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

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Objective: To evaluate the heterogeneity in the clinical expression in a family with GCK-MODY.

Research design and methods: Members (three generations) of the same family presented either with overt neonatal hyperglycemia, marked postprandial hyperglycemia or glucosuria. HOMAIR, insulinogenic and disposition indices were calculated. OGTT results in the glucokinase(GCK)-mutation carriers from this 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 glucokinase gene. During OGTT, glucose (p=0.02) and insulin (p=0.009) response at 2-hours as well as 2h glucose increment (GCK_{261} vs. 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.
Inactivating heterozygous mutations in the glucokinase gene (GCK) cause a form of monogenic diabetes with autosomal dominant inheritance (GCK-MODY) (1,2). GCK-MODY has generally been considered a phenotypically homogenous mild form of diabetes, which does not lead to marked hyperglycaemia or diabetic complications and does not need treatment (2-5).

Phenotypic heterogeneity within carriers of the same GCK mutation has been observed only in one family (6). Here we report a new GCK-MODY (GCK-G261R) family characterised by marked prandial hyperglycaemia, and unusual high levels of postprandial insulinemia.

RESEARCH DESIGN AND METHODS

The proband (Online Appendix Figure A1, which is available at http://care.diabetesjournals.org) was a firstborn child from a Finish family with neonatal plasma glucose (PG) of 10 mmol/l. At two 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 HbA1c of 5.8 % without insulin treatment, but due to high postprandial PG (10-11 mmol/l), rapid-acting meal-time 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 around 6-7 mmol/l, but stayed at about 10 mmol/l for nearly two weeks after intake of larger quantities of carbohydrates and returned to 6-7 mmol/l when carbohydrates were restricted. She takes nateglinide 60 mg before meals. All available family members were offered an oral glucose tolerance test (OGTT) and/or genetic testing for the mutation after genetic counselling.

OGTT (except subjects <15 years) with samples drawn at 5, 0, 30, 60, 90 and 120 min was performed to determine PG and serum insulin. Insulin resistance and β-cell function was estimated using the Homeostasis Model Assessment (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(GCK261) from the European MODY Consortium Database (EMCD) (3) were compared with that of carriers of other missense and other types of GCK mutations (insertions, deletions, etc...) (GCKm) and normoglycaemic controls from the Botnia study (Table 1). The 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 (age: 0.2-72 yrs) with abnormal fasting glucose. Fasting PG ranged from 6.0 to 7.6 mmol/l. The 2-hour PG 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-hour increment in PG (2hAPG) higher than 3 mmol/l and half higher than 6 mmol/l. Fasting insulin was 4.1-9.9 mU/l and 2h-insulin 28.8–61.9 mU/l. There was no
Clinical Heterogeneity in GCK-MODY

relationship between age and glucose or insulin concentrations.

The GCK\textsubscript{261} mutation carriers from our family, like those from the EMCD, had a significantly higher glucose and insulin response compared with GCK\textsubscript{m} carriers (Table 1). Fasting PG and insulin were similar in all groups, however, the 2h-PG and insulin and 2hAPG values were significantly higher in GCK\textsubscript{261} carriers than in GCK\textsubscript{m} carriers (Table 1). The glucose response during OGTT was higher at all time points in GCK\textsubscript{261} carriers compared also with GCK\textsubscript{m} carriers (data not shown). In 61 and 35% of GCK\textsubscript{261} carriers, 2hAPG was larger than 3 and 4.6 mmol/l respectively. HOMA\textsubscript{IR}, I/G30 and DI values were higher (not significant) in the GCK\textsubscript{261} carriers (Table 1) indicating possibly higher degree of \(\beta\)-cell compensation (Table 1).

The results from the functional studies showed that the mutations GK-G261R/E lead to a severely effected protein, with an almost negligible enzyme activity, indicating that these GK-mutants can not contribute to \(\beta\)-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 the Online Appendix, Table A1, available at http://care.diabetesjournals.org).

CONCLUSION

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 2hAPG 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 2hAPG values and age. A similar 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\textsubscript{261} carriers has previously been observed in GCK-L184P carriers (6). However, while the insulin response was attenuated in GCK-L184P carriers, in GCK\textsubscript{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 2h glucose in GKG\textsubscript{261} carriers could be reduced hepatic glycogen synthesis due to the lost of activity of GKG\textsubscript{261}. Hence, the marked insulin response could be due to larger \(\beta\)-cell compensation in GCK\textsubscript{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 post-challenge glucose values, possibly resulting from a marked liver component of the disease.

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REFERENCES
Table 1: Clinical characteristics of patients with glucokinase inactivating mutation \( GCK_{261} \) and functional studies of recombinant human wild-type and mutants' glucokinase.

<table>
<thead>
<tr>
<th></th>
<th>( GCK_{261} )</th>
<th>( P_1 )</th>
<th>Other missense</th>
<th>( P_2 )</th>
<th>Other GCK</th>
<th>( P_3 )</th>
<th>NGT</th>
<th>( P_4 )</th>
</tr>
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<tbody>
<tr>
<td>( N (M/F) )</td>
<td>23 (13/10)</td>
<td>144 (73/71)</td>
<td>82 (42/40)</td>
<td>45 (20/25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( BMI (kg/m^2) )</td>
<td>21.80 (7.0)</td>
<td>NS</td>
<td>20.00 (5.42)</td>
<td>NS</td>
<td>21.30 (5.73)</td>
<td>NS</td>
<td>23.7 (6.2)</td>
<td>NS</td>
</tr>
<tr>
<td>( Age (yrs) )</td>
<td>20.00 (27.0)</td>
<td>NS</td>
<td>19.00 (27.00)</td>
<td>NS</td>
<td>29.00 (28)</td>
<td>NS</td>
<td>41.6 (31)</td>
<td>0.001</td>
</tr>
<tr>
<td>( 0 \text{ min. PG (mmol/l)} )</td>
<td>7.00 (0.69)</td>
<td>NS</td>
<td>6.70 (0.90)</td>
<td>NS</td>
<td>6.80 (1.01)</td>
<td>0.17</td>
<td>5.0 (0.7)</td>
<td>NS</td>
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<tr>
<td>( 120 \text{ min. PG (mmol/l)} )</td>
<td>10.90 (4.13)</td>
<td>0.02</td>
<td>8.60 (2.58)</td>
<td>0.046</td>
<td>8.60 (2.98)</td>
<td>0.38</td>
<td>5.5 (1.8)</td>
<td>NS</td>
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<tr>
<td>( 2 \text{ hours } \Delta \text{ PG (mmol/l)} )</td>
<td>4.12 (3.25)</td>
<td>0.003</td>
<td>2.00 (2.05)</td>
<td>0.046</td>
<td>2.50 (2.20)</td>
<td>0.233</td>
<td>0.6 (1.8)</td>
<td>0.004</td>
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<td>( 0 \text{ min. P-ins. (mU/l)} )</td>
<td>10.00 (7.44)</td>
<td>NS</td>
<td>8.00 (6.00)</td>
<td>NS</td>
<td>9.00 (5.00)</td>
<td>0.41</td>
<td>7.7 (7.7)</td>
<td>NS</td>
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<td>( 120 \text{ min. P-ins. (mU/l)} )</td>
<td>55.2 (28.05)</td>
<td>0.009</td>
<td>25.00 (23.50)</td>
<td>0.002</td>
<td>24.00 (13.50)</td>
<td>0.11</td>
<td>30.2 (27.4)</td>
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<td>( \text{Incremental I/G30} )</td>
<td>8.71 (7.46)</td>
<td>0.106</td>
<td>6.09 (3.74)</td>
<td>0.513</td>
<td>4.21 (5.55)</td>
<td>0.672</td>
<td>23.7 (25.4)</td>
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<td>( \text{HOMAIR} )</td>
<td>3.47 (2.31)</td>
<td>0.568</td>
<td>2.68 (2.45)</td>
<td>0.318</td>
<td>2.21 (1.92)</td>
<td>0.259</td>
<td>1.7 (1.4)</td>
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<td>( \text{Disposition Index} )</td>
<td>3.23 (2.25)</td>
<td>NS</td>
<td>2.64 (2.02)</td>
<td>NS</td>
<td>1.98 (2.18)</td>
<td>0.76</td>
<td>15.5 (13.5)</td>
<td>0.004</td>
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</table>

Upper panel: Clinical characteristics, glucose and insulin values of patients with glucokinase inactivating mutation \( GCK_{261} \), other missense \( GCK \) inactivating mutations and other types of \( GCK \) inactivating mutations (insertions, deletions, frame shifts, etc.). Data are median (interquartile range). * For insulin data: N=11 (\( GCK_{261} \)), 36 (other missense \( GCK \) mutations), 45 (other type of \( GCK \) mutations) and 45 (NGT controls) P1: \( GCK_{261} \) mutations vs. other missense \( GCK \) mutations; P2: \( GCK_{261} \) mutations vs. other type of \( GCK \) mutations; P3: other missense \( GCK \) mutations vs. other type of \( GCK \) mutations; P4: \( GCK_{261} \) mutations vs. controls. P<0.05 – 0.00001 are considered statistically significant. HOMAIR = fasting serum insulin * fasting serum 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. Disposition Index (DI) = Insulinogenic Index / HOMAIR. Lower panel: Results of the functional studies of GK-WT and mutants GK-G261R and GK-G261E. Data are means of the three independent analyses. AI, the activity index for the enzyme was calculated as previously described (8). Glucose \( S_{0.5} \) of GK-G261R mutations was carried out with 25 mmol/l of MgATP, and the ATP-Km measurement was performed at glucose concentration of 500 mmol/l.