Brief Genetics Report
Cosegregation of MIDD and MODY in a Pedigree
Functional and Clinical Consequences
Camilla Cervin,1 Brita Liljestrom,2,3,4 Tiinamaija Tuomi,2,4 Seija Heikkinen,2,4 Juha S. Tapanainen,5 Leif Groop,1 and Corrado M. Cilio1,6

The aim of this study was characterization of a family carrying two mutations known to cause monogenic forms of diabetes, the M626K mutation in the HNF1α gene (MODY3) and the A3243G in mtDNA. β-Cell function and insulin sensitivity were assessed with the Botnia clamp. Heteroplasmy of the A3243G mutation and variants in type 2 diabetes susceptibility genes were determined, and transcriptional activity, DNA binding, and subcellular localization of mutated HNF1α were studied. Thirteen family members carried the mutation in mtDNA; 6 of them also had the M626K mutation, whereas none had only the M626K mutation. The protective Ala12 allele in peroxisome proliferator-activated receptor (PPARγ) was present in two non-diabetic individuals. Carriers of both mtDNA and HNF1α mutations showed an earlier age at onset of diabetes than carriers of only the mtDNA mutation (median 22 vs. 45 years) but no clear difference in β-cell function or insulin sensitivity. In vitro, the M626K mutation caused a 53% decrease in transcriptional activity in HeLa cells. The mutated protein showed normal nuclear targeting but increased DNA binding. These data demonstrate that several genetic factors might contribute to diabetes risk, even in families with mtDNA and HNF1α mutations. Diabetes 53:1894–1899, 2004

Over the last decade, the molecular causes of several monogenic forms of diabetes have been described, including maturity-onset diabetes of the young (MODY) and maternally inherited diabetes and deafness (MIDD) (1). Both MODY and MIDD lead to impaired insulin secretion, which, especially in the case of MODY, causes diabetes at a young age (2). MODY is characterized by autosomal-dominant inheritance, whereas MIDD is maternally transmitted.

In Finland, the most common form of monogenic diabetes is MODY3, which results from mutations in the hepatocyte nuclear factor (HNF)-1α gene (3). HNF1α is a transcription factor expressed in several tissues, including liver, kidney, pancreas, and gut (4–6). The protein regulates a number of liver-specific genes and genes involved in glucose metabolism (e.g., GLUT2, α-type pyruvate kinase) (7,8). HNF1α is composed of three functional domains: an NH2-terminal dimerization domain, a DNA-binding domain, and a COOH-terminal transactivation domain (9). Two distinct regions within the transactivation domain are required for a high level of transcription, AD I (from amino acid 547 to 628) and AD II (from 281 to 318) (10). MODY3 is characterized by postprandial hyperglycemia, low urine threshold for glucose, and absence of macrovascular complications (11). The A3243G mutation in the mitochondrial tRNA-Leu(UUR) gene associated with MIDD is also associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) in some pedigrees (12). Differences in heteroplasmy, the fraction of the total mitochondrial DNA (mtDNA) carrying the mutation, in different tissues could explain part of the phenotypic variation (13). Common clinical features of MIDD are diabetes neurosensory hearing loss, a normal or low BMI, short stature, and presence of macular dystrophy. High prevalence of neuromuscular and psychiatric disturbances has also been reported (14). These defects have been ascribed to mitochondrial defects in oxidative phosphorylation (15).

The aim of this study was to investigate the pathophysiology of diabetes in a family carrying a mutation in both the ADI region of HNF1α (M626K) and the A3243G mutation in mtDNA.

Thirteen family members had detectable amounts of the mtDNA mutation (individuals I:1 and I:3–III:1) (Fig. 1); 6 of them carried both the mtDNA mutation and the HNF1α mutation (I:1, I:4, II:3, II:4, II:5, and II:6). The family shows a clear maternal transmission of the 3243 mutation in mtDNA. Although one woman (I:2) did not have detectable amounts of the mtDNA mutation in her peripheral blood lymphocytes (PBLs), we cannot exclude that she carried small amounts of the mutation because heteroplasmy levels have been shown to decrease faster in PBLs than in...
other tissues upon aging (16). In β-cells, this decline is thought to enhance cell death and thereby contribute to the reduction in insulin secretion (17,18). Heteroplasmy correlated strongly with age at onset of diabetes in our pedigree (r = 0.9, n = 7; P = 0.02).

Three of the six patients with both mutations showed a very low degree of heteroplasmy (5% or less); two of them were healthy at the age of 44–48 years and the third had onset of diabetes at age 58 years. The other three carriers of both mutations had a higher degree of heteroplasmy (median 42%) and an earlier age at onset of diabetes (median 22 years). It is unlikely that the degree of heteroplasmy per se would determine the development of diabetes, as three nondiabetic family members with only the mtDNA mutation had a similar degree of heteroplasmy. The finding that among insulin-treated diabetic individuals, those with only the mtDNA mutation developed diabetes later than those with both mutations (45 vs. 22 years, P = 0.05) suggests that the effect of the two mutations might be additive. Caution is, however, warranted in this interpretation given the small number of subjects available. Unfortunately, none of the family members had only the HNF1α mutation, so we could not evaluate its separate effect. Individual III:7 showed marked sensory neural symptoms despite a low degree of heteroplasmy. This could be explained by a difference in the degree of heteroplasmy in PBLs and the neural tissue. Alternatively, the symptoms might solely be due to asphyxia during delivery (emergency cesarean section) and not MIDD.

Variants in the genes encoding peroxisome proliferator-activated receptor PPARγ (P12A), Calpain10 (SNP43/44), and Kir6.2 (E23K) were genotyped to study whether they potentially would modify the phenotype. It is of interest that the protective Ala allele at codon 12 of the PPARγ gene was observed in two of the nondiabetic carriers of the mtDNA mutation. The risk allele Lys at codon 23 of Kir6.2 was found in 3 of 14 family members, of whom 1 was nondiabetic. The GG risk genotype of SNP 43 was seen in 5 of the 14 family members, and 4 of them had diabetes. The C allele of SNP 44 was seen in 13 family members, 7 of them with diabetes. Family member I:2 neither carried the HNF1α mutation nor had detectable amounts of the mtDNA mutation, but she developed diabetes at the age of 60 years. However, she carried risk variants in two type 2 diabetes susceptibility genes, PPARγ and Kir6.2, in addition to being obese. Compared with the healthy control subjects, she had relatively normal glucose uptake and insulin response to intravenous glucose (Table 1).

In general, the carriers (n = 6) of both mtDNA and HNF1α mutations tended to have higher fasting insulin concentrations but lower first-phase insulin response and lower peak insulin levels during an intravenous glucose tolerance test than those carrying only the mtDNA mutation (n = 8). This could indicate a decreased ability to upregulate their insulin secretion in response to glucose. In support of this, individuals with both mutations tended to have a lower C-peptide response to glucagon (serum C-peptide at 6 min = 0.66, 0.10, and 0.59 nmol/l in individuals I:4, II:6, and II:5, respectively) than those with

FIG. 1. Pedigree of the family with mutations in mtDNA and HNF1α gene showing data for sex (males in squares, females in circles), presence of diabetes (filled symbols for diabetes, open symbols for normal glucose tolerance, and question mark for glucose tolerance unknown). Age at onset of diabetes and genotypes (Mt+ indicates a carrier of the mtDNA mutation, Mt- indicates a noncarrier of the mtDNA mutation, HNF-m indicates a M626K/HNF1α mutation carrier, and HNF-wt indicates a noncarrier of the HNF1α mutation) are shown underneath the symbol. In addition, the following subjects also had other signs and symptoms: I:3, hearing loss, hypertension, and coronary heart disease; I:4, short stature, hearing loss, hypertension, cardiomyopathy and mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS); I:5, myocardial infarction and asthma; I:6, myocardial infarction; I:7, hypertension; II:1; glaucoma; II:2, hypertension and asthma; II:3, sporadic headache; II:4, short stature, hearing loss, sporadic headache, impaired cognitive function, and epilepsy; II:6, short stature, hearing loss, mild mental retardation, pigment epithelium degeneration, facial palsy, and muscle spasticity; and III:7, severe mental retardation.
<table>
<thead>
<tr>
<th>Individual no.</th>
<th>I:2</th>
<th>I:6</th>
<th>I:7</th>
<th>I:3</th>
<th>I:5</th>
<th>II:1</th>
<th>II:2</th>
<th>III:1</th>
<th>I:4</th>
<th>II:6</th>
<th>I:5</th>
<th>I:1</th>
<th>II:4</th>
<th>II:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
</tr>
<tr>
<td>HNF1α-M626K</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
<td>MM</td>
</tr>
<tr>
<td>Heteroplasmy (%)</td>
<td>5</td>
<td>17</td>
<td>13</td>
<td>16</td>
<td>19</td>
<td>28</td>
<td>34</td>
<td>8</td>
<td>19</td>
<td>42</td>
<td>45</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>5</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>60</td>
<td>43</td>
<td>45</td>
<td>48</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>21</td>
<td>22</td>
<td>24</td>
<td>58</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age at investigation (years)</td>
<td>65</td>
<td>51</td>
<td>52</td>
<td>61</td>
<td>61</td>
<td>46</td>
<td>41</td>
<td>17</td>
<td>56</td>
<td>30</td>
<td>26</td>
<td>65</td>
<td>44</td>
<td>48</td>
</tr>
<tr>
<td>Treatment</td>
<td>Diet</td>
<td>Insulin</td>
<td>Oral + insulin</td>
<td>Insulin</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Insulin</td>
<td>Insulin</td>
<td>Insulin</td>
<td>Insulin</td>
<td>Diet</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31</td>
<td>27</td>
<td>28</td>
<td>22</td>
<td>29</td>
<td>20</td>
<td>20</td>
<td>16</td>
<td>21</td>
<td>23</td>
<td>19</td>
<td>31</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>6.0</td>
<td>10</td>
<td>6.2</td>
<td>6.6</td>
<td>6.9</td>
<td>5.7</td>
<td>5.4</td>
<td>—</td>
<td>7.6</td>
<td>8.8</td>
<td>7.9</td>
<td>7.0</td>
<td>5.1</td>
<td>5.5</td>
</tr>
<tr>
<td>Fasting serum insulin (mU/l)</td>
<td>8.9</td>
<td>22</td>
<td>12</td>
<td>15</td>
<td>6.8</td>
<td>7.3</td>
<td>33</td>
<td>26</td>
<td>9.6</td>
<td>13</td>
<td>8.4</td>
<td>30</td>
<td>6.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Glucose uptake (mg · kg lean body mass⁻¹ · min⁻¹)</td>
<td>6.9</td>
<td>2.3</td>
<td>—</td>
<td>4.1</td>
<td>4.2</td>
<td>14</td>
<td>8.1</td>
<td>4.1</td>
<td>—</td>
<td>3.6</td>
<td>—</td>
<td>11</td>
<td>22</td>
<td>10 ± 2.7</td>
</tr>
<tr>
<td>CGO (mg · kg lean body mass⁻¹ · min⁻¹)</td>
<td>2.8</td>
<td>1.2</td>
<td>—</td>
<td>2.6</td>
<td>0.5</td>
<td>4.6</td>
<td>3.9</td>
<td>—</td>
<td>—</td>
<td>2.2</td>
<td>—</td>
<td>3.6</td>
<td>6.2</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>CLO (mg · kg lean body mass⁻¹ · min⁻¹)</td>
<td>0.3</td>
<td>1.7</td>
<td>—</td>
<td>0.5</td>
<td>1.6</td>
<td>0.5</td>
<td>0.5</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>0.6</td>
<td>0.3</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>Glucose storage (mg · kg lean body mass⁻¹ · min⁻¹)</td>
<td>4.0</td>
<td>1.1</td>
<td>—</td>
<td>1.5</td>
<td>3.7</td>
<td>9.2</td>
<td>4.1</td>
<td>—</td>
<td>1.4</td>
<td>—</td>
<td>—</td>
<td>7.3</td>
<td>16</td>
<td>7.6 ± 2.6</td>
</tr>
<tr>
<td>FPIR (µU²/ml²)</td>
<td>303</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>413</td>
<td>451</td>
<td>302</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>275</td>
<td>104</td>
<td>461 ± 338</td>
</tr>
<tr>
<td>Peak insulin (mU/l)</td>
<td>70</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>83</td>
<td>98</td>
<td>75</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>62</td>
<td>22</td>
<td>99 ± 60</td>
</tr>
<tr>
<td>Disposition index (glucose uptake/FPIR)</td>
<td>0.02</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>—</td>
<td>0.4</td>
<td>0.21</td>
<td>0.05 ± 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting serum C-peptide (nmol/l)</td>
<td>0.86</td>
<td>1.0</td>
<td>0.51</td>
<td>0.46</td>
<td>1.4</td>
<td>0.57</td>
<td>0.51</td>
<td>—</td>
<td>0.37</td>
<td>0.06</td>
<td>0.58</td>
<td>0.44</td>
<td>0.43</td>
<td>0.39 ± 0.13</td>
</tr>
<tr>
<td>Serum C-peptide (6 min) (nmol/l)</td>
<td>—</td>
<td>—</td>
<td>0.80</td>
<td>0.80</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.66</td>
<td>0.10</td>
<td>0.59</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ΔSerum C-peptide</td>
<td>—</td>
<td>—</td>
<td>0.29</td>
<td>0.34</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.29</td>
<td>0.04</td>
<td>0.01</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Allele data:
- Kir6.2-E23K
- PPAR-P12A
- Calpain-SNP43
- Calpain-SNP44

Serum C-peptide (6 min) is the concentration 6 min after an intravenous injection of 0.5 mg glucagon. Δserum C-peptide is calculated as (serum C-peptide [6 min] – fasting serum C-peptide). CGO, clamp glucose oxidation; CLO, clamp lipid oxidation; FPIR, first-phase insulin response. *n = 9; †n = 2,293.
only the mtDNA mutation (0.80 and 0.80 nmol/l in individuals I:7 and I:3). Due to the small number of individuals, it is difficult to apply any statistical tests to the data.

To characterize the functional consequences of the M626K/HNF1α mutation in vitro, we studied the ability of the mutated protein to activate the human GLUT2 promoter. Transcriptional activity of the mutated HNF1α in HeLa cells (lacking endogenous HNF1α) was reduced by 53% compared with the wild type (0.23 ± 0.04 vs. 0.51 ± 0.08; P < 0.001) (Fig. 2A). On the other hand, transcriptional activity was normal when tested in MIN6 cells (Fig. 2B) that express the corresponding mouse HNF1α, suggesting that the endogenous protein can rescue the observed defect of the mutation. To directly test whether the mutation could have a dominant-negative effect on wild-type human HNF1α, increasing amounts of the M626K/HNF1α were transfected together with wild-type HNF1α in HeLa cells. No dominant-negative effect was observed.

**FIG. 2.** Transcriptional activation by wild-type (wt) and mutant HNF1α. Luciferase activity was normalized by the activity of the internal control pRL-TK and the reporter construct containing the GLUT2 promoter. The results presented are from three independent experiments (mean ± SEM). *P < 0.05. A: HeLa cells were transfected with 50 ng wild type, M626K-HNF1α/pcDNA3.1, or empty vector (neg). B: MIN6 cells were transfected with 100 ng wt, M626K-HNF1α/pcDNA3.1, or empty vector (neg). C: Wild-type HNF1α/pcDNA3.1 (50 ng) and M626K-HNF1α/pcDNA3.1 (0, 50, 100, and 200 ng) were cotransfected to examine a possible dominant-negative effect by the mutated protein. Total amount of DNA added was adjusted to 250 ng with empty vector.
FIG. 3. Electrophoretic mobility shift assay demonstrating DNA-binding ability of wild-type and mutated HNF1α to the binding sequence of the GLUT2 promoter.

The experiment was performed on full-length human HNF1α cDNA using the QuickChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). The oligonucleotide used in mutagenesis was 5'-CGCAGGATGCCAGGCGCTCTCC-3', with the mutated nucleotide underlined corresponding to the M626K substitution. Wild-type and mutant HNF1α were subcloned into a pcDNA3.1 expression vector (Invitrogen, Carlsbad, CA) and used for transfections.

Transactivation assay. HeLa cells (1.5 x 10^5) were transfected with Lipofectamine Plus Reagent (Life Technologies, Rockville, MD), with indicated amounts of wild-type or mutant HNF1α-pcDNA3.1 together with 0.5 or 2 µg GLUT2/pGL3 basic luciferase vector (Promega, Madison, WI) and 25 or 100 ng pGL-3TK internal control vector (Promega), respectively. The transcriptional activity was measured after 24 h (HeLa) or 48 h (MIN6) using the Dual Luciferase Assay System (Promega) on a Victor3 1420 Multilabel counter (PerkinElmer, Waltham, MA).

Electrophoretic mobility shift assay. HeLa cells were transfected with 5 µg wild-type or mutant HNF1α-pcDNA3.1. Blotting was performed as described (24) using anti-HNF1α (Santa Cruz Biotechnology) and horseradish peroxidase conjugate anti-goat IgG (Santa Cruz Biotechnology).

Coimmunoprecipitation of HNF1α and PPARγ. HeLa cells (1.5 x 10^5) or MIN6 cells (3 x 10^5) were transfected with Lipofectamine (Life Technologies, Rockville, MD), with indicated amounts of wild-type or mutant HNF1α-pcDNA3.1 together with 0.5 or 2 µg GLUT2/pGL3 basic luciferase vector (Promega, Madison, WI) and 25 or 100 ng pGL-3TK internal control vector (Promega), respectively. The transcriptional activity was measured after 24 h (HeLa) or 48 h (MIN6) using the Dual Luciferase Assay System (Promega) on a Victor3 1420 Multilabel counter (PerkinElmer, Waltham, MA).

Acknowledgments. This work was supported in Sweden by the Swedish Research Council (31/10-8858), Juvenile Diabetes Research Foundation—Wallenberg (JD-12812), the Novo Nordisk Foundation, the Swedish Diabetes Research Foundation, the Albert Påhlsson Foundation, and the European Community (BM4-CT95-0662). Support from Finland was from the Sigrid Juselius Foundation, the Academy of Finland, the Folkhalsan Research Foundation, the Finnish Diabetes Research Found-
and the Helsinki University Central Hospital. C.M.C. is supported by the Juvenile Diabetes Research Foundation. This study is part of the Molecular Medicine Research Program of Helsinki University.

We thank Dr. Graeme J. Bell for sharing hHNF1α/pcDNA and Dr. Jun Takeda for the GLUT2prom/pGl3-basic vector. We are indebted to the members of the affected family for their participation and to Dr. Carol Forsblom and the local health care personnel for their skillful support.

REFERENCES


