Exons<sup>a</sup> | cDNA mutation<sup>b</sup> | Aminoacid change<sup>c</sup> | Domain localization /Secondary structure | Effect on protein 3D-structure | References |
|--------------|------------------|---------------------------|-----------------------------|-----------------------------------|--------------------------------|------------|
| M001         | 8                | c.868G>T                   | p.Glu290X Stop codon, truncated protein | Large domain/ α7 helix | Truncated protein | Present study |
| M006         |                  |                           |                            |                                   |                               |            |
| M002         | 2                | c.115delA                  | p.Lys391X6 Frameshift and stop codon | Large domain/ α2 helix | Truncated protein | Prisco et al 2000 |
| M003         | 9                | c.1174C>A                  | p.Arg392Ser Positively charged Arg → polar Ser | Large domain/ α11 helix | Disruption of H-bond and salt-bridge network | Present study |
| M005         | 10               | c.1358C>A                  | p.Ser453X Stop codon | Small domain/ α13 helix | Truncated protein Loss of C-term segment of α13 helix | Massa et al 2001 |
| M009         | 4                | c.409C>G                   | p.His137Asp Polar His → negatively charged Asp | Small domain/Last residue of α13 helix | Loss of α13 helix capping Variation of local interactions | Present study |
| M013         | 5                | c.485G>A                   | p.Gly162Asp Apolar Gly → negatively charged Asp | Small domain/ α strand 7 | Introduction of a negative charge in a hydrophobic environment | Present study |
| M017         | 4                | c.349C>G                   | p.Phe123Leu Aromatic hydrophobic Phe → hydrophobic Leu | Small domain/ α3 helix | Reorganization dictated by an introduction of small cavity into a hydrophobic environment | Present study |
| M018         | 3                | c.317_319delAGA            | p.Gln106_Met107delinsLeu | Small domain/ Edge α strand 4 | Perturbation of the β-sheet | Present study |
| M019         | 2                | c.210A>C                   | p.Glu70Asp Negatively charged Glu → negatively charged Asp | Connection/Loop spatially near α13 helix | Weakness of salt-link interaction with K458 (α13 helix) | Present study |
| M022<sup>d</sup> | 5              | c.502A>G                   | p.Thr168Ala Polar Thr → apolar Ala | Small domain/Loop | Loss of H-bond between T168 and glucose | Present study |
| M023<sup>e</sup> |       |                           |                            |                                   |                               |            |
| M024<sup>f</sup> | 7              | c.793G>A                   | p.Glu265Lys Negatively charged Glu → positively charged Lys | Large domain/Loop | E265 is between R36 and R43, positively-charged K, provokes a dramatic rearrangement | Bellane-Chantelot C et al 1998 |
| M025<sup>f</sup> |       |                           |                            |                                   |                               |            |
| M028<sup>f</sup> | 4              | c.394G>A                   | p.Asp132Asn Negatively charged Asp → polar Asn | Small domain/ α3 helix | Mild structural alterations | Present study |

<sup>a</sup>GenBank accession n° (AH005826)
<sup>b</sup>The reference cDNA sequence was obtained from GenBank (NM_000162) and +1 corresponds to the A of the ATG translation initiation codon
<sup>c</sup>Swissprot accession n° P30527
<sup>d</sup>, <sup>e</sup>, <sup>f</sup>Sibling pairs
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of the domain thereby contributing to its stability. The substitution of a phenylalanine with a leucine is not dramatic in terms of hydrophobicity, however, it introduces a cavity in the hydrophobic core. This may affect inner surface complementarity thereby influencing the structural stability and the dynamical behaviour of the domain with consequences at a functional level.

In mutant p.Asp132Asn (detected in 2 related patients), the acidic negatively charged aspartate, located in the α3 helix that belongs to the small domain, is changed into the uncharged asparagine. Aspartate 132 is a poorly conserved amino acid and this variant probably provokes only mild structural alterations. In fact, the 2 siblings bearing this mutation had normal glucose tolerance. The p.His137Asp mutation (detected in 1 patient) altered a moderately conserved amino acid. The activity of hepatic GCK is regulated by the glucokinase regulatory protein (GKRP). This would act as an allosteric inhibitor of GCK that specifically binds to the super-open form. Indeed, mutational analyses [30] have shown that two GCK fragments, 51–52 and 141–144, are involved in such interactions. Sequence 141–144 follows the α3 helix that is terminated by His 137. Histidine, by interacting with the carbonyl of Phe133, is involved in helix capping. Mutation p.His137Asp introduces a negative charge in the region and, in

![Figure 1. Distribution of the GCK mutations.](image1)

The structure of GCK in the closed form (PDB code: 1v4s) is shown as cyan and red ribbons that represent the small and large domain, respectively. Orange ribbons show the α13 helix. Yellow spheres are the mutation sites. Red and blue circles indicate clusters of mutations in the large and small domain, respectively.

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![Figure 2. Structural features of mutations p.Phe123Leu and p.Asp168Ala GCK.](image2)

The structure of GCK in the closed form (PDB code: 1v4s) is represented by cyan ribbons. A) Mutation p.Phe123Leu. Phe123 is structured inside the hydrophobic core of the small domain. Yellow sticks represent hydrophobic residues that constitute the core. Phe123 and Leu123 are represented by red sticks (left and right panel, respectively). B) The sticks represent residue Thr168 and glucose. The right panels show a close-up view of the glucose-binding cleft for the wild-type (top) and for the Ala168 mutant (bottom).

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Figure 3. Structural features of mutations p.His137Asp, p.Arg392Ser and p.Gly162Asp GCK. The structure of GCK in the closed form (PDB code:1v4s) is shown as cyan ribbons. A) p.His137Asp. Loop 141–144, which is involved in GKRP binding, is in red. His137 is at the end of the α3 helix. His137 is a capping residue of the helix, which is terminated by the interaction between side chain of His137 and Phe133. Asp137 (right) is not able to replace His137 interactions but adds a new interaction with Lys104. B) p.Gly162Asp. Yellow sticks represent hydrophobic residues that constitute the core. The location of Gly162 is marked in red (left of the panel). Asp162 is on the right of the panel. C) p.Arg392Ser. Residues Asp42, Glu236, Asn240 and Arg392 are represented by yellow sticks. H-bonds are shown in green. The wild-type enzyme and the p.Arg392Ser mutant are on the left and right of the panel, respectively.

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our simulations, Asp137 does not exert a capping function, but strongly interacts with Lys104 by making a salt bridge. Accordingly, p.His137Asp may affect the conformational properties of fragment 141–144 thereby indirectly influencing the binding with GKRP. The p.His137Arg mutation has been described in association with diabetes [31].

The p.Gly162Asp mutation (detected in 1 patient) alters a highly conserved amino acid. Gly162 is located on the β-sheet that encloses the small domain hydrophobic core. p.Gly162Asp is one of the most dramatic mutations we identified because it introduces a negative residue inside the hydrophobic core. p.Gly162Asp very probably influences the stability of the core thereby altering the structure and dynamics of the domain. This scenario is indicative of functional impairment of the enzyme.

The p.Thr168Ala mutation (detected in 2 related patients) affected a conserved amino acid. The glucose-binding cleft is located at the interface between small and large domains. It is constituted by residues Glu256 and Glu290 from the large domain, Thr168 and Lys169 from the small domain, and Asn204 and Asp205 from the interconnecting region. Binding a glucose molecule requires a precise pattern of H-bonds between the substrate and GCK. Thr168 binds glucose, therefore the p.Thr168Ala substitution prevents the formation of the H-bond and probably perturbs the enzyme’s binding affinity and efficiency. Mutation p.Thr168Ala has been described in patients affected by diabetes [32]; it greatly increased Vmax and resulted in a complete loss of cooperative behaviour associated with glucose binding, the 2 siblings bearing this mutation had normal glucose tolerance and impaired glycosylated hemoglobin. Glutamate 290 is a highly conserved residue involved in glucose binding. The p.Glu290X mutation (detected in 2 unrelated patients) introduces a stop codon and generates a truncated protein of only 209 amino acids, which is thus unable to function.

The p.Arg392Ser mutation (detected in 1 patient) alters a conserved amino acid. Arg392, is located on the α11 helix in the large domain and is involved in a local H-bond/salt bridge network. Arg392 is positively charged and makes a salt bridge with the negative residues Asp42 (22) and Ghu236. The H-bond network extends to two water molecules and residue Asn240. These residues, which are far in sequence, are relevant for the tertiary structure of the domain, in fact serine is unable to replace the wild-type Arg392 interactions. The p.Arg392Cys mutation was reported in co-segregation with hyperglycemia in pregnancy [33].

Three patients carried already known GCK mutations: p.Lys396X6, p.Ser433X and p.Glu263Lys. All these mutations were described in association with hyperglycemia. In particular, the Ser → Leu mutation at residue 433 was recently found to reduce GCK activity in a GCK MODY patient [34]. In our GCK MODY patients, the distribution of mutation sites in the GCK protein (59%, 33% and 8% in the small domain, large domain and in the connection region, respectively) differed from the distribution observed in European Caucasians and in other ethnic groups (41%, 58% and 1% in the small domain, large domain and in the connection region, respectively) [3,35]. Consequently, the GCK small domain may be a hot spot for MODY mutations typical of Southern Italy. Interestingly, almost all the mutation sites we describe are in regions involved in structural rearrangements required for catalysis. This finding supports the notion that mutations may affect GCK function, which is intimately related to interdomain region [7]. Our data confirm the association between low triglyceride values and GCK mutations and support a low rate of cardiovascular complications in GCK MODY diabetes [36]. Interestingly, the two patients (M001 and M013) with the lowest BMI z scores also had the lowest FPIR values, which is in line with the finding that, at low levels, insulin does not exert an anabolic effect [37].

Massa et al. [25] did not find an association between phenotype and genotype in GCK MODY patients. Two of our unrelated patients, M001 and M006, who both carried the p.Glu290X mutation, had a low birth weight but a different diabetic phenotype as evaluated by OGTT, FPIR tests and triglyceride level. In contrast, among the three pairs of siblings, each with the same mutation, two pairs (M022-M023, M028-M029) had almost identical metabolic phenotypes. In the third pair of siblings (M024-M025, aged 12 and 5 years respectively), the elder child was diabetic and the younger had impaired glucose tolerance. In these two patients, the p.Glu263Lys mutation provoked a dramatic rearrangement of GCK, which indicates a more severe prognosis. Thus, both the severity of the GCK mutation and the genetic background seem to play a relevant role in the GCK MODY phenotype.

In conclusion, all mutations detected in our diabetic children from south Italy co-segregated with the diabetic status in one or two family members of each patient and were not detected during the screening of 200 normal chromosomes. The new deletion and missense mutations produced an amino acid substitution at positions that are well conserved among several species. Finally, our data show that molecular screening is useful in the diagnosis of MODY because it allows one to confirm the diagnosis and to predict the prognosis as well as the clinical course of the patient.

Acknowledgments

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Table 3. Biochemical and phenotypic characteristics of the children affected by GCK MODY

| PATIENT CODE | SEX | BIRTH WEIGHT (kg) | AGE AT DIAGNOSIS (years) | BMIa z-scores | FPGb (mmol/L) | 120 min OGTTc (mmol/L) | FPIRd (μU/ml) | HbA1ce (%) | TRIGLYCERIDES (mmol/L) | AFFECTED FAMILY MEMBER |
|--------------|-----|------------------|--------------------------|---------------|--------------|------------------------|--------------|-----------|------------------------|------------------------|
| M001         | F   | 1.80             | 10                       | -3.59         | IFG          | NGT                   | 28.4         | 6.1       | 0.65                   | F                      |
| M002         | F   | 2.70             | 1                        | - --7         | IFG          | n.a.                  | 33.4         | 6.4       | 2.26†                   | M                      |
| M003         | F   | 3.30             | 8                        | 0.67          | IFG          | NGT                   | 112.7        | 6.7       | 0.99                   | M                      |
| M005         | F   | 2.95             | 9                        | 0.26          | IFG          | DM                    | 88.0         | 6.5       | 0.45                   | F                      |
| M006         | M   | 2.05             | 6                        | 0.72          | IFG          | IGT                   | 69.1         | 5.9       | 0.36                   | M                      |
| M009         | M   | 3.25             | 6                        | 1.56          | DM           | NGT                   | 39.4         | 5.6       | 0.80                   | F                      |
| M012         | M   | 2.97             | 7                        | -1.80         | DM           | IGT                   | 246.0        | 5.9       | 0.64                   | M                      |
| M016         | M   | 3.50             | 6                        | -0.59         | IFG          | NGT                   | n.a.         | n.a.      | 0.57                   | M                      |
| M018         | F   | 2.80             | 14                       | -0.60         | IFG          | NGT                   | 130.5        | 6.5       | 0.49                   | F                      |
| M019         | F   | 3.55             | 10                       | 1.46          | IFG          | IGT                   | 140.0        | 5.9       | 0.54                   | M                      |
| M022         | F   | 2.95             | 8                        | 1.60          | IFG          | NGT                   | n.a.         | 6.2       | 0.66                   | F                      |
| M023         | M   | 3.40             | 9                        | 0.67          | IFG          | NGT                   | 156.0        | 6.4       | 0.82                   | F                      |
| M024         | F   | 2.75             | 12                       | 1.29          | IFG          | DM                    | 104.0        | 6.5       | 0.40                   | F                      |
| M025         | F   | 2.50             | 5                        | 1.56          | IFG          | IGT                   | 35.5         | 6.2       | 0.29                   | F                      |
| M026         | F   | 2.85             | 12                       | 1.42          | IFG          | NGT                   | n.a.         | 5.4       | 0.52                   | M                      |
| M027         | M   | 2.91             | 7                        | 0.74          | IFG          | NGT                   | n.a.         | 5.5       | 0.56                   | M                      |

BMIa: BMI z-scores: (calculated in children aged 2–18 years), see Materials and methods
FPG: Fasting plasma glucose; impaired fasting glucose [IFG] = 5.6–6.9 mmol/L; diabetes mellitus [DM] = ≥7.0 mmol/L
OGTT: Oral glucose tolerance test (normal glucose tolerance [NGT] <7.8 mmol/L; impaired glucose tolerance [IGT] = 7.8–11.0 mmol/L; DM = ≥11.1 mmol/L)
FPIR: First phase insulin response (reference value: ≥60.0 μU/ml)
HbA1c: glycosylated haemoglobin (reference value: 4.3–5.9%)
Not available because patient M002 was <2 years old
Not fasting nursing
Not available

†Not fasting nursing

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Author Contributions
Conceived and designed the experiments: AD NT LS AZ. Performed the experiments: AD NT LS MC VC GD. Analyzed the data: AD NT LS AZ.

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