RESEARCH PAPER

Genotype-Phenotype Characteristics of Turkish Children With *Glucokinase* Mutations Associated Maturity-Onset Diabetes of the Young

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Objective: To investigate phenotype-genotype correlations in Turkish children with *glucokinase* gene mutations leading to Maturity-onset diabetes in young (GCK-MODY). **Methods:** Retrospective analysis of 40 patients (16 girls) aged under 18 with GCK-MODY. **Results:** Mean (SD) serum fasting blood glucose level was 6.79 (0.59) mmol/L and the mean (SD) HbA1c level at diagnosis was 6.3% (0.5). Sixteen different variations were detected in the *GCK* genes of the 40 cases; 33 missense mutations, 6 deletions, and one nonsense mutation. The birthweight of infants with deletion mutation was significantly lower than that of infants with other mutations [2460 (353.66) g *vs* 2944.11 (502.08) g]. **Conclusion**: GCK-MODY patients with deletion mutation inherited from mothers had lower birthweight and higher fasting blood glucose than those with other inherited mutations but similar HbA1c values.

Keywords: Gestational diabetes mellitus, Next generation sequencing.

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Maturity-onset diabetes of the young (MODY) is a rare form of diabetes inherited in an autosomal dominant manner and developing secondary to beta cell dysfunction. MODY accounts for 1.1-4.2% of diabetic children and has a reported prevalence of 2.4-4.6 per 100,000 [1,2]. GCK-MODY (MODY2) and HNF1A-MODY (MODY3) constitute 90% of all MODY cases [3,4]. Heterozygous, inactivating mutations in the *glucokinase (GCK)* gene cause GCK-MODY, while homozygous or combined heterozygous mutations lead to permanent neonatal diabetes mellitus [5]. GCK mutations are commonly encountered in countries such as Spain, France, and Italy, where blood glucose screening is routinely performed, it is also reported as the leading cause of MODY in the Turkish population [4]. The purpose of this study was to investigate the genotype-phenotype correlations of patients with GCK-MODY followed-up in three different centers in Turkey.

METHODS

Data was retrieved from hospital records for 40 MODY patients with GCK mutations (16 girls) aged under 18 years, who had presented to the department of pediatric endocrinology between 2013 and 2018. All selected cases were variant carriers in the *GCK* gene. Parents with no history of diabetes mellitus were tested for fasting plasma glucose and glycosylated hemoglobin (HbA1c). *GCK* gene mutation analysis was also performed on the parents of children with GCK-MODY. Demographic features, laboratory findings and treatments received were retrieved from hospital.

DNA was isolated from samples as per standard technique. The *GCK* gene was sequenced using the Next generation sequencing (MISEQ-Illumina) method. The pathogenicity of the detected variants in the GCK gene was determined by combined evaluation of bioinformatics, in-silico analysis of the detected variants with segregation studies, and the laboratory and clinical findings. The study was approved by the local ethics committee.

Statistical analyses: All statistical analyses were performed on IBM SPSS Statistics for Windows software, version 23.0 (IBM Corp., Armonk, NY, USA). Relations between the GCK gene variants and both clinical and laboratory parameters were evaluated using the chi-square test. P values <0.05 were considered statistically significant.

RESULTS

The mean (SD) age at diagnosis was 8.6 (4.25) years. The mean (SD) fasting blood glucose level was 6.79 (0.59) mmol/L. The mean (SD) fasting C-peptide level was 1.3

(1.4) ng/mL, the mean (SD) insulin level was 7.44 (4.95) µU/mL, and the mean (SD) HbA1c level at diagnosis was 6.34 (0.56)%. Thirty patients presented with fasting hyperglycemia, while 10 patients were admitted with symptoms of hyperglycemia. The mean (SD) HbA1c value was 6.48 (0.41)% at the last follow-up, and the mean (SD) length of follow-up was 2.14 (1.72) years.

Sixteen different variants were detected in the *GCK* gene of the 40 cases; 33 were missense mutations, six were deletions, and one was a nonsense mutation. The most common mutations were p.Met393Thr (15/40) and p.Ile189Val (6/40). Three of the cases were homozygous, and 37 were heterozygous. We detected a new variant that had not been previously described, named c.537delG / p.Asn180ThrfsTer25 in exon 5 of the *GCK* gene. GCK-MODY was present in the mother or father in 32 of the 40 cases in this study, while the parents in the other eight cases had no GCK-MODY diagnosis. Deletion mutation was determined in six of the fathers with GCK-MODY and missense mutation in 13. Fourteen of the mothers had a missense mutation in the *GCK* gene, and five had deletion. Nine infants (9/40) were small for gestational age. The infants with deletion mutation had statistically significantly lower birthweight than infants with other mutations (***Table* I**).

The mean (SD) insulin requirement in the final trimester of pregnancy among mothers with deletion mutation (0.48 (0.13) U/kg) was higher than that of mothers with other mutations (0.20 (0.13) U/kg) (P=0.07). Permanent neonatal diabetes was diagnosed in one case with homozygous deletion mutation, and glycemic control was achieved with insulin pump treatment. GCK-MODY was diagnosed in two cases with homozygous missense mutation, and medical treatment was not required in these.

Table I Characteristics of Children With GCK-MODY (N=40)

Characteristics	*Deletion mutation (n=6)*	*Other mutation (n=34)*
Age (y)	8 (5.76)	10.5 (4.90)
Age diagnosis (y)	4.4 (4.7)	9.3 (3.77)
Birthweight, g[#]	2460 (353.66)	2944.1 (502.1)
[*]FBG (mmol/L)[‡]	7.4 (0.40)	6.68 (0.56)
[*]HbA1c (%)	6.13 (0.98)	6.38 (0.46)
C-peptide, ng/mL	2.44 (3.76)	1.24 (0.85)
Insulin, µU/mL	9.16 (7.57)	7.27 (4.79)
HDL, mmol/L	1.05 (0.27)	1.29 (0.26)
LDL, mmol/L	1.90 (0.63)	2.09 (0.62)
Cholesterol, mmol/L	3.60 (0.44)	3.74 (0.77)
Triglyceride, mmol/L	1.20 (0.67)	0.85 (0.37)

All values in mean (SD); [*]values at diagnosis; [#]P=0.016, [‡]P=0.007; HDL: High density lipoprotein, LDL: Low density lipoprotein.

DISCUSSION

Patients with GCK-MODY are mostly asymptomatic. The fasting blood glucose level and HbA1c level at first admission in the present study was consistent with the literature [6]. Pharmacological therapy is not recommended in GCK-MODY, except during pregnancy, because of the low effect of blood glucose lowering therapy and the absence of complications in diabetics with this mutation [8].

Most GCK-MODY mutations consist of missense (65%), nonsense, frameshift or splice site mutations [2]. Rare causes include GCK pancreatic islet promoter mutations [9] and partial or complete gene deletions [10]. Consistent with the previous literature, the most common GCK mutation in the present study was missense mutation. High blood glucose is reported to induce glucokinase through post-translation mechanisms, and the clinical phenotype is therefore similar in cases with GCK-MODY irrespective of the severity of the mutations [11]. However, recent studies have demonstrated that the phenotype may significantly differ in patients with GCK-MODY depending on the type of the mutation [12]. Fasting blood sugar in our deletion mutation group was significantly higher than in patients with other mutations, a finding consistent with previous studies revealing a relationship between genotype and phenotype. Velho, *et al*. [13] reported that missense mutations exhibit varying effects on glucokinase activity, and that glucose affinity can be affected, ranging from a small change to complete inactivity. In contrast, we found that children with heterozygous missense mutation GCK-MODY exhibited similar phenotype characteristics to those of children with GCK-MODY with other mutations, and that they presented with mild fasting hyperglycemia. Although homozygous mutations in the *GCK* gene are known to cause permanent neonatal diabetes, few cases with this mutation are diagnosed with mild fasting hyperglycemia, and protein instability has been implicated in this difference in phenotypes [14].

Maternal hyperglycemia during pregnancy is the primary risk factor for fetal macrosomia caused by fetal hyperinsulinism [15]. However, if the fetus inherits the *GCK* mutation from the mother, aggressive insulin treatment directed toward maternal euglycemia may cause fetal growth retardation [16]. In our study, the mean birthweight of the patients with deletion mutation inherited from the mothers was significantly lower than that of patients with nonsense and missense mutations

WHAT THIS STUDY ADDS?

Patients with *glucokinase* gene mutation associated maturity-onset diabetes in young (MODY) who inherited the deletion mutation from their mothers, had lower birthweight and higher fasting blood glucose than those with other mutations.

inherited from mothers. The need for blood glucose-lowering treatment in pregnant women with GCK-MODY with gene deletion may explain the lower birth weight in the infants with the same mutation.

In conclusion, our study shows that patients with deletion mutation inherited from mothers had lower birth weight and higher fasting blood glucose, but similar HbA1c values, compared to patients with other inherited mutations, and that homozygous gene mutations in the *GCK* gene result in phenotypic characteristics ranging from neonatal diabetes to GCK-MODY.

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