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Early-onset Type II diabetes mellitus in Italian families due to mutations in the genes encoding hepatic nuclear factor 1α and glucokinase

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Abstract

Aims/hypothesis. Maturity-onset-diabetes of the young (MODY) is caused by mutations in at least five different genes. Our aim was to determine the prevalence of the most common MODY genes in Italian families with early-onset Type II (non-insulin-dependent) diabetes mellitus.
Methods. We screened 28 Italian early-onset Type II diabetic families (diagnosis < 35 years) for mutations in the hepatic nuclear factor-4α, (MODY1), glucokinase (MODY2) and hepatic nuclear factor-1α (MODY3). Both strands of exons, flanking introns and minimal promoter regions of the above-mentioned genes were amplified using polymerase chain reaction and were sequenced directly.
Results. We identified four different mutations, three of which are not described, (*W113X*, *G42P43fsCC* → *A*, *H514R*) and four new polymorphisms (*G184G*, *T513T*, *IVS3-nt47delG*, *IVS1- nt53C* → *G*) in the hepatic nuclear factor-1α gene, two new potential mutations (*G44S*, *IVS4nt + 7C* → *T*) and three new polymorphisms (*promoter-nt84C* → *G, IVS9 + nt8C* → *T*, *IVS9 + nt49G* → *A*) in the glucokinase gene, and a new polymorphism (*IVS1c-nt11T* → *G*) in the hepatic nuclear factor-4α gene.
Conclusion/interpretation. Mutations in the hepatic nuclear factor-1α and glucokinase are associated with Type II diabetes in 14 % and 7 % of Italian families, respectively. Our findings provide an impetus for screening Italian MODY and early-non Type II diabetic families for mutations in the above mentioned genes to identify relatives at risk who could benefit from primary prevention care. [Diabetologia (2001) 44: 1326–1329]

Keywords Type II diabetes mellitus, genetics, hepatocyte nuclear factor-1α, hepatocyte nuclear factor-4α, glucokinase, MODY, Italian, mutation, polymorphism.

Maturity-onset diabetes of the young (MODY), a monogenic subset of Type II (non-insulin-dependent) diabetes mellitus, is caused by mutations in at least five different genes: HNF-4α-MODY1 [1], GCK-MODY2 [2], HNF-1α-MODY3 [3], IPF-1-MODY4 [4], and HNF-1β-MODY5 [5]. MODY is an autosomal dominant disorder with an onset typically under 25 years of age, and usually accounts for 2 % to 5 % of all patients with Type II diabetes but a true estimate is challenging because patients with MODY are often wrongly diagnosed as having Type I or Type II diabetes. Late-onset Type II diabetes is a polygenic heterogeneous disorder whose pathogenesis is not clear. We regard Type II diabetes as a genetically continuous spectrum of early-onset to late-onset diabetes, partially due to mutations in the MODY genes. The prevalence of HNF-1α, GCK and HNF-4α mutations in MODY patients varies among different populations. Up to now four missense mutations in the MODY2 [6, 7] and none in the MODY3 gene have been identified in Italian MODY patients. The aim of our study

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Abbreviations: HNF, hepatocyte nuclear factor; GCK, glucokinase; IPF-1, insulin promoter factor-1; NN, normal and normal; NM, normal and mutant.

is to report the prevalence of MODY1, MODY2 and MODY3 mutations among Italian early-onset Type II diabetic families.

Subjects and methods

Subjects. A total of 28 early-onset Type II diabetic families with autosomal dominant inheritance and the probands diagnosed under the age of 35 were ascertained through Santo Spirito Hospital, Sacro Cuore University in Rome and University of Studies in Siena, Italy. Affection status was determined by National Diabetes Data Group (NDDG) criteria. Among these families, 15 fit the clinical criteria for MODY. Standard OGTT tests were done in family members who were not affected. Patients were classified according to age of onset of diabetes, treatment and complications. The C-peptide concentrations were measured 6 min after a glucagon stimulation test (1000 ng i. v.) in the affected patients carrying the gene mutation (NM). The control group consisted of 50 unrelated Italian healthy subjects. These studies were carried out in accordance with the Declaration of Helsinki (1996) and all subjects gave their informed consent.

Methods. Both strands of 10 exons, flanking intronic sequences, and minimal promoter regions of the HNF-1α gene of the 28 probands were screened for mutations [3], excluding the allelic drop-out of exon 2. Both strands of exon 1a, exons 2–10, flanking intronic sequences and the promoter of HNF-4α [1] and the GCK gene [2] were screened in all probands, excluding HNF-4α eight families with no allelic cosegregation with disease at markers *D20S424, D20S88, D20S49* and the already defined MODY3 families Italy-1, Italy-2, Italy-3, and Siena-1, and excluding GCK in four families with no allelic cosegregation with disease at markers GCK1, GCK2 and GCK3 and the already identified MODY3 families. Each identified mutation sequence was cloned into pGEM-4Z/pGEM-T Easy and clones derived from both alleles were sequenced. The proband mutation was screened in other family members to define the cosegregation of the mutation with disease. As a negative control, we screened 50 unrelated healthy Italian subjects for all mutations identified and, for the HNF-1α *H514R* mutation, we also screened 60 unrelated Italian Type II diabetic patients. As HNF-1α *W113X* and HNF-1α *G42P43fsCC* $\rightarrow$ *A154X* create restriction sites for the enzymes Afl III and Ppu MI, respectively, we checked control sequences by using these enzymes. HNF-1α *H514R*, GCK *G44S* and *GCK IVS4nt + 7C* $\rightarrow$ *T* were amplified in the control groups by PCR and direct sequencing. A heterozygous sequence change was considered a mutation when it resulted in a truncated protein, altered the reading frame or caused a replacement of a conserved amino acid. It was also considered a mutation when it cosegregated with disease within the pedigree and when it was not present in 100 normal chromosomes of unrelated Italian healthy subjects.

Results

We found four mutations in the HNF-1α gene and two potential mutations in the GCK gene (Fig. 1 and Table 1). First generation affected Italy-5 family members do not share the missense mutation; we excluded a MODY3 mutation in the first and second generation family members who have glycosuria under normoglycaemic conditions. We also excluded the possibility of non-paternity in this family by multiple genotyping and the possibility of sample switch by withdrawing blood twice from the subjects. All mutations were excluded in the above-mentioned control groups. No mutations were identified in the HNF-4α gene.

We found 14 common MODY3 polymorphisms, four new MODY3 polymorphisms, three new MODY2 polymorphisms and one new and one common MODY1 polymorphism (Table 1).

Clinical data were obtained for the NM MODY3 and MODY2 patients. The NM unaffected Italy-1 family members have higher basal insulin concentrations, lower basal blood glucose and higher basal insulin-to-glucose ratios compared to the NN Italy-1 family members, though these data do not reach statistical significance, and, as expected, impaired maximal insulin response to glucose stimulus after 60, 90, and 120 min (data not shown). The NM affected patients have low basal and glucose-stimulated insulin secretion (data not shown). The I.2 NM Italy-2 patient has a basal glucose concentration of 7.2 mmol/l, thus she has diabetes according to the new World Health Organisation (WHO) classification criteria. Italy-5 family members I.1, II.2 and II.3 have glycosuria under the normal renal threshold. The average BMI of all these NM unaffected family members is 24.5 Kg/m^2 (n = 14).

Discussion

Mutations in the HNF-1α and GCK genes are responsible for diabetes in 14% and 7%, respectively, of the Italian early-onset Type II diabetic families (and of 16% and 8%, respectively, of the Italian subset of MODY families) whereas HNF-4α gene mutations are not common in Italians. Northern Caucasian MODY cohorts have a higher percentage of MODY3, whereas among French families with MODY, MODY3 is found in less than 25% [9] and MODY2 in 56% [2]. The relatively low percentage of MODY3 in Italian families (14%) and the difference in percentages of MODY2 and MODY3 between Italians and French is probably due to the selection criteria for age at onset and to a different family phenotype in the studies, very mild in the French [2] and more severe in the Italians.

Our study indicates that the prevalence of MODY among Type II diabetic patients is much higher (at least 10% of Type II diabetes, because our patients are early-onset Type II diabetic patients) than has been usually described.

Whereas the HNF-1α *–58 A* $\rightarrow$ *C* mutation is located in the promoter and affects gene transcription [8, 10], the other three mutations are located in the coding region. The *W113X* HNF-1α mutation causes a premature transcriptional stop at amino acid 113 leading to a protein retaining the amino-terminal

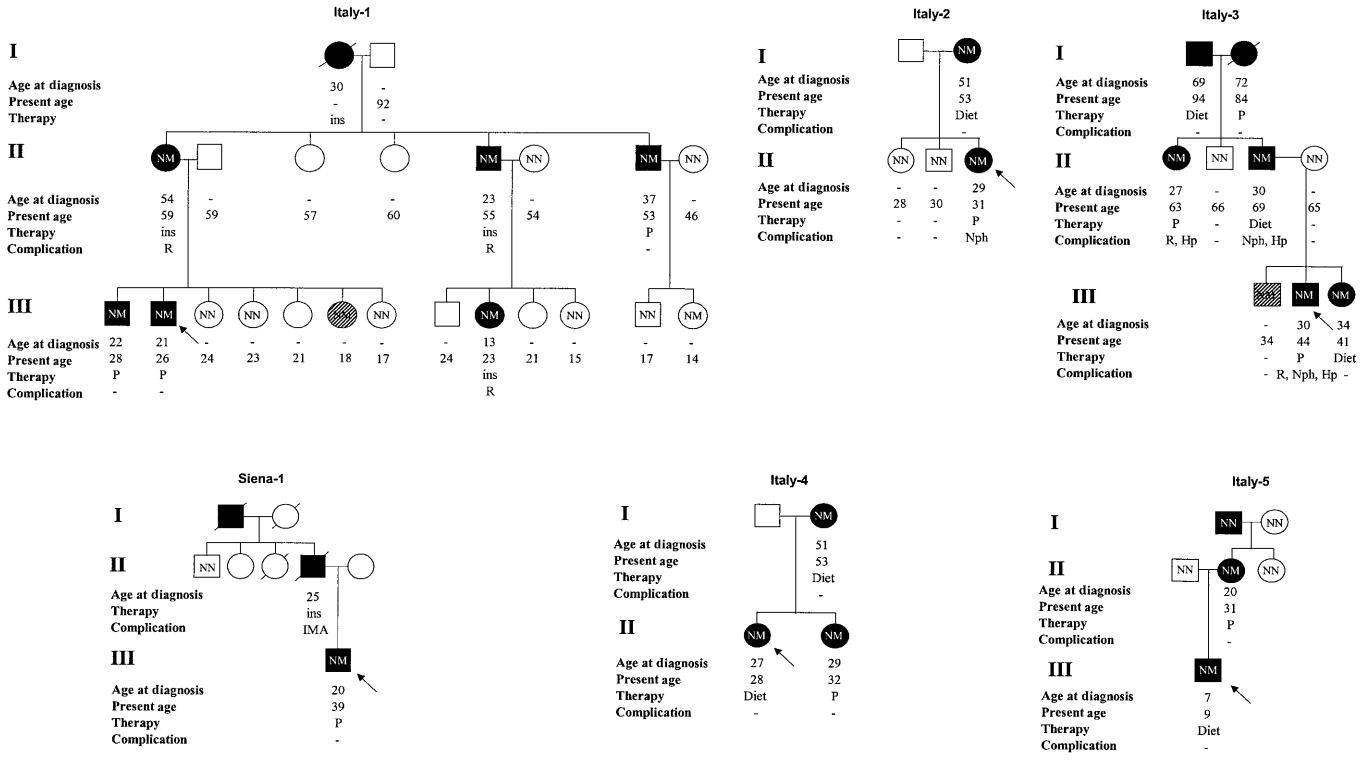

Fig. 1. ↖ proband; ■ non-insulin-dependent diabetes; ▨ impaired glucose tolerance; R, retinopathy; N, neuropathy; Nph, nephropathy; Hp, hypertension; IMA, acute myocardial infarct; ins, insulin; P, pills; NN, normal; NM, mutant

dimerization domain without either a DNA binding domain or a transactivation domain. The *G42P43fsCC → A154X* HNF-1α frameshift mutation generates an abnormal reading frame at amino acid 43, whereas translation stops at amino acid 154 yielding an abnormal protein lacking a functional DNA binding domain. These two HNF-1α mutations might affect critical functions of the dimeric protein, affecting binding and transcription of target genes. Both mutations are present in the B-domain shared by HNF-1α and HNF-1β genes that form heterodimers that bind DNA and stimulate gene transcription. Mutational alterations of these highly conserved HNF-1 regions might affect RNA stability. These two mutations might cause diabetes by a dosage and/or haploinsufficiency mechanism. The *H514R* HNF-1α family mutation alters amino acid 514 histidine, in the transcriptional activation domain and thereby might interfere with protein conformational structure. The wild sequence is conserved in the HNF-1α gene of mouse, rat, chicken, and hamster and in the HNF-1β gene of pig, mouse, rat, hamster and frog (GDB Alignment). Nevertheless, it is not possible to exclude a "private" polymorphism.

The NM unaffected Italy-1 family members have an average BMI of 21.31 kg/m^2 thus excluding that high basal insulin levels are secondary to obesity. Higher basal insulin concentrations might be the result of an initial beta-cell functional compensation in the NM Italy-1 members due to the HNF1α gene haploinsufficiency [10]; therefore NM Italy-1 members are probably better compensated at an earlier stage of disease, compared to MODY3 patients carrying mutations with dominant/negative effects. The C-peptide responses of MODY3 and MODY2 patients after glucagon stimulation, (values > 0.6 nmol/l) indicate the presence of residual beta-cell function, excluding misdiagnosis for Type I diabetes.

The GCK *G44S* variant may be acquired in the second generation. The GCK *G44S* is located in a conserved region in rat, mouse, human liver and pancreatic GCK sequence (GDB Alignment). The diabetes in the GCK *G44S* Italy-5 family is mild insofar as insulin response after a glucose stimulus is preserved. Glycosuria in Italy-5 patients could contribute to the milder form of diabetes and indicates that there is a compound form of metabolic disease which is also due to a mutation in a gene affecting renal glucose threshold. Therefore, we excluded mutations in the MODY3 gene that could cause glycosuria. The GCK *IVS4nt + 7C → T* genetic variant, located in a conserved region of the human and rat gene (GDB Alignment) might alter consensus sequences important in the splicing process of exon 3 and exon 4 but requires further analysis.

By screening Italian early-onset Type II diabetic families for mutations in HNF-1α and GCK genes, it is possible to select patients at risk for diabetes (maximal penetrance 0.95 for MODY3, minimal penetrance 0.50 for MODY2) and start preventive care.

Table 1. Gene mutations

Family	HNF-1 α gene mutation site	Nucleotide change	Amino acid change	Designation
Italy-1	*Promoter/-58*	*CCA to CCC*	–	*Promoter-58A to C*
Italy-3	*Exon 1/43*	CC to A	Frameshift	*G42P43fsCC to A154X*
Siena-1	*Exon 2/113*	*TGG* to *TGA*	Trp to Stop	*W113X*
Italy-2	*Exon 8/514*	*CAC* to *CGC*	His to Arg	*H514R*
Family	**GCK gene mutation site**	**Nucleotide change**	**Amino acid change**	**Designation**
Italy-4	*Intron 4*	*C* to *T*	–	*IVS4nt +7C to T*
Italy-5	*Exon 2*	*GGC* to *AGC*	Gly to Ser	*G44S*
Gene polymorphisms				
HNF-1a	Codon/nucleotide	Nucleotide change	Frequence	
Exon 1	*17*	*CTC* (Leu) to *CTG* (Leu)	*G* – 0.60, *C* – 0.40	
	27	*ATC* (Iso) to *CTC* (Leu)	*A* – 0.79, *C* – 0.21	
	98	*GCC* (Ala) to *GTC* (Val)	*C* – 0.93, *T* – 0.07	
Exon 3	*184*[a]	*GGG* (Gly) to *GGT* (Gly)	*G* – 0.96, *T* – 0.04	
Exon 4	*288*	*GGG* (Gly) to *GGC* (Gly)	*G* – 0.73, *C* – 0.27	
Exon 7	*459*	*CTG* (Leu) to *TTG* (Leu)	*C* – 0.64, *T* – 0.36	
	487	*AGC* (Ser) to *AAC* (Asn)	*G* – 0.59, *A* – 0.41	
Exon 8	*513*[a]	*ACG* (Thr) to *ACA* (Thr)	*G* – 0.99, *A* – 0.01	
Intron 1	*nt-91*	A to *G*	*A* – 0.89, *G* – 0.11	
	nt-42	G to *A*	*G* – 0.79, *A* – 0.21	
	nt-53[a]	C to *G*	*C* – 0.96, *G* – 0.04	
Intron 2	*nt-51*	T to *A*	*T* – 0.74, *A* – 0.26	
	nt-23	C to *T*	*C* – 0.50, *T* – 0.50	
Intron 3	*nt-47*[a]	GGG to *GG*	*GGG* – 0.96, *GG* – 0.04	
Intron 5	*nt-47*	C to *T*	*C* – 0.80, *T* – 0.20	
Intron 7	*nt +7*	G to *A*	*G* – 0.53, *A* – 0.47	
Intron 9	*nt +44*	C to *T*	*C* – 0.91, *T* – 0.09	
	nt-24	T to *C*	*T* – 0.55, *C* – 0.45	
GCK	Codon/nucleotide	Nucleotide change	Frequence	
Promoter	*nt-84*[a]	*C* to *G*	*C* – 0.87, *G* – 0.13	
intron 9	*nt-8*[a]	*C* to *T*	*C* – 0.86, *T* – 0.14	
	nt+49[a]	*G* to *A*	*G* – 0.97, *T* – 0.03	
HNF-4 α	Codon/nucleotide	Nucleotide change	Frequence	
Intron 1c	*nt-11*[a]	*T* to *G*	*C* – 0.56, *T* – 0.44	
	nt-5	*C* to *T*	*G* – 0.69, *T* – 0.31	

HNF-1α/*n* = 54 chromosome; T513T is in the control group; GCK/*n* = 40 chromosomes; HNF-4α/*n* = 32 chromosomes
[a]New polymorphism

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