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ORIGINAL ARTICLE

Glucokinase Gene Mutations (MODY 2) in Asian Indians

Sekar Kanthimathi, MSc, MPhil, PhD,[1] Suresh Jahnavi, MSc,[1]
Kandasamy Balamurugan, MSc,[1] Harish Ranjani, MSc, PhD,[1]
Jagadesan Sonya, MSc, MPhil,[1] Soumik Goswami, MD, DM,[2]
Subhankar Chowdhury, MD, DM, DTM&H, MRCP,[2]
Viswanathan Mohan, MD, PhD, DSc, FRCP, FNA,[1,3] and Venkatesan Radha, MSc, PhD[1]

Abstract

Background and Aim: Heterozygous inactivating mutations in the glucokinase (*GCK*) gene cause a hyperglycemic condition termed maturity-onset diabetes of the young (MODY) 2 or GCK-MODY. This is characterized by mild, stable, usually asymptomatic, fasting hyperglycemia that rarely requires pharmacological intervention. The aim of the present study was to screen for *GCK* gene mutations in Asian Indian subjects with mild hyperglycemia.

Subjects and Methods: Of the 1,517 children and adolescents of the population-based ORANGE study in Chennai, India, 49 were found to have hyperglycemia. These children along with the six patients referred to our center with mild hyperglycemia were screened for MODY 2 mutations. The *GCK* gene was bidirectionally sequenced using BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA) chemistry. *In silico* predictions of the pathogenicity were carried out using the online tools SIFT, Polyphen-2, and I-Mutant 2.0 software programs.

Results: Direct sequencing of the *GCK* gene in the patients referred to our Centre revealed one novel mutation, Thr206Ala (c.616A>G), in exon 6 and one previously described mutation, Met251Thr (c.752T>C), in exon 7. In silico analysis predicted the novel mutation to be pathogenic. The highly conserved nature and critical location of the residue Thr206 along with the clinical course suggests that the Thr206Ala is a MODY 2 mutation. However, we did not find any MODY 2 mutations in the 49 children selected from the population-based study. Hence prevalence of *GCK* mutations in Chennai is <1:1,517.

Conclusions: This is the first study of MODY 2 mutations from India and confirms the importance of considering *GCK* gene mutation screening in patients with mild early-onset hyperglycemia who are negative for *β*-cell antibodies.

Introduction

MATURITY-ONSET DIABETES OF THE YOUNG (MODY) is an autosomal, dominantly inherited form of diabetes that is characterized by an early age of onset, usually younger than 25 years, and primarily due to pancreatic *β*-cell dysfunction. There are several types of MODY based on the genetic mutation, now termed MODY 1–MODY 11.[1] *Glucokinase* (*GCK*) was the first gene for MODY to be identified in French and United Kingdom pedigrees in 1992.[2,3]

GCK is a key regulator of glucose-stimulated insulin secretion in pancreatic *β*-cells and glycogen synthesis in the liver hepatocytes.[4] Variations in the catalytic activity of GCK, encoded by the *GCK* gene, are associated with abnormal glycemia. A modest decrease in enzyme activity due to heterozygous

mutations in the *GCK* gene results in mild fasting hyperglycemia, termed MODY 2 or GCK-MODY (MIM:125851).[5] About 644 *GCK* mutations have been reported so far in the literature. Most of the missense mutations are inactivating mutations causing hyperglycemia.[6] GCK-MODY is characterized by persistent mild, asymptomatic hyperglycemia, good pancreatic *β*-cell reserve, absence of autoimmune markers of type 1 diabetes, dominant mode of inheritance, and a potential for insulin withdrawal when the mutation is defined.[7,8] Most cases are detected only during routine medical screening, during pregnancy, or during family screening when MODY is suspected.[9]

Patients with *GCK* mutations rarely need any pharmacological treatment, and the majority are managed on diet and exercise.[10] The prevalence of GCK-MODY as a percentage of all MODY cases has been reported from several European

[1]Madras Diabetes Research Foundation, Chennai, India.
[2]Department of Endocrinology and Metabolism, The Institute of Post-Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital, Kolkata, India.
[3]Dr. Mohan's Diabetes Specialities Centre, WHO Collaborating Centre for Non Communicable Diseases Prevention & Control, IDF Centre of Education, Chennai, India.

populations and also from Chile (50%), Brazil (19%), and Israel (40%). However, there are no reports on GCK-MODY from India, which has the second largest number of people with diabetes in the world.[11,12] The aim of the present study was to look for *GCK* gene mutations in Asian Indian subjects.

Subjects and Methods

The subjects for the present study were drawn from two sources. The first was from an epidemiological study, the Obesity Reduction And Non communicable disease awareness through Group Education (ORANGE) study, carried out in children and adolescents in Chennai, India, with the aim of looking for prevalence of glucose intolerance among these age groups. The ORANGE study was carried out in children and adolescents residing in 10 corporation zones, selected to be representative of Chennai city in Tamilnadu. The methodology of the study has been published elsewhere.[13] In total, 1,517 children and adolescents in the age group 6–19 years were screened under the ORANGE study. Patients with impaired fasting glucose (fasting plasma glucose level of 100–125 mg/dL), impaired glucose tolerance (2-h post-glucose load of 140–199 mg/dL), or both, according to the World Health Organization Consultation Group report,[14] were classified under the prediabetes group. Those with a fasting plasma glucose level of ≥126 mg/dL and/or 2-h post-glucose load of ≥200 mg/dL, according to the World Health Organization Consultation Group report,[14] were classified as having diabetes.

Forty-nine children and adolescents were found to have any degree of hyperglycemia among the 1,517 ORANGE study subjects screened (3.2%).[13] Of them, nine had more
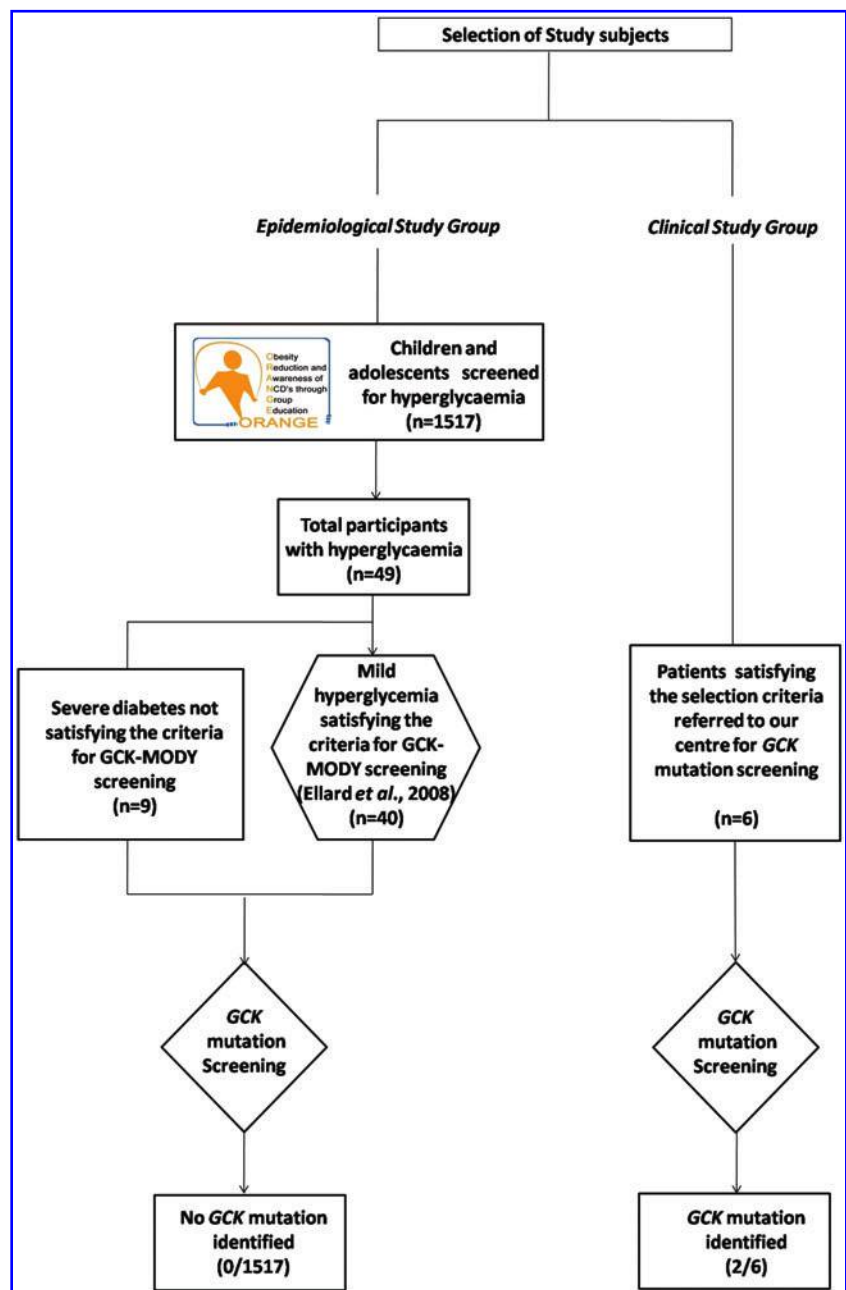

FIG. 1. Study subject selection for glucokinase (*GCK*) gene mutation screening. MODY, maturity-onset diabetes of the young. Ellard et al.[10] Color images available online at www.liebertpub.com/dia

severe forms of diabetes, whereas the remaining 40 children satisfied the selection criteria[10] of having fasting hyperglycemia between 5.5 and 8 mmol/L (99–144 mg/dL) and an oral glucose tolerance test showing a 2-h increment of ≤4.6 mmol, glycosylated hemoglobin (HbA1c) level of <7.0%, no autoimmune markers of type 1 diabetes (glutamic acid decarboxylase and insulinoma antigen 2), and with a first-degree family history of diabetes. We screened all 49 for *GCK* gene mutations (Fig. 1). The nine subjects with severe diabetes were found to be negative for the mutation in the most common MODY genes, hepatocyte nuclear factor *HNF4A* (MODY 1) and *HNF1A* (MODY 3) (data not shown). Because we had undertaken screening of all three common MODY genes (*HNF4A*, *HNF1A*, and *GCK*), we included these nine patients for the *GCK* gene mutation screening as well.

In the second part of the study, we screened for GCK-MODY in six patients with nonprogressive mild hyperglycemia, referred to our Centre (Fig. 1). The patients with fasting hyperglycemia between 5.5 and 8 mmol/L (99–144 mg/dL), persistent (at least three separate occasions) and stable over a period of months or years, an oral glucose tolerance test showing a 2-h increment of ≤4.6 mmol, and HbA1c typically just above the upper limit of normal and rarely exceeding 7.5% were considered to have nonprogressive mild hyperglycemia.

The study was approved by the institutional ethical committee. Informed consent was obtained from all study subjects and from the parents, if the subject was younger than 18 years of age, and, in addition, an assent was also obtained from these children.

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood using a standard phenol-chloroform procedure. Exons 1a and 2–10 (β-cell GCK isoform), flanking intronic regions, and β-cell promoter of the *GCK* gene were bidirectionally sequenced in 55 subjects, using BigDye® Terminator v3.1 chemistry (Applied Biosystems, Foster City, CA) as previously described.[15] In silico predictions of the pathogenicity were carried out using the online tools SIFT (http://sift.jcvi.org/www/SIFT_dbSNP.html), Polyphen-2 (http://genetics.bwh.harvard. edu/pph/), and I-Mutant 2.0 software.[16]

Results

Among the 49 children with hyperglycemia, detected after screening 1,517 subjects in the ORANGE study, none of them harbored GCK-MODY (MODY 2) mutations. So the prevalence of GCK-MODY is less than 1:1,517 children in Chennai.

In the clinical samples, of the six patients tested, we identified two heterozygous *GCK* gene mutations in two unrelated clinically diagnosed MODY patients: one previously described, Met251Thr (c.752T >C), and one novel mutation, Thr206Ala (c.616A >G).

The mutation Met251Thr (c.752T >C) in the exon 7 of the *GCK* gene was identified in a nonobese 7-year-old girl, diagnosed with mild hyperglycemia at the age of 2 years (fasting glucose level, 115–127 mg/dL [6.3–7.0 mmol/L]; 2-h postprandial, 125–140 mg/dL [6.9–7.7 mmol/L]; HbA1c, 6.5–6.9% [47.5–51.9 mmol]). Anti-glutamic acid decarboxylase and anti-insulinoma antigen 2 antibodies were negative. She was on diet and exercise and is continuing with the same. She was the first-born baby of nonconsanguineous parents, delivered at term (cesarean delivery) with a normal birth weight of 2.6 kg (5th percentile) and length of 47 cm. She had a history of

neonatal hypoglycemia (two episodes) but was treated symptomatically. The girl also had a history of neonatal hypocalcemia (serum calcium level, 7.8 mg/dL) and was given calcium gluconate (intravenously). Her mother was diagnosed as having gestational diabetes mellitus (fasting glucose level, 110–135 mg/dL [6.1–7.5 mmol/L]; 2-h postprandial, 146 mg/dL [8.1 mmol/L]) and was on insulin treatment during pregnancy. The proband's mother and maternal grandfather had normal dilated fundoscopy, serum creatinine, and spot urine microalbumin creatinine ratio. Her maternal grandfather was recently diagnosed with diabetes (fasting glucose level, 138 mg/dL [7.6 mmol/L]; 2-h postprandial, 200 mg/dL [11.0 mmol/L]; HbA1c, 6.9% [51.9 mmol/mol]), whereas her father, maternal grandmother, and maternal uncle did not have diabetes.

Genetic testing was offered to the proband's family. Direct sequencing analysis showed the presence of the Met251Thr mutation in the proband's mother (who had gestational diabetes mellitus) and her maternal grandfather in the heterozygous state (Fig. 2). The mutation was absent in other family members without diabetes (father, maternal grandmother, and maternal uncle) screened.

The *in silico* analysis of the Met251T mutation based on SIFT (score 0.000) and Polyphen-2 (score 0.99) predicted that this substitution at position 251 from Met to Thr could be pathogenic. This mutation leads to a decrease (DDG, −1.53 kcal/mol) in stability of the protein as predicted by I-Mutant 2.0. (The DDG value is calculated from the unfolding Gibbs free energy value of the mutated protein minus the unfolding Gibbs free energy value of the wild type [in kcal/mol]).

The patient with the novel heterozygous mutation Thr206Ala (c.616A >G) in exon 6 of the *GCK* gene is a nonobese 34-year-old male, diagnosed with impaired glucose tolerance at the age of 7 years, who has mild hyperglycemia (fasting glucose level, 141 mg/dL [7.8 mmol/L]; 2-h postprandial, 149 mg/dL [8.2 mmol/L]; HbA1c, 6.5–7.1%
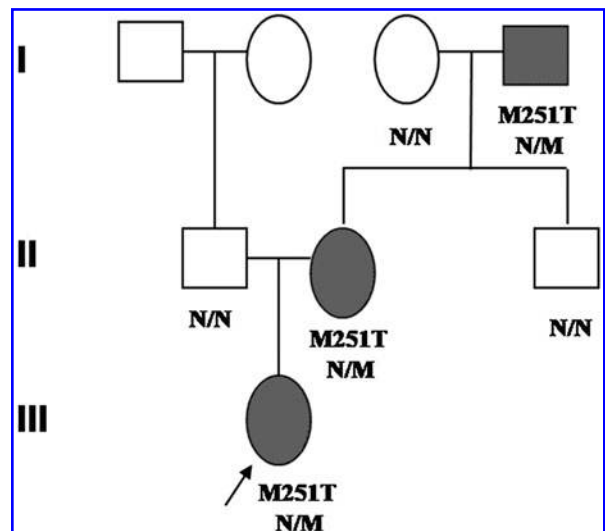
FIG. 2. Family pedigree showing diabetes status and inheritance of the glucokinase mutation Met251Thr (c.752T >C). The proband is indicated with an arrow. Solid circles and squares indicate diabetes. Open circles and squares indicate normal glucose tolerance. N/M, heterozygous; N/N, homozygous normal.

[47.5–54.1 mmol/mol]). Serum anti-glutamic acid decarboxylase and anti-insulinoma antigen 2 antibodies were negative. Fasting C-peptide levels showed fairly good pancreatic β-cell reserve (fasting, 0.9 ng/mL [0.29 nmol/L]; stimulated, 2.8 ng/mL [0.93 nmol/L]). The patient was managed with diet and exercise until 18 years of age, and currently he is on an oral hyperglycemic agent (a sulfonylurea drug, Reclide [Dr. Reddy's Laboratories Ltd., Hyderabad, India], 80 mg/day). He was born as the second child to nonconsanguineous parents and has a strong family history of diabetes—both parents, paternal aunt, and paternal uncle had diabetes (Fig. 3). Because the parents' blood samples were unavailable to perform genetic tests, the co-segregation of this novel mutation with the phenotype could not be studied in this family. Mutation validation of this novel variant was done by screening 100 healthy individuals without diabetes, and none of them was found to carry the variation. No other coding variants in *GCK*, *HNF1A*, and *HNF4A* genes were detected in the proband.

In silico analysis based on SIFT (score 0.010) and Polyphen-2 (score 0.996) predicted that the Thr206Ala substitution at position 206 from Thr to Ala would be pathogenic. This mutation leads to decrease in protein stability (DDG, −0.71 kcal/mol) as predicted by I-Mutant 2.0.

Discussion

Our study shows that GCK-MODY is an uncommon condition in Chennai, representative of urban India. The OR-ANGE study was primarily meant to study the prevalence of glucose intolerance in Chennai. Unfortunately, as routine screening of children for diabetes is not recommended because of ethical reasons, we were unable to study a much larger sample size, which could have provided the exact prevalence of GCK-MODY in our population. Hence, we can only conclude that it is less than 1:1,517 children in this study.

Mild fasting hyperglycemia-associated *GCK* mutations have been identified in many populations, with the majority being found in European white populations, and GCK-MODY is reported to constitute a high percentage of all MODY subjects.[6] There is some discrepancy in the literature on the prevalence of GCK-MODY as a percentage of all MODY patients in European whites. In the United Kingdom, the prevalence is reported as 20%,[17] and in France it appears to be 56%.[5] Other studies[6] have reported the prevalence as follows: Italy, 41–61%; Spain, 25–80%; Czech Republic, 31%; Norway, 12%; Denmark, 10%; Chile, 50%; an Israeli population, 40%; and Brazil, 19%.

Few studies have done screening of the *GCK* gene mutation in large populations. Therefore the exact prevalence of GCK-MODY in different geographic locations and ethnic groups is not known.[18] The prevalence of GCK-MODY is difficult to determine as the hyperglycemia is mild and the disease is asymptomatic. In the white population, approximately 2% of the population are diagnosed as having gestational diabetes, and of these, approximately 2–5% have a *GCK* mutation.[19] This would suggest a population prevalence of 0.04–0.1%.
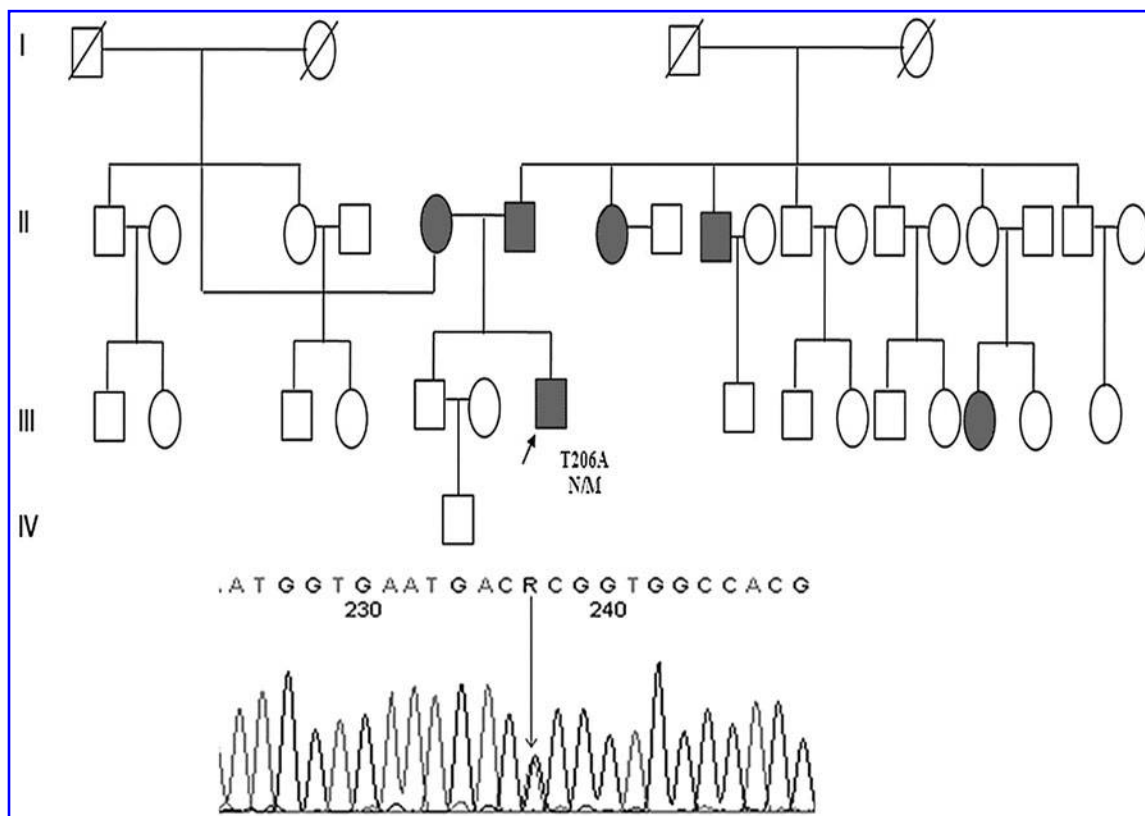
FIG. 3. **(Top panel)** Family pedigree showing diabetes status and **(bottom panel)** chromatogram showing the novel glucokinase mutation Thr206Ala (c.616A>G). The proband is indicated with an arrow. Solid circles and squares indicate diabetes. Open circles and squares indicate normal glucose tolerance. A diagonal line indicates relatives who had died. N/M, heterozygous.

Screening of the clinical patients with mild fasting hyperglycemia revealed the presence of two heterozygous MODY2 mutations: Met251Thr and Thr206Ala. Among the two, Thr206Ala is a novel mutation.

The heterozygous *GCK* mutation Met251Thr (c.752T>C) found in one of the patients has been reported earlier in a French family.[6] There are two other mutations that have been reported in the same nucleotide position: Met251Ile in an Italian family and Met251Val in Czech and French families.[6] So far no functional studies have been done for these mutations. The amino acid methionine at position 251 is evolutionarily conserved among human, rat, and mouse species (placental mammals). The *in silico* analysis based on SIFT and Polyphen-2 predicted that this substitution at position 251 from Met to Thr could be pathogenic. This mutation is likely to decrease the protein stability as predicted by I-Mutant software. The Met251Thr mutation co-segregated with diabetes in the family. The proband, her mother, and maternal grandfather continue to have nonprogressive mild hyperglycemia over the years without pharmacotherapy. The pathogenicity of the mutation is suggested by the typical MODY 2 clinical symptoms and the co-segregation of the phenotype with the genotype.

There is strong evidence in GCK-MODY that both the fetal and mother's *GCK* genotypes influence the fetal growth.[20] Therefore, *GCK* mutation screening in female patients is of particular importance, as it is critical to know the exact diagnosis, because during pregnancy they are often overtreated with insulin. Owing to its role in fetal growth, this overtreatment in an affected mother carrying an affected fetus will result in reduced birth weight.[20] Had the mother of the proband been tested for *GCK* gene mutation during her pregnancy, her treatment would likely have been different.

The Thr206Ala (c.616A>G) mutation identified has not been reported so far, although four different substitutions were previously described for this residue. The Thr206Pro mutation (c.616A>C; American family), Thr206Met (c.617C>T; Sardinian, Italian, Danish, Spanish, American, German, and French populations), Thr206Arg (c.617C>G; Canadian family), and Thr206Lys (c.617C>A; family of unknown origin) have been reported in MODY patients.[6] The proband with 27 years of stable hyperglycemia without any complications managed only with diet for 11 years and later on with oral hypoglycemic agents, confirming the characteristics of GCK-MODY. Residue Thr206 is located in the glucose-binding site of GCK,[21,22] and the high conservation of Thr206 among species suggests that it has an important functional role. This variation was not found in over 200 chromosomes from control subjects without diabetes. The in silico analysis showed that this missense mutation is likely to be pathogenic. Enzymatic assay studies have shown that Thr206Met strongly affects the kinetic properties of GCK,[23] and Thr206Pro substitution was found to distort the conformation of the glucose-binding pocket.[24]

Pathogenicity of identified missense *GCK* mutations can be better understood using functional studies in addition to family and bioinformatic studies.[25] Although the functional effects of these mutations have not been studied directly, a set of bioinformatic tools (SIFT, Polyphen-2, and I-Mutant) was used to predict their impact on protein function. SIFT and Polyphen-2 gave similar results predicting both the mutations (Met251Thr, Thr206Ala) to be deleterious. I-Mutant predicted them to be least stable and deleterious,

suggesting a possibility of conformational change at their structural level.

We have earlier reported on the prevalence of MODY 3[26] and MODY 1[27] in our clinic population. In a cohort of 96 clinically diagnosed MODY subjects with the severe form of diabetes referred to our center, we found that 9% had MODY 3, whereas 3% had MODY 1. None of the subjects had GCK-MODY (MODY 2) in that cohort, perhaps because their clinical profile was different from GCK-MODY (i.e., more severe diabetes, progressing to complications and needing drug therapy including insulin for glycemic control). In the present study, we screened six patients with mild hyperglycemia, where the clinical profile was suggestive of GCK-MODY, and we identified MODY2 mutations in two of the six patients. Thus, while screening for GCK-MODY, the right clinical phenotype of mild hyperglycemia has to be selected.

In conclusion, we describe two studies, one epidemiological and the other a clinic study where we screened participants for GCK-MODY mutations. We detected two patients with nonprogressive mild hyperglycemia harboring mutations in the *GCK* gene, including a novel missense mutation, Thr206Ala (c.616A>G). The highly conserved nature and critical location of the residue Thr206 along with the clinical course suggested that this novel variation is indeed a MODY 2 mutation. To our knowledge, this is the first report of GCK-MODY mutations from India. The present study highlights that *GCK* mutation screening may be considered in patients with chronic mild early-onset hyperglycemia and negative β-cell antibodies. This could potentially help clinicians to classify the subtype of MODY diabetes, to help screen other family members, to predict the prognosis, and to offer correct treatment to patients. The genetic analysis for GCK-MODY is currently offered at 50 dollars (U.S.) at our center. In most cases, patients with *GCK* gene mutations are managed on diet and exercise. The mutation screening could thus help patients to save on the costs of drugs, monitoring, and follow-up expenses. These long-term benefits may outweigh the cost of the genetic testing, which is currently quite high.

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Address correspondence to:
Venkatesan Radha, PhD
Department of Molecular Genetics
Madras Diabetes Research Foundation
No. 4, Conran Smith Road
Gopalapuram, Chennai-600 086, India

E-mail: radharv@yahoo.co.in
drradha@mdrf.in

Web site: www.mdrf.in
www.drmohansdiabetes.com