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# Genetic epidemiology of MODY in the Czech republic: new mutations in the MODY genes *HNF-4α*, *GCK* and *HNF-1α*

**S. Pruhova[1], J. Ek[2], J. Lebl[1], Z. Sumnik[3], F. Saudek4, M. Andel[5], O. Pedersen[2], T. Hansen[2]**

[1] Department of Paediatrics, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic
[2] Steno Diabetes Centre and Hagedorn Research Institute, Gentofte, Copenhagen, Denmark
[3] 2nd Department of Paediatrics, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic
[4] Diabetes Centre, Institute of Clinical and Experimental Medicine, Prague, Czech Republic
[5] 2nd Department of Internal Medicine, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic

## Abstract

*Aims/hypothesis.* The aim of this study was to examine the prevalence and nature of mutations in HNF4α/MODY1, GCK/MODY2 and HNF-1α/MODY3 genes in Czech subjects with clinical diagnosis of MODY.
*Methods.* We studied 61 unrelated index probands of Czech origin (28 males, 33 females) with a clinical diagnosis of MODY and 202 family members. The mean age of probands was 22.7±12.0 years (range, 6–62) and the mean age at the first recognition of hyperglycaemia was 14.7±6.0 years (range, 1–25). The promotor and coding regions inclusive intron exon boundaries of the *HNF-4α*, *GCK* and *HNF-1α* genes were examined by PCR-dHPLC (*HNF-1α* and *GCK*) and direct sequencing.
*Results.* We identified 20 different mutations in the *HNF-4α*, *GCK* and *HNF-1α* in 29 families (48% of all families studied), giving a relative prevalence of 5% of MODY1, 31% of MODY2 and 11.5% of MODY3 among the Czech kindred with MODY. Three of 3, 10 of 11 and 1 of 6 of the mutations identified in *HNF-4α*, *GCK* and *HNF-1α* respectively, were new.
*Conclusion/interpretation.* Of the families 48% carried mutations in the MODY1–3 genes and of the identified mutations 70% were new. In 52% of Czech families with clinical characteristics of MODY, no mutations were found in the analysed genes. This finding shows that the majority of MODY mutations in a central European population are local and that other MODY genes could be responsible for autosomal dominant transmission of diabetes mellitus. [Diabetologia (2003) 46:291–295]

**Keywords** MODY, genetics, mutation, glucokinase, HNF-1α, HNF-4α, MODY X.

Maturity Onset Diabetes of the Young is a genetically heterogeneous form of diabetes mellitus, characterised by an autosomal dominant inheritance, by early age at onset and by a primary defect in beta-cell function. Six known MODY subtypes are caused by mutations in genes encoding the hepatocyte nuclear factor-4α (*HNF-4α*), glucokinase (*GCK*), hepatocyte nuclear factor-1α (*HNF-1α*), insulin promoter factor-1 (*IPF-1*), hepatocyte nuclear factor-1β (*HNF-1β*) and NeuroD1 respectively [1].

The relative prevalence of distinct MODY subtypes differs substantially in studies in various populations [2, 3, 4], mutations in *GCK* representing from 8 to 63% and *HNF-1α* mutations for 13 to 64% of all subjects with MODY [5]. Mutations in the *HNF-4α, IPF-1, HNF-1β* and *NeuroD1* have been recognised in single families only, while additional unknown MODY genes ("MODY X") may cause between 16 and 45% of cases of MODY [3].

We initiated a study of genetic epidemiology of MODY in the Czech republic, as no data on the rela-

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*Corresponding author:* J. Lebl, Department of Paediatrics, 3rd Faculty of Medicine, Charles University, Vinohradska 159, 100 81 Prague 10, Czech Republic
E-mail: lebl@fnkv.cz
*Abbreviations:* GCK, Glucokinase; HNF-1α, hepatocyte nuclear factor-1alpha; HNF-4α, hepatocyte nuclear factor-4alpha; IPF-1 insulin promoter factor-1; HNF-1β, hepatocyte nuclear factor-1beta; dHPLC, denatured high performance liquid chromatography; RFLP, restriction fragment length polymorphism; OHA, oral hypoglycaemic agents.

**Table 1.** Mutations, silent mutations, intronic variants and polymorphisms in the *HNF4α*, *GCK, HNF-1α* gene in Czech subjects with MODY

| Subject | Location | Codon/nt | Nucleotide change | Designation | Frequency |
|---|---|---|---|---|---|
| *HNF4α* gene | | | | | |
| Mutations | | | | | |
| cz 205 | Exon 4 | 125 | CGG(Arg)→TGG(Trp) | Arg125Trp[a] | |
| cz 243 | Exon 4 | 121 | GTC(Val)→ATC(Ile) | Val121Ile[a] | |
| cz 162 | Exon 7 | 244 | CGG(Arg)→CAG(Gln) | Arg244Gln[b] | |
| Polymorphisms | | | | | |
| | Exon 1c | 49 | ATG(Met)→GTG(Val) | Met49Val | A 0.73, G 0.27 |
| | Exon 2 | 58 | GCC(Ala) →GCT(Ala) | A58 C/T | C 0.98, T 0.02 |
| | Intron 2 | nt-5 | C→T | IVS2nt-5 C/T | C 0.79, T 0.21 |
| | Exon 4 | 130 | ACT(Thr)→ATT(Ile) | Thr130Ile | C 0.98, T 0.02 |
| | Exon 7 | 273 | GAT(Asp)→GAC(Asp) | A 273 T/C | T 0.99, C 0.01 |
| *GCK* gene | | | | | |
| Mutations | | | | | |
| cz050, cz054, cz196, cz245 | Exon 2 | 40 | GAG(Glu)→AAG(Lys) | Glu40Lys[a] | |
| cz063, cz180 | Exon 2 | 44 | GGC(Gly)→GAC(Asp) | Gly44Asp[a] | |
| cz086 | Exon 4 | 150 | TTC(Phe)→TTA(Leu) | Phe150Leu[a] | |
| cz013, cz066, cz118 | Exon 6 | 220 | GAG(Cys)→TAG(Stop) | Cys220Stop[a] | |
| cz015, cz112 | Exon 6 | 226 | GTG(Val)→ATG(Met) | Val226Met | Velho 1997 |
| cz097 | Exon 6 | 251 | ATG(Met)→GTG(Val) | Met251Val[a] | |
| cz098 | Exon 7 | 252 | TGC(Cys)→CGC(Arg) | Cys252Arg[a] | |
| cz168 | Exon 7 | 268 | GAG(Glu)→TAG(Stop) | Glu268Stop[a] | |
| cz042 | Exon 7 | 294 | GGT(Gly)→GAT(Asp) | Gly294Asp[a] | |
| cz114 | Exon 8 | 318 | GGG(Gly)→AGG(Arg) | Gly318Arg[a] | |
| cz056, cz225 | Exon 10 | 434 | TGC(Cys)→TAC(Tyr) | Cys434Tyr[a] | |
| Silent mutation and intronic variants: | | | | | |
| | Exon 6 | 215 | TAC(Tyr)→TAT(Tyr) | Y 215 C/T | C 0.99, T 0,01 |
| | Intron 2 | nt-8 | G→A | IVS 2nt-8 G/A | G 0.99, A 0.01 |
| | Intron 2 | nt+11 | G→A | IVS 2nt+11G/A | G 0.99, A 0.01 |
| | Intron 9 | nt-53 | del ATTCATTACC | IVS 9nt-53 del | |
| *HNF 1α* gene | | | | | |
| Mutations | | | | | |
| cz 023 | Exon 3 | 200 | CGG(Arg)→GGG(Gly) | Arg200Gly[a] | |
| cz 092 | Exon 3 | 203 | CGT(Arg)→CAT(His) | Arg203His | Awata 1998 |
| cz 060 | Exon 3 | 229 | CGA(Arg)→TGA(Stop) | Arg229Stop | Kaisaki 1997 |
| cz 040, cz 028 | Exon 4 | 272 | CGC(Arg)→CAC(His) | Arg272His | Kaisaki 1997 |
| cz 230 | Exon 4 | 288 | GGG(Gly)→TGC(Cys) | Gly288Cys[c] | |
| cz 132 | Exon 4 | 291 | | P291fsinsC | Yamagata 1996 |
| cz 082 | Exon 6 | 379 | | P379fsdelCT | Yamagata 1996 |
| Polymorphisms | | | | | |
| | Exon 1 | 17 | CTC(Leu)→CTG(Leu) | L17C/G | C 0.53, G 0.47 |
| | Exon 1 | 27 | ATC (Ile)→CTC(Leu) | I/L 27 | A 0.64, C 0.36 |
| | Exon 1 | 98 | GCC(Ala)→GTC(Val) | Ala98Val | C 0.97, T 0.03 |
| | Intron 2 | nt-23 | C→T | IVS2nt-23C/T | C 0.74, T 0.26 |
| | Exon 4 | 288 | GGG(Gly)→GGC(Gly) | G 288G/C | G 0.74, C 0.26 |
| | Exon 7 | 459 | CTG(Leu)→TTG(Leu) | L459C/T | C 0.65, T 0.35 |
| | Exon 7 | 487 | AGC(Ser)→AAC(Asn) | S/N 487 | G 0.33, A 0.67 |
| | Intron 7 | nt+7 | G→A | IVS 7nt+7G/A | G 0.35, A 0.65 |
| | Exon 8 | 515 | ACG(Thr)→ACA(Thr) | Thr515Thr | G 0.83, A 0.17 |
| | Intron 9 | nt-24 | T→C | IVS9nt-24 T/C | T 0.65, C 0.35 |

nt, indicates the nucleotide location relative to the splice donor (+) or acceptor site (−)

[a] new mutation which co-segregated with diabetes within the family and which was not found in 90 chromosomes from 45 unrelated healthy Czech Caucasian subjects

[b] new mutation not tested for co-segregation

[c] new mutation without co-segregation with diabetes within the family

tive prevalence of the different MODY subtypes and on the spectrum of mutations in MODY genes have been published from central or east European countries with a predominantly Slavonic population.

## Subjects and methods

*Subjects.* A total of 61 unrelated probands between 6 and 62 years of age (median 18; 28 males, 33 females) with a clinical diagnosis of MODY and 202 members of their families participated. Hyperglycaemia in probands was firstly recognised at age 1 to 25 years (median 15), 1 to 43 years (median 3) prior to this study.

The probands were previously diagnosed to suffer from diabetes mellitus or IFG and all had a family history of diabetes mellitus or another form of hyperglycaemia (gestational diabetes mellitus, "impaired glucose tolerance") in at least two consecutive generations. The patients were referred from paediatric endocrinologists from the whole country (38 patients) and from clinics for adults from Prague (23 patients). Informed consent was obtained from all study participants. The protocol was approved by the Ethics Committee of the 3rd Faculty of Medicine, Charles University, Prague, and was in accordance with the Helsinki declaration II.

*Clinical studies.* All patients underwent a structured assessment including detailed family history. A fasting blood sample was taken for measurements of glucose, C-peptide and glycosylated haemoglobin ($HbA_{1c}$). Retinopathy was evaluated by an ophthalmologist at the diabetes centre. Nephropathy was diagnosed by testing for microalbuminuria and proteinuria.

*Genetic analysis.* All exons, the intron-exon boundaries and the promoter regions of the *HNF-1α* and *GCK* gene were examined using dHPLC (denatured High Performance Liquid Chromatography) and direct sequencing [6]. The *HNF-4α* gene and the P1 promoter was analysed by direct sequencing using ABI PRISM Dye Primer Cycle Sequencing Kit with Amplitaq DNA Polymerase FS. The published primers were used [7] except for exon 1c, where we used primers: F 5′GCCAATTTCCAGCAAAAGTC and R 5′CTTGCCGTCTCTCTGAACCT. The PCR amplifications of exon 1c were done by using a previously described PCR protocol [7] using 1.5 mg $MgCl_2$ and an annealing temperature of 60°C.

The prevalence of variants identified as putative mutations was estimated in 45 unrelated healthy Czech Caucasian subjects by PCR-restriction fragment length polymorphism (RFPL) for variants identified in the *HNF-4α* gene and by dHPLC in the *HNF-1α* gene and the *GCK* gene, respectively. The *HNF-4α* gene variant Arg125Trp (CGG→TGG) was detected using enzyme *BsrB*1, the variant Arg244Gln (CGG→CAG) using enzyme *BsaJ*1 and for detection of the variant Val121Ile (GTC→ATC) was used *Fok*1 (New England Biolabs, Beverly, Mass., USA).

## Results

*Screening of the HNF-4α gene.* Three new mutations and five polymorphisms were identified (Table 1). The Arg125Trp and Val121Ile variants co-segregated with diabetes in five and two family members, respectively. In the case of the Arg244Gln variant, only the patient's DNA was available for investigation. None of the mutations was identified in 45 unrelated healthy Czech Caucasian subjects. Clinical features of the probands are given in Table 2.

*Screening of the GCK gene.* We identified 11 different mutations (Table 1) in 19 patients, all within the coding region of the gene. All mutations co-segregated with hyperglycaemia among affected family members with the exception of Val226Met, which was only found in the proband. However, this mutation has already been reported to be associated with MODY [8]. Clinical fea-

**Table 2.** Clinical characteristics of subjects with mutations in the *HNF-4α*, *GCK* and *HNF-1α* genes and in MODY X subjects

| | Subjects with *HNF-4α* mutations | Subjects with *GCK* mutations | Subjects with *HNF-1α* mutations | MODY X |
|---|---|---|---|---|
| Number | 3 | 19 | 7 | 32 |
| Age at diagnosis (years) | 15.0±5.3 | 11.3±4.7 | 14.8±4.9 | 16.8±5.6 |
| Age (years) | 26.3±9.5 | 16.2±4.5 | 25.0±9.1 | 26.2±14.0 |
| Sex (F/M) | 3/0 | 8/11 | 4/3 | 18/14 |
| Duration of DM (years) | 17.0±13.1 | 4.9±3.4 | 10.7±9.3 | 9.1±10.6 |
| BMI ($kg/m^2$) | 21.7±2.7 | 19.8±3.2 | 22.4±2.3 | 24.6±6.2 |
| Fasting b-glucose(mmol/l) | 10.7±4.4 | 6.9±0.8 | 6.2±1.1 | 8.9±3.8 |
| $HbA_{1c}$ | 8.8±3.2 | 6.3±0.8 | 7.0±1.2 | 7.5±2.5 |
| Total s-cholesterol(mmol/l) | 5.1±1.3 | 4.2±0.7 | 4.7±0.9 | 4.9±1.6 |
| HDL s-cholesterol(mmol/l) | 1.3±0.2 | 1.3±0.4 | 1.2±0.3 | 1.2±0.5 |
| Complications: | | | | |
| NP | 1 | 0 | 1 | 7 |
| ESRF | 0 | 0 | 1 | 3 |
| PDR | 2 | 0 | 1 | 3 |
| Current therapy (diet/OHA/insulin) | 0/2/1 | 18/0/0 | 2/1/4 | 12/7/14 |

Data are n or mean±SD (range). The values for fasting glucose and insulin are current values measured with the subjects on diabetes treatment. NP, diabetic neuropathy; ESRF, end-stage renal failure; PDR, proliferative diabetic retinopathy; OHA, oral hypoglycaemic agent

tures of subjects with *GCK* mutations are summarised in Table 2. Furthermore, we identified a silent mutation in exon 6 and a nine nucleotide deletion in intron 9 which did not co-segregate with diabetes, and two single base intron polymorphisms (Table 1).

*Screening of the HNF-1α gene.* We identified 10 polymorphisms and 5 previously described mutations located in the coding region of the gene (Table 1). One variant Arg272His was identified in two unrelated probands. In addition, one new missense mutation Arg200Gly was found. All identified variants co-segregated with diabetes (Table 2).

## Discussion

We have screened for mutations in the MODY genes *HNF-4α*, *GCK* and *HNF-1α* in Czech Caucasian families with clinically diagnosed MODY diabetes.

We identified three new mutations in the *HNF-4α* gene: Arg125Trp, Val121Ile and Arg244Gln. All amino acids altered by these missense mutations are conserved across rat, mouse, hamster and frog. The mutation Arg125Trp is located two codons upstream from the known mutation Arg127Trp in the exon 4 [9] and alters a conserved amino acid that is located in the T-box, a region of the receptor implicated in dimerization and DNA binding. The variant co-segregated with diabetes within the family suggesting that the variant Arg125Trp is a new disease-causing mutation. The variant Val121Ile was identified in a 16-year-old lean girl who was diagnosed to be mildly hyperglycaemic at 14 years and in her mother with mild Type 2 diabetes treated with OHA.

Within 19 families, we detected a total number of 11 mutations in the *GCK* gene. Ten of them are new including eight missense and two nonsense mutations. Some of the new mutations were detected in two or more apparently unrelated families: Glu40Lys (four times), Gly44Asp (twice), Cys434Tyr (twice), Cys220Stop (three times), probably reflecting Czech founder mutations. Also a previously described mutation Val226Met [8] was identified in two families. None of the mutations were found in normal chromosomes. The youngest subject with *GCK* mutation was an 8-month-old boy with fasting glycaemia ranging between 6.1 to 7.2 mmol/l. No signs of microvascular complications were found in any subject with a *GCK* mutation.

Seven patients with six different mutations and one patient with an uncharacteristic variant in the *HNF-1α* gene were found (Table 1). The new Arg200Gly mutation leads to a change in codons that are conserved in the genomes of human, chicken, mouse, rat and hamster and are therefore assumed to be of functional importance. The Arg200Gly is located in the DNA-binding domain and could affect DNA recognition and/or binding. This mutation was observed in one allele of a patient and not found in any of the 45 healthy control subjects. A new Gly288Cys mutation was found in a 14-year-old girl with mild hyperglycaemia. Detailed investigation of the family did not show co-segregation with diabetes.

The clinical features in seven probands with mutations in *HNF-1α* ranged from mild hyperglycaemia in children and youngsters to severe diabetes mellitus on insulin therapy in most subjects with a disease duration of more than 10 years. Some stage of diabetic complications was detected in 71% of patients. Among them, a 47-year-old man carrying the Arg272His mutation already developed end-stage renal failure and underwent combined renal-pancreas transplantation at the age of 45 years.

After complete screening for the *HNF-4α*, *GCK* and *HNF-1α* genes in 61 families with clinically diagnosed MODY, we identified 20 different mutations and variants in 29 families (48%) and observed a relative prevalence of 5% of MODY1, 31% of MODY2 and 11.5% of MODY3. The high relative prevalence of MODY2 compared to some other studies could reflect not only a specific genetic background but also the mode of recruitment of our study cohort, as 62% of probands were referred by paediatricians. We have not tested for mutations in the genes causing MODY4–6 as these according to previous reports are not likely to be responsible for diabetes in a substantial proportion of affected families [1].

We have identified gene mutations (70% of them new) in 48% of families with clinical characteristics of MODY. These findings show that the majority of MODY mutations in the Czech population are local and support the hypothesis that other genes might be involved in autosomal dominant transmission of diabetes mellitus.

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