β-Cell Genes and Diabetes: Quantitative and Qualitative Differences in the Pathophysiology of Hepatic Nuclear Factor-1α and Glucokinase Mutations

Ewan R. Pearson, Gilberto Velho, Penny Clark, Amanda Stride, Maggie Shepherd, Timothy M. Frayling, Michael P. Bulman, Sian Ellard, Phillipe Froguel, and Andrew T. Hattersley

Mutations in the β-cell genes encoding the glycolytic enzyme glucokinase (GCK) and the transcription factor hepatocyte nuclear factor (HNF)-1α are the most common causes of maturity-onset diabetes of the young (MODY). Studying patients with mutations in these genes gives insights into the functions of these two critical β-cell genes in humans. We studied 178 U.K. and French MODY family members, including 45 GCK mutation carriers and 40 HNF-1α mutation carriers. Homeostasis model assessment of fasting insulin and glucose showed reduced β-cell function in both GCK (48% controls, $P = 0.0001$) and HNF-1α (42% controls, $P < 0.0001$). Insulin sensitivity was similar to that in controls in the GCK subjects (93% controls, $P = 0.78$) but increased in the HNF-1α subjects (134.5% controls, $P = 0.005$). The GCK patients showed a similar phenotype between and within families with mild lifelong fasting hyperglycemia (fasting plasma glucose [FPG] 5.5–9.2 mmol/l, interquartile [IQ] range 6.6–7.4), which declined slightly with age (0.017 mmol/l per year) and rarely required pharmacological treatment (17% oral hypoglycemic agents, 4% insulin). HNF-1α patients showed far greater variation in fasting glucose both between and within families (FPG 4.1–18.5 mmol/l, IQ range 5.45–10.4), with a marked deterioration with age (0.06 mmol/l per year), and 59% of patients required treatment with tablets or insulin. Proinsulin-to-insulin ratios are increased in HNF-1α subjects (29.5%) but not in GCK (18.5%) subjects. In an oral glucose tolerance test, the 0- to 120-min glucose increment was small in GCK patients ($2.4 \pm 1.8$ mmol/l) but large in HNF-1α patients ($8.5 \pm 3.0$ mmol/l, $P < 0.0001$). This comparison shows that the clear clinical differences in these two genetic subgroups of diabetes reflect the quantitative and qualitative differences in β-cell dysfunction. The defect in GCK is a stable defect of glucose sensing, whereas the HNF-1α mutation causes a progressive defect that alters β-cell insulin secretion directly rather than the sensing of glucose. 

*Diabetes* 50 (Suppl. 1):S101–S107, 2001

Maturity-onset diabetes of the young (MODY) is caused by mutations in a small number of β-cell genes. Five genes have been identified to date. These encode the glycolytic enzyme glucokinase (GCK) and the transcription factors hepatic nuclear factor (HNF)-1α, HNF-1β, and HNF-4α and the insulin promoter factor (IPF)-1 (1). The two most common forms of MODY are due to mutations in the GCK and HNF-1α genes (2,3,3a). These mutations are heterozygous, and the failure of the nonmutated allele or the other β-cell genes to compensate indicates the critical role of the proteins encoded by these genes. Studying the pathophysiology of diabetes due to a known etiology, such as these discrete monogenic subgroups, can give important insights into the pathophysiology of type 2 diabetes.

GCK is a hexokinase-like enzyme catalyzing the phosphorylation of glucose to glucose-6-phosphate, the first step in the metabolism of glucose. It is expressed principally in pancreatic β-cells and hepatocytes, and it has the critical features of a rate-determining enzyme: there is no product inhibition and a relatively high $K_m$. The critical role of GCK as the pancreatic β-cell sensor was recognized before mutations in the gene were shown to cause diabetes (4–6). The first mutation was reported in 1992 (7), and more than 60 mutations of the GCK gene have now been described in many populations, although the majority have been found in France (1,8). Patients with GCK mutations have mild fasting hyperglycemia (5.5–9.0 mmol/l) from early childhood and probably from birth (2,9). This results principally from β-cell dysfunction (9–12), although hepatic glucose synthesis is also impaired (13). The β-cell defect has been characterized as a defect in glucose sensing, in keeping with the known function of GCK (12). Small studies have suggested that GCK-mutation
subjects have a small increment during an oral glucose tolerance test (OGTT) (14), and this is supported by the fact that despite all subjects having a fasting plasma glucose (FPG) >5.5 mmol/l, only 46% of a large French series had diabetes by World Health Organization criteria (2).

The transcription factor HNF-1α was identified as a MODY gene in 1996 (15) through a positional-cloning approach after the localization of the gene to the long arm of chromosome 12 (16). This was rapidly confirmed as a common cause of MODY in U.K. (3,3a), German (17), French (18), Danish (19), Italian (20), Finnish (21), North American (21), and Japanese (22) pedigrees. Subjects with a mutation in the HNF-1α gene usually develop diabetes in adolescence or early adulthood. The diabetes frequently requires treatment with oral agents or insulin (3,16). The mutation in the HNF-1α gene causes a defect in β-cell function (23–25). The exact β-cell defect has yet to be determined, but is not due to a defect in glucose sensing (23) and probably involves altered metabolic signaling pathways in the β-cell (26).

An elevated proinsulin-to-insulin ratio characterizes a β-cell defect in type 2 diabetes, impaired glucose tolerance, and normal glucose tolerance (27–29). There are limited studies in MODY subjects. In two small studies, proinsulin-to-insulin ratios were normal in GCK patients (30,31), and this was suggested to be a specific feature of a glucose-sensing defect (30). In a single study in patients with HNF-1α mutations, there was a proportional reduction in both total proinsulin (intact proinsulin was not measured) and insulin concentrations (24). These results would suggest that in both common types of MODY, despite a clear β-cell defect, the proinsulin-to-insulin ratio is normal.

The study of the pathophysiology of β-cell function in monogenic diabetes is a useful tool, because differences in function can be attributed to a single gene and thus more easily interpreted than in polygenic diabetes. To date there has been no direct comparison of the pathophysiology, its variation with age, the proinsulin-to-insulin ratio, or the increment to an oral glucose load in the two most common forms of MODY. The aim of this study was to use simple fasting measures on a large number of subjects with GCK or HNF-1α mutations to assess how the pathophysiology of these subtypes differ. We describe both quantitative and qualitative differences in the β-cell dysfunction in these two subgroups.

**RESEARCH DESIGN AND METHODS**

Subjects were taken from 38 families with known HNF-1α and GCK mutations from the U.K. and French MODY collections. For the initial fasting study, 174 subjects were studied, including 40 HNF-1α and 45 GCK mutation carriers; 22% of HNF-1α mutation carriers and 4% of GCK carriers were excluded because they were treated with insulin. The control subjects in each group were the non–mutation carriers within the families. Subjects with an HNF-1α mutation show much greater variation in fasting glucose both between and within families, with a more rapid deterioration in glycemia with age. Figure 1B shows FPG plotted against age in HNF-1α mutation carriers. Fasting glucose varied between 4.1 and 18.5 mmol/l (IQ range 5.45–10.4 mmol/l). There is a significant increase in FPG with age of the subject (r = 0.426, P = 0.002), at a rate of 0.06 mmol/l per year.

**β-Cell function and insulin sensitivity.** β-Cell function is reduced in both the GCK (48% controls, P < 0.001) and the HNF-1α (42% controls, P < 0.001) group (Fig. 2). The insulin sensitivity in the GCK group was not significantly different from the control group (93% controls, P = 0.784), but was significantly increased in the HNF-1α group compared with control subjects (134.5% controls, P = 0.005) (Fig. 2). This difference in insulin sensitivity between the HNF-1α and control subjects remained significant after correction for BMI, so it is not explained by the increase in BMI seen in the HNF-1α control subjects.

**Variation in β-cell function and insulin sensitivity with age.** Figure 3 shows β-cell function plotted against age in subjects with GCK mutations (Fig. 3A) and HNF-1α mutations (Fig. 3B). There was a steady decline in β-cell function in the immunometric assay (antibodies A6/3B1) (1), with time-resolved fluorescence as the detection system. The assay was calibrated against the IRP coded 84/611. The coefficient of variation between assays was <14% over the concentration range 6–102 pmol/l.

**RESULTS**

**Variation of FPG with age.** The GCK subjects showed similar degrees of fasting glycemia both within and between families. Figure 1A shows fasting glucose plotted against age in subjects with GCK mutations. There was mild fasting hyperglycemia throughout life (plasma glucose 5.5–9.2 mmol/l, interquartile (IQ) range 6.6–7.4 mmol/l). Fasting glucose deteriorates with the age of the subject (r = 0.325, P = 0.023), but the rate of deterioration is small (0.017 mmol/l per year). Subjects with an HNF-1α mutation show much greater variation in fasting glucose both between and within families, with a more rapid deterioration in glycemia with age. Figure 1B shows FPG plotted against age in HNF-1α mutation carriers. Fasting glucose varied between 4.1 and 18.5 mmol/l (IQ range 5.45–10.4 mmol/l). There is a significant increase in FPG with age of the subject (r = 0.426, P = 0.002), at a rate of 0.06 mmol/l per year.

**Clinical characteristics of GCK and HNF-1α mutation groups with their respective family control subjects**

<table>
<thead>
<tr>
<th>Glucokinase group</th>
<th>45</th>
<th>42</th>
<th>HNF-1α group</th>
<th>40</th>
<th>51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td></td>
<td></td>
<td>Mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>54</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>OHAs</td>
<td>9</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2</td>
<td>(P = 0.35)</td>
<td>23.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.9</td>
<td>(P = 0.91)</td>
<td>37.3</td>
<td>(P = 0.047)</td>
<td></td>
</tr>
</tbody>
</table>

OHAs, oral hypoglycemic agents. P values represent significance compared with control group (*P < 0.05).
GCK group with age ($r = -0.479, P = 0.01$). β-cell function declined more rapidly in the HNF-1α group ($r = -0.429, P = 0.002$). There was no significant change in insulin sensitivity with age in the HNF-1α group ($P = 0.131$), but there was a gradual increase in insulin sensitivity with age in the GCK group ($r = 0.373, P = 0.01$).

Proinsulin-to-insulin ratios. Figure 4A shows the fasting proinsulin-to-insulin ratio for the GCK and HNF-1α groups and their control groups. There was no significant increase in proinsulin-to-insulin ratio in the GCK group ($P = 0.184$), whereas in the HNF-1α group, proinsulin-to-insulin ratio was twice that of the control group ($P = 0.0004$). This increase in proinsulin-to-insulin ratio occurred before the fasting glucose level exceeded the diagnostic level for diabetes (7 mmol/l). This is shown in Fig. 4B, where the HNF-1α group is divided according to fasting glucose <7.0 mmol/l and ≥7.0 mmol/l. The <7 mmol/l group has a proinsulin-to-insulin ratio 171% of the control group ($P = 0.021$), whereas the diabetic group shows a proinsulin-to-insulin ratio 226% of the control group ($P < 0.0001$).

OGTT. The plasma glucose values during OGTT were markedly different between patients with GCK and HNF-1α mutations (Table 2). Fasting values in the two groups were similar, but despite this, there were clear differences in the 2-h values (GCK 9.0 ± 1.8 mmol/l vs. HNF-1α 15.5 ± 3.6 mmol/l, $P < 0.001$). The OGTT increment (120-min minus fasting) in the HNF-1α patients was also larger than that in the GCK patients (8.5 ± 3.0 vs. 2.4 ± 1.8 mmol/l, $P < 0.0001$). In this series, an increment of <4 mmol/l was 90.9% sensitive and 97.7% specific for a GCK mutation.

DISCUSSION

Mutations in the GCK and HNF-1α genes cause primary β-cell dysfunction. Using simple fasting and OGTT assessment, this study shows both a quantitative and a qualitative difference in the pathophysiology of these two subtypes of MODY. Use of a simple approach rather than more sophisticated methodology (e.g., glucose clamps and graded glucose infusions) has some advantages, including easy replication in clinical practice and the ability to study large numbers of subjects across a range of ages. However, the use of HOMA in determining β-cell function and insulin sensitivity may not be valid across the whole range of glycemia. Care must be taken when comparing HOMA values between diabetic and nondiabetic subjects, although recent comparative trials have shown it offers good discrimination compared with the insulin tolerance test and the frequently sampled intravenous glucose tolerance test (36,37).
Quantitative differences in β-cell function. In keeping with previous reports, β-cell dysfunction is the primary defect in both HNF-1α (23–25) and GCK (9–12) function. In both genetic subgroups, there is an increase in FPG with increasing subject age. This age-related increase is far more marked in the HNF-1α subjects. The deterioration of fasting glucose with age is a result of faster deteriorating β-cell function with the HNF-1α than with the GCK mutation. The deterioration in glycemia and β-cell function in HNF-1α subjects is probably an underestimate, since 22% of subjects were excluded because of insulin treatment, which would principally be reserved for patients with marked hyperglycemia as a result of poor β-cell function. β-Cell function has previously been shown to deteriorate with age in a single U.K. GCK family (9). A large cross-sectional study has not been reported in HNF-1α subjects; however, reduction in β-cell function was shown when comparing diabetic and nondiabetic subjects with HNF-1α mutations (23–25).

Quantitative differences in insulin sensitivity. Interestingly, the HNF-1α mutation carriers were significantly more insulin sensitive than control subjects. This was not entirely explained by their lower BMI, and the mechanism is uncertain. There have been some reports in the literature that might support this finding. There was an increase in insulin sensitivity in HNF-1α mutation carriers compared with control subjects, as determined by fasting insulin levels with similar fasting glucose, and a nonsignificant increase in insulin-stimulated glucose metabolism (24); although in a French study using euglycemic-hyperinsulinemic clamps, there was a nonsignificant trend of lower insulin sensitivity in diabetic but not nondiabetic mutation carriers (25). In addition, recent case reports (38,39) demonstrate marked sensitivity to the hypoglycemic effect of sulfonylureas in HNF-1α subjects, which could be explained, at least in part, by increased insulin sensitivity. Despite the marked hyperglycemia, there was no evidence that HNF-1α mutation carriers became more insulin resistant with age. The GCK subjects had a slight but nonsignificant reduction in insulin sensitivity compared with control subjects. Clement et al. (40) have previously shown, in a larger GCK cohort, that mutation carriers with diabetes were more insulin resistant than sub-

### Table 2

Fasting plasma glucose values during a 75-g OGTT in patients with GCK and HNF-1α mutations

<table>
<thead>
<tr>
<th></th>
<th>Glucokinase</th>
<th>HNF-1α</th>
<th>P (GCK vs. HNF-1α)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests (subjects)</td>
<td>21 (17)</td>
<td>11 (11)</td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>6.6 ± 0.6</td>
<td>6.8 ± 1.9</td>
<td>0.76</td>
</tr>
<tr>
<td>120 min</td>
<td>9.0 ± 1.8</td>
<td>15.5 ± 3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Increment (120–0 min)</td>
<td>2.4 ± 1.8</td>
<td>8.5 ± 3.0</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means ± SD. Comparisons were made using an unpaired Student’s t test.
jects with a normal or impaired glucose tolerance based on the 2-h glucose value.

**Quantitative differences in β-cell function: proinsulin-to-insulin ratio.** In HNF-1α subjects, the proinsulin-to-insulin ratio is markedly increased compared with control subjects—in contrast to GCK subjects, in whom it is normal. This difference is not explained purely in terms of degree of glycemia, because HNF-1α subjects have an increased proinsulin-to-insulin ratio when fasting blood glucose is <7 mmol/l. Our findings do not support the results of Lehto et al. (24), who reported a proportionate decrease in insulin and total proinsulin levels in diabetic and non-diabetic mutation carriers when compared with non-mutation carriers in the same families. One possible explanation is in the assays used: Lehto et al. (24) used radioimmunoassay to measure insulin and total proinsulin (both will have considerable cross-reaction with split proinsulin); in contrast, our immunoassays were specific for intact insulin and intact proinsulin without significant cross-reaction with split proinsulin. Our results in GCK subjects are in line with those of previous smaller studies (30,31). In type 2 diabetes, there is disproportionate hyperproinsulinemia, with an increased proinsulin-to-insulin ratio. We have previously shown that even at the levels of glycemia and obesity seen in GCK subjects, the proinsulin-to-insulin ratio is increased in type 2 diabetes (30). The mechanism for the increase in proinsulin-to-insulin in type 2 diabetes is not clearly understood. There is increasing evidence in nondiabetic (29), impaired glucose tolerant (29), and diabetic (29) subjects that a raised proinsulin-to-insulin ratio is a feature of β-cell stress (i.e., functional insufficiency) rather than insulin resistance. We propose that the raised proinsulin-to-insulin ratio in HNF-1α reflects a similar β-cell stress. However, at present, we have not performed sufficient studies to exclude the possibility that hepatic uptake of insulin could be enhanced and/or renal excretion of proinsulin diminished. Our contrasting observations in two genetic subgroups with a similar degree of β-cell dysfunction suggest that hyperproinsulinemia is a feature of some but not all types of β-cell dysfunction.

We have previously proposed (30) that normal proinsulin-to-insulin ratio in the presence of β-cell defect may be a feature of the glucose-sensing defect found in GCK patients (12). If GCK deficiency is primarily an abnormality in glucose sensing, then hyperglycemia would not result in chronic hyperstimulation of the β-cell. This is in contrast to what is seen in the majority of type 2 diabetic patients, where the defect is not in the sensing of glucose by the β-cell. In HNF-1α mutation subjects, raised proinsulin-to-insulin ratio is a feature of an intrinsic β-cell defect that does not result from impaired glucose sensing but rather from reduced insulin secretion of insulin in response to hyperglycemia (23). The exact intrinsic β-cell defect(s) caused by HNF-1α mutations is not known. HNF-1α deficient mice have been shown to have decreased flux through the glycolytic pathway and therefore decreased ATP production in response to glucose (41). A primary defect in metabolism, hence before the sulfonylurea receptor in β-cell stimulus-secretion coupling, could also explain the sensitivity of these subjects to the hypoglycemic effect of sulfonylurea therapy (19,38,39).

**Qualitative differences in β-cell function: response to an oral glucose load.** Our results show a striking difference in the response to an oral glucose load for the two genetic subgroups. HNF-1α mutation carriers have a greatly elevated 2-h glucose value and increment in OGTT compared with GCK subjects with a similar fasting glucose. This difference is seen despite their having a quantitatively similar β-cell defect, as measured by HOMA, and less insulin resistance. This suggests that our previous hypothesis that an elevated fasting glucose but small increment on OGTT was a general feature of β-cell dysfunction (14) is incorrect. We now suggest that a small increment in OGTT is a specific feature of β-cell dysfunction due to GCK mutations. This can be used to detect gestational diabetic subjects with GCK mutations (32). The relatively normal 2-h value in GCK subjects probably represents the preservation of first-phase insulin secretion to a bolus glucose load (11). Because GCK deficiency is a glucose-sensing disorder, the maximum insulin secretion to a bolus is not reduced, so a defect in first-phase insulin is only seen when a slow infusion rather than a bolus is administered (42). In contrast, in HNF-1α mutations, maximal insulin secretion is markedly impaired (23), so an oral bolus of glucose results in both reduced first-phase and reduced second-phase insulin secretion, glucose persistently rising throughout the OGTT. It is therefore likely that a small increment from an elevated fasting glucose level is, like a normal proinsulin-to-insulin ratio, a feature of a glucose-sensing defect.

**Clinical implications.** There are clear clinical implications that arise from the differences we have shown in the pathophysiology of these two subgroups. GCK mutations cause mild hyperglycemia with very little progression. The fact that the β-cell glucose-sensing defect is present in the fetal β-cell (43) would suggest that hyperglycemia is probably present from birth. Diet, as indicated by the OGTT results and the lack of impact of obesity (9), has little effect on glycemia because the defect is one of glucose sensing. Treatment with oral hypoglycemic agents or insulin is rarely needed. In keeping with the mild hyperglycemia found in these patients, diabetic complications, particularly microvascular ones, are rare and pharmacological treatment is rarely needed (2,8–10).

In contrast, subjects with HNF-1α mutations are probably born with normal glucose tolerance but have a more rapidly progressive deterioration in β-cell function. Diagnosis of diabetes is usually made in adolescence or young adulthood with the development of symptoms (44). The HNF-1α defect causes impaired β-cell response to simple carbohydrate (as shown by the response to an OGTT); therefore, diet can have considerable effect on glycemic control. Lehto et al. (24) showed that glucose-tolerant mutation carriers were slimmer than diabetic carriers, demonstrating that obesity may considerably alter glycemic control. Treatment with oral hypoglycemic agents or insulin is often needed, especially in middle and old age (3a), but the insulin sensitivity of these subjects (and possible β-cell responsiveness to sulfonylureas [19]) puts them at higher risk of hypoglycemia (38,39) than subjects with type 2 diabetes.

In conclusion, this comparison shows that the clear clinical differences in these two genetic subgroups of diabetes reflect quantitative and qualitative differences in β-cell dysfunction. The defect in GCK is a relatively stable defect of glucose sensing, whereas the HNF-1α mutation causes a progressive defect that alters insulin secretion directly rather than altering the sensing of glucose.
REFERENCES


34. Harris MP, Levy JC, Morris RJ, Turner RC: Comparison of tests of beta-cell function across a range of glucose tolerance from normal to diabetes.