Diabetologia (1998) 41: 1528–1531

Diabetologia

Short communication

Mutations in the hepatocyte nuclear factor-1α gene in Caucasian families originally classified as having Type I diabetes

A.M. Møller, L.T. Dalgaard, F. Pociot, J. Nerup, T. Hansen, O. Pedersen

Steno Diabetes Center and Hagedorn Research Institute, Gentofte, Copenhagen, Denmark

Summary Mutations in the hepatocyte nuclear factor-1α (HNF-1α) gene are the cause of maturity-onset diabetes of the young type 3 (MODY3), which is characterised by a severe impairment of insulin secretion and an early onset of the disease. Also at onset of diabetes some MODY patients show similar clinical symptoms and signs as patients with Type I (insulin-dependent) diabetes mellitus. The objective of this study was to estimate the prevalence of MODY3 patients misclassified as Type I diabetic patients. From a large population-based sample of unrelated Danish Caucasian Type I diabetic patients with an affected first degree relative, 39 patients (6.7%) who did not carry any high-risk HLA-haplotypes, i.e. DR3 or DR4 or both were examined by single-strand conformational polymorphism scanning and direct sequencing of the coding region and the minimal promoter of the HNF-1α gene. Four of the 39 Type I diabetic patients (10%) were identified as carrying mutations in the HNF-1α gene. One patient carried a missense mutation (Glu48Lys) in exon 1, two patients carried a missense mutation (Cys241Gly) in exon 4 and one patient carried a frameshift mutation (Pro291fsdelA) in exon 4. The mutations were all identified in heterozygous form, segregated with diabetes, and were not identified in 84 unrelated, healthy subjects. Furthermore, family history in three of the four families showed diabetes in four consecutive generations, suggestive of an autosomal dominant inheritance. In conclusion, about 10% of Danish diabetic patients without a high-risk HLA-haplotype, originally classified as having Type I diabetes could have diabetes caused by mutations in the HNF-1α gene. Clinical awareness of family history of diabetes and mode of inheritance might help to identify and reclassify these diabetic subjects as MODY3 patients. [Diabetologia (1998) 41: 1528–1531]

Keywords HNF-1α, Mutations, IDDM, MODY3, Misclassification, non-DR3 and non-DR4 genotypes.

Introduction

Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous form of diabetes, characterised by an autosomal dominant inheritance, early age of onset (< 25 years of age) and a primary defect in beta-cell function. Four known MODY subtypes, MODY1, MODY2, MODY3 and MODY4 are caused by mutations in genes encoding the hepatocyte nuclear factor-4α (HNF-4α), glucokinase, hepatocyte nuclear factor-1α (HNF-1α) and the insulin promoter factor-1 (IPF-1), respectively [1, 2, 3, 4]. Mutations in the hepatocyte nuclear factor-1β (HNF-1β), gene could cause an autosomal dominant form of diabetes with early onset [5]. Whereas the diabetic phenotype of MODY2 is characterised by mild elevations in fasting and postprandial blood glucose concentrations, the MODY1 and MODY3 subtypes present more severe phenotypes characterised by se-

Received: 25 May 1998 and in revised form: 13 July 1998

Corresponding author: A.M. Møller, Ms. Sci., Steno Diabetes Center, Niels Steensens Vej 2, DK-2820 Gentofte, Copenhagen, Denmark

Abbreviations: HNF-1α; hepatocyte nuclear factor-1α; HNF-4α, hepatocyte nuclear factor-4α; MODY, maturity-onset diabetes of the young; HLA, human leucocyte antigen; SSCP, single strand conformational polymorphism; PCR, polymerase chain reaction; GAD65, glutamine acid decarboxylase; IA-2, protein tyrosine phosphatase

vere fasting hyperglycaemia, frequent need of insulin treatment and frequent occurrence of microvascular complications. In the Danish Caucasian population about 50% of MODY families carry mutations in the HNF-1α gene [6].

The most important susceptibility locus for Type I (insulin-dependent) diabetes mellitus is the region encoding the human leucocyte antigen (HLA) class II alleles of which the DR3 and DR4 haplotypes show the strongest association with Type I diabetes. Because of the early onset, the severe hyperglycaemia and the frequent need for insulin treatment of patients with MODY3 we hypothesised that some diabetic patients classified as having Type I diabetes in fact could have MODY3. Consequently, we have examined a subgroup of nuclear families without the high-risk HLA haplotypes for mutations in the HNF-1α gene.

Subjects and methods

From a large population-based family sample with Type I diabetes all the HLA-DR3 or HLA-DR4 or both (all subtypes) negative, unrelated patients (6.7%; $n = 39$, 23 males and 16 females) were selected. Their mean age at onset was 12.7 years (range 3–20 years) and their mean disease duration was 19.9 years (range 2–52 years). They were all clinically diagnosed as having Type I diabetes according to the World Health Organisation criteria and required insulin from the time of diagnosis. Information on diabetic complications (retinopathy, nephropathy or neuropathy) was obtained by questionnaire at the time of examination. Eight were glumatic acid decarboxylase (GAD65) antibody positive, four were protein tyrosine phosphatase (IA-2) antibody positive and two were positive for both GAD65 and IA-2 antibodies, i.e. 36% were positive for at least one antibody.

The control subjects, 84 unrelated Danish Caucasians, randomly traced in the Danish Central Population Register were screened for mutations in the HNF-1α gene. They underwent a standard (75 g) oral glucose tolerance test (OGTT) and were normal glucose tolerant by definition of the World Health Organisation.

The minimal promoter (the 700 bp upstream from the start codon) and the 10 exons and flanking introns were examined by combined heteroduplex and single strand conformational polymorphism (SSCP) analysis at two different experimental settings as described previously [7]. Polymerase chain reaction (PCR) amplification of the mimimal promoter was performed using the following primers: 5′-tgtaaaacgacggccagtagccagcactgttcttg-3′ and 5′-caggaaacagctatgaccgtcccacccccgaaaga-3′, 5′-tgtaaaacgacggccagtgcatgatgcccctacaag-3′ and 5′-caggaaacagctatgaccgttctgttttacattggag-3′; 5′-tgtaaaacgacggccagtataaatatgaaccttggag-3′ and 5′-caggaaacagctatgaccagaaaccatggctcggc-3′; and the 10 exons and flanking introns were amplified using specific primers as described previously [7]. Variants identified by the SSCP/heteroduplex scanning were examined by direct sequencing from both ends using ABI PRISM Dye Primer Cycle Sequencing Kit with Amplitaq DNA Polymerase FS and ABI PRISM 373 (Perkin Elmer, Foster City, Calif., USA).

In the screening for specific mutations in MODY family members and control subjects the Glu48Lys mutation was detected by amplification of exon 1 followed by digestion with the restriction enzyme *Hinf*I. The Cys241Gly in exon 4 was detected by amplification of exon 4 followed by digestion with *Nsi*I. The frameshift mutation in codon 291 was detected by PCR and the size of the γ-^{33}PPCR product was evaluated by denaturing gel electrophoresis.

Haplotype analysis of the nuclear families with the Cys 241Gly mutation was done with the following markers: D12S366, D12S321, D12S807, D12S820, D12S342. These markers were all positioned within the distance of 4 cM from the HNF-1α gene. The heterozygosity of the markers was between 0.65 and 0.81.

Results

Analysis by SSCP-heteroduplex and sequencing of the 10 exons and the minimal promoter of the HNF-1α gene in 39 unrelated patients with Type I diabetes who were negative for HLA-DR3 or DR4 or both showed four patients with mutations in the HNF-1α gene. Two missense mutations in exon 1, Glu48Lys and in exon 4, Cys241Gly were identified in one and two families, respectively. A frameshift mutation in exon 4, Pro291fsdelA was identified in one family.

The Glu48Lys mutation was identified in both the diabetic proband and in her sister and father. The proband and the sister had overt diabetes and both the father and mother had impaired glucose tolerance as measured by an oral glucose tolerance test (Fig. 1). Both parents of the father had Type II diabetes diagnosed at an age of 69 (grandfather) and 55 years (grandmother), respectively. There was no known diabetes in the mother's family. The two sisters had diabetes diagnosed at the age of 4 and 5 years, respectively. No diabetic complications were reported at examination. The proband, but not the sister, had antibodies to glumatic acid decarboxylase (GAD65); none of them had IA-2 antibodies.

Two Type I diabetic probands who were assumed to be unrelated carried a Cys241Gly mutation in exon 4. This mutation segregated with diabetes in both families and the age of diagnosis was in the range of 9 to 20 years in the affected subjects (Fig. 1). Haplotype analysis using five informative genetic markers around the HNF-1α gene showed that the two nuclear families had the same at-risk haplotype segregating with diabetes. None of the examined diabetic patients carrying the mutation had GAD65 or IA-2 antibodies. Furthermore, at the time of examination none of the mutation carriers reported any diabetic complications.

In exon 4 we identified a novel frameshift mutation Pro291fsdelA that also segregated with diabetes in the family. None of the affected subjects had antibodies against GAD65 or IA-2. Diabetes was diagnosed in these subjects at the age of 9 and 12 years, respectively, and they did not report diabetic complications at the time of examination.

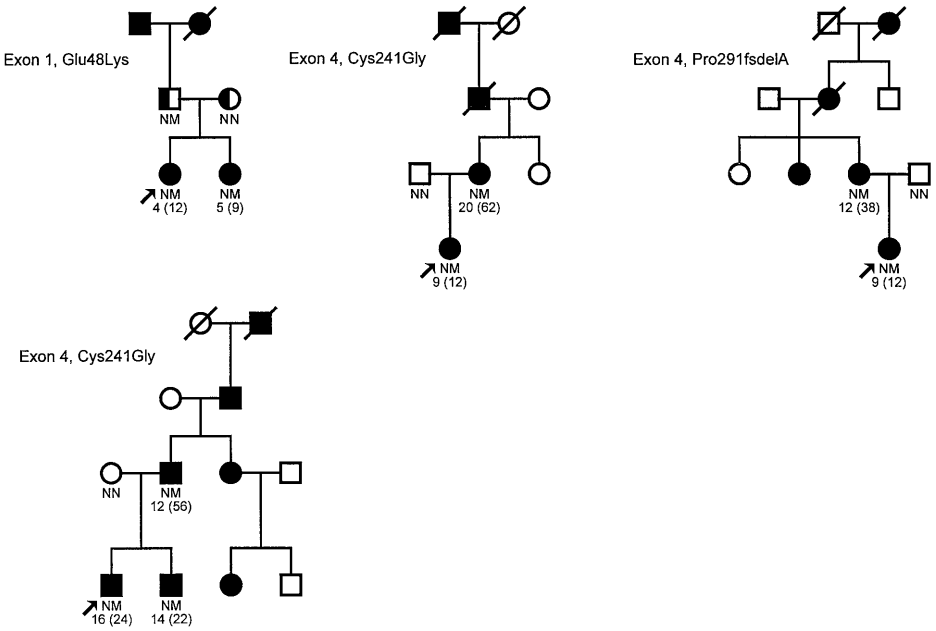

Fig. 1. MODY families with mutations in the HNF-1α gene. Patients with diabetes are indicated by black symbols, non-diabetic subjects by white symbols and subjects with impaired glucose tolerance are indicated by half-filled symbols. Males are indicated with square symbols, females with round symbols. Subjects who were deceased by the time of examinations are indicated by a line drawn through the symbol. Arrow indicate the proband from each pedigree who was examined for mutations. N and M denote normal and mutated HNF-1α alleles, respectively. Age of diagnosis (age of examination) is indicated as well

Neither the Glu48Lys, Cys241Gly nor the Pro291fsdelA mutation were identified in 84 unrelated healthy subjects.

Discussion

The Glu48Lys mutation identified in exon 1 of the HNF-1α gene results in an amino acid replacement in the N-terminal part of the encoded protein 17 amino acids downstream of the dimerisation domain. This mutation was present in two diabetic sisters of whom one had antibodies against GAD65 but not against IA-2 and the other sister had no antibodies against neither GAD65 nor IA-2. This existence of GAD65 antibodies in one of the diabetic sisters is not in opposition to diabetes being non-autoimmune since about 2% of the normal population have GAD65 antibodies [8]. Instead, the sister being diabetic without autoantibodies indicates that the diabetes in this family is perhaps caused by a common genetic defect and has possibly a primarily non-autoimmune origin. Also the father in this family carried the mutation, though he was non-diabetic at the age of 43 years, possibly due to incomplete penetrance.

The Cys241Gly mutation in exon 4 was identified in two Type I diabetic patients, who were assumed to be unrelated, and segregated with diabetes in both pedigrees. In both families four generations were known to have diabetes and in one of the families the affected grandfather of the proband had 11 siblings of which 8 were known to have had diabetes (not shown in Fig. 1), all suggestive of an autosomal dominant mode of inheritance. Haplotype analysis using five informative genetic markers around the HNF-1α gene showed that the two nuclear families had the same at-risk haplotype segregating with diabetes suggesting that the disease was inherited from a common ancestor. These two families may also be related to another Danish family (the Jutland family) which has been shown previously to have diabetes caused by the Cys241Gly mutation [9]. The Cys 241Gly mutation is positioned in a region encoding the homeobox domain which has been shown to be important in DNA binding [10].

Both the novel Glu48Lys and the Cys241Gly mutation change codons that are conserved in the genomes of human, chicken, mouse, rat and hamster and are therefore assumed to be of functional importance.

The novel frameshift mutation, Pro291fsdelA is positioned in a mutational hotspot region of the gene following an eight basepair polyC tract and is predicted to generate a truncated protein of 341 amino acids. This mutation also segregated with diabetes in the nuclear family in which it was identified and could therefore, in accordance with previously identified mutations in the HNF-1α gene, be assumed to cause MODY.

The present findings substantiate the findings in atypical Japanese Type I diabetic patients where 5.5% of the patients carried mutations in the HNF-

1α gene which segregated with diabetes in the respective families [11]. The results highlight the difficulties in distinguishing MODY3 patients treated with insulin from patients with Type I diabetes on the basis of clinical observations alone. Both subtypes of diabetes have an early onset – usually before 25 years of age – and severe hyperglycaemia. Therefore, to avoid the misclassification of MODY3 patients as Type I diabetic patients, patients with a family history of diabetes compatible with an autosomal mode of inheritance and without GAD65 or IA-2 antibodies or both could be examined for mutations in the HNF-1α gene. These findings have major implications for genetic counselling and for ongoing genetic dissection of Type I diabetes.

Acknowledgements. This study was supported by grants from the University of Copenhagen, the Velux Foundation, the Danish Diabetes Association, EEC grant (BMH4-CT-950662) and the Danish Medical Research Council. The authors thank B. Mottlau for dedicated and careful technical assistance and G. Lademann for secretarial support.

References

1. Yamagata K, Furuta H, Oda N et al. (1996) Mutations in the hepatocyte nuclear factor-4α gene in maturity-onset diabetes of the young (MODY1). Nature 384:458–460
2. Froguel P, Zouali H, Vionnet N et al. (1993) Familial hyperglycemia due to mutations in glucokinase. Definition of a subtype of diabetes mellitus. N Engl J Med 328:697–702
3. Yamagata K, Oda N, Kaisaki PJ et al. (1996) Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY3). Nature 384:455–458
4. Stoffers DA, Ferrer J, Clarke WL et al. (1996) Early-onset type-II diabetes mellitus (MODY4) linked to IPF-1. Nat Genet 17:138–139
5. Horikawa Y, Iwasaki N, Hara M et al. (1997) Mutations in the hepatocyte nuclear factor-1β gene (TCF2) associated with MODY. Nat Genet 17:384–385
6. Hansen T, Eiberg H, Rouard M et al. (1997) Novel MODY3 mutations in the hepatocyte nuclear factor-1α gene. Diabetes 46:726–730
7. Urhammer SA, Rasmussen SK, Kaisaki PJ et al. (1997) Genetic variation in the hepatocyte nuclear factor-1α gene in Danish Caucasians with late-onset NIDDM. Diabetologia 40:473–475
8. Bingley PJ, Bonifacio E, Williams AJK et al. (1997) Prediction of IDDM in general population. Diabetes 46:1701–1710
9. Kaisaki PJ, Menzel S, Lindner T et al. (1997) Mutations in the hepatocyte nuclear factor-1α gene in MODY and early-onset NIDDM. Diabetes 46:528–535
10. Frain M, Swart G, Monaci P et al. (1989) The liver-specific transcription factor LF-B1 contains a highly diverged homeobox DNA binding domain. Cell 59:145–157
11. Yamada S, Nishigori H, Onda H et al. (1997) Identification of mutations in the hepatocyte nuclear factor (HNF)-1α gene in japanese subjects with IDDM. Diabetes 46:1643–1647