Clinical Heterogeneity in Monogenic Diabetes Caused by Mutations in the Glucokinase Gene (GCK-MODY)

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RESEARCH DESIGN AND METHODS — Members (three generations) of the same family presented either with overt neonatal hyperglycemia, marked postprandial hyperglycemia, or glucosuria. Homeostasis model assessment of insulin resistance (HOMA$_{IR}$) and insulinogenic and disposition indexes were calculated. Oral glucose tolerance test (OGTT) results in the GCK mutation carriers from the same family were compared with those from other subjects with GCK mutations in the same codon (GCK$_{261}$), with other missense and other types of GCK mutations in different codons from the European MODY Consortium database (GCK$_{m}$).

RESULTS — Mutation G261R was found in the GCK gene. During the OGTT, glucose ($P = 0.02$) and insulin ($P = 0.009$) response at 2 h as well as at the 2-h glucose increment (GCK$_{261}$ versus other missense GCK mutations, $P = 0.003$) were significantly higher in GCK$_{261}$ than in GCK$_{m}$ carriers.

CONCLUSIONS — Differing from other GCK$_{m}$ carriers, the glucose and insulin response to oral glucose was significantly higher in GCK$_{261}$ carriers, indicating clinical heterogeneity in GCK-MODY.

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RESEARCH DESIGN AND METHODS — The proband (online appendix Fig. A1, available at http://care.diabetesjournals.org/cgi/content/full/dc09-0681/DC1) was a firstborn child from a Finnish family with neonatal plasma glucose of 10 mmol/l. At 2 years of age, without treatment, she presented preprandial and postprandial capillary glucose of 6.5–6.8 and 8.6 mmol/l, respectively. Her younger sister had random glucose between 7 and 11.5 mmol/l as a neonate. Their mother was diagnosed with gestational diabetes and treated with insulin. After the pregnancy, she had an A1C of 5.8% without insulin treatment, but due to high postprandial plasma glucose (10–11 mmol/l), rapid-acting mealtime insulin was started. Since the second pregnancy, she is treated with diet alone. The maternal grandmother presented with hyperglycemia and glucosuria at age of 22 years and gestational diabetes during four pregnancies. She was treated with diet during the first pregnancy and with insulin during three later pregnancies, after which she had been without treatment. Her fasting capillary glucose level was normally ~6–7 mmol/l but stayed at ~10 mmol/l for nearly 2 weeks after intake of larger quantities of carbohydrates and returned to 6–7 mmol/l when carbohydrates were restricted. She takes 60 mg of nateglinide before meals. All available family members were offered an oral glucose tolerance test (OGTT) and/or genetic testing for the mutation after genetic counseling.

OGTT (except subjects <15 years) with samples drawn at –5, 0, 30, 60, 90, and 120 min was performed to determine plasma glucose and serum insulin. Insulin resistance and β-cell function was estimated using the homeostasis model assessment of insulin resistance (HOMA$_{IR}$) and the insulinogenic indexes (IG30), respectively. The disposition index (DI) was used to assess β-cell compensation. These results and those from 15 subjects with GCK mutations in position 261 (GCK$_{261}$) from the European MODY Consortium Database (EMCD) (3) were compared with that of carriers of other missense and other types of GCK

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Clinical characteristics of patients with glucokinase inactivating mutation GCK261 and functional studies of recombinant human wild-type and mutants’ gk

|                | GCK261- mutations | Other missense: GCK mutations | Other GCK mutation types | Normal glucose tolerance: control subjects |
|----------------|-------------------|-------------------------------|--------------------------|--------------------------------------------|
| n (male/female)| 23 (13/10)        | 144 (73/71)                   | 82 (42/40)               | 45 (20/25)                                 |
| BMI (kg/m²)   | 21.80 (7.0)       | 20.00 (5.42)                  | 21.30 (5.73)             | NS                                         |
| Age (years)   | 20.00 (27.0)      | 19.00 (27.00)                 | 29.00 (28)               | NS                                         |
| 0-min plasma glucose (mmol/l) | 7.00 (0.69) | 6.70 (0.90)                   | 6.80 (1.01)              | 0.17                                       |
| 120-min plasma glucose (mmol/l) | 10.90 (4.13) | 8.60 (2.58)                   | 8.60 (2.98)              | 0.38                                       |
| 2hΔPG (mmol/l) | 4.12 (3.25)      | 2.00 (2.05)                   | 2.50 (2.20)              | 0.233                                      |
| 0-min plasma insulin (mU/l) | 10.00 (7.44) | 8.00 (6.00)                   | 9.00 (5.00)              | 0.41                                       |
| 120-min plasma insulin (mU/l) | 55.2 (28.05) | 25.00 (23.30)                 | 24.00 (13.50)            | 0.11                                       |
| Incremental I/G30 | 8.71 (7.46) | 6.09 (3.74)                   | 4.21 (5.55)              | 0.672                                      |
| HOMAIR | 3.47 (2.31) | 2.68 (2.45)                   | 2.21 (1.92)              | 0.259                                      |
| DI | 3.23 (2.25) | 2.64 (2.02)                   | 1.98 (2.18)              | 0.76                                       |
| Proteins studied | Glucose S₂₅ | Hill number (unit less) | ATPₖₐₚ (mmol/l) | Turnover rate (Kcat) (sec⁻¹) | Activity index (AI) | T-GSIR (mmol/l) |
| gk-WT | 7.55 ± 0.23 | 1.74 ± 0.04 | 0.41 ± 0.03 | 62.3 ± 4.75 | 1.45 ± 0.11 | 5 |
| gk-G261R | 68.61 ± 16.15 | 1.53 ± 0.11 | 0.63 ± 0.10 | 17.03 ± 4.11 | 0.04 ± 0.001 | 7 |
| gk-G261E | 334.73 ± 26.78 | 1.92 ± 0.06 | 2.99 ± 0.37 | 3.72 ± 0.32 | 0.00 | 7 |

Upper panel: data are median (interquartile range). Clinical characteristics, glucose, and insulin values of patients with GCK-inactivating mutation GCK261, other missense GCK-inactivating mutations, and other types of GCK mutations (insertions, deletions, frameshifts, etc.). For insulin data: n = 11 (GCK261), 36 (other missense GCK mutations), 45 (other types of GCK mutations), and 45 (normal glucose tolerant controls). P₁: GCK261 mutations vs. other missense GCK mutations; P₂: GCK261 mutations vs. other types of GCK mutations; P₃: other missense GCK mutations vs. other types of GCK mutations; P₄: GCK261 mutations vs. control subjects. P < 0.05–0.00001 are considered statistically significant. HOMAIR = fasting serum insulin × fasting glucose/22.5. Insulinogenic index (I/G30) = serum insulin at 30 min / serum insulin at 0 min / serum glucose at 30 min / serum glucose at 0 min. DI = insulinogenic index/HOMAIR. Lower panel: data are means of the three independent analyses. Results of the functional studies were approved by the institutional ethics committee. Written consent was obtained from the adults and from the parents of the children. DNA extraction, microsatellite genotyping, direct sequencing, and functional analysis of the glucokinase protein (gk), with and without gk activator, were performed as described (7–10).

RESULTS—The mutation G261R (exon 7) on GCK was found in the proband and in nine family members (aged 0.2–72 years) with abnormal fasting glucose. Fasting plasma glucose ranged from 6.0 to 7.6 mmol/l. The 2-h plasma glucose ranged from 9.3 to 14.5 mmol/l in the carriers, three of them presented values exceeding 13 mmol/l. All but one of the carriers had a 2-h increment in plasma glucose (2hΔPG) higher than 3 mmol/l and half higher than 6 mmol/l. Fasting insulin was 4.1–9.9 mU/l and 2-h insulin 28.8–61.9 mU/l. There was no relationship between age and glucose or insulin concentrations.

The GCK261 mutation carriers from our family, like those from the EMCD, had a significantly higher glucose and insulin response compared with GCKm carriers (Table 1). Fasting plasma glucose and insulin were similar in all groups; however, the 2-h plasma glucose and insulin and 2hΔPG values were significantly higher in GCK261 carriers than in GCKm carriers (Table 1). The glucose response during OGT was higher at all time points in GCK261 carriers compared also with GCKm carriers (data not shown). In 61 and 35% of GCK261 carriers, 2hΔPG was >3 and 4.6 mmol/l, respectively. HOMAIR, I/G30, and DI values were higher (not significant) in the GCK261 carriers (Table 1), indicating possibly higher degree of β-cell compensation (Table 1).

The results from the functional studies showed that the mutations gk-G261R/E lead to a severely affected protein, with an almost negligible enzyme activity, indicating that these gk mutants cannot contribute to β-cell and hepatic glucose phosphorylation. The effect of the gk activator on the inactivating gk-G261R mutation was similar to that on the gk-WT (see online appendix Table A1).

CONCLUSIONS—The clinical phenotype of carriers from our family was heterogeneous. The proband and her sister presented with neonatal hyperglycemia, their mother with gestational diabetes, and the maternal grandmother with glucosuria. Many carriers had much higher 2hΔPG values than what is usually seen in GCK-MODY. In three carriers (one child and two adults), it exceeded 13 mmol/l and in another young carrier 12 mmol/l, indicating no relationship between high 2hΔPG values and age. A sim-

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Clinical heterogeneity in GCK-MODY

ilar pattern was seen in other carriers of the same mutation, while those with other GCK mutations in the MODY database had a lower glucose response during OGTT. Of note, a similar pattern of glucose response as in GCK_{261} carriers has previously been observed in GCK-L184P carriers (6). However, while the insulin response was attenuated in GCK-L184P carriers, in GCK_{261} carriers it was high and significantly different from that seen with other GCK mutations (11). Glucokinase is required for glycogen synthesis in liver (12). One explanation for the high 2-h glucose in GCK_{261} carriers could be reduced hepatic glycogen synthesis due to the lost of activity of GCK_{261}. Hence, the marked insulin response could be due to larger β-cell compensation in GCK_{261} carriers. Nonetheless, possible additional genetic defects could be also involved.

In summary, the clinical phenotype of patients with GCK-MODY can be heterogeneous and patients carrying severe inactivating GCK mutations can have high postchallenge glucose values, possibly resulting from a marked liver component of the disease.

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