Mutations in the hepatocyte nuclear factor-1 α gene in Chinese MODY families: prevalence and functional analysis

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Abstract

Aims/hypothesis. Maturity-onset diabetes of the young is an autosomal dominant form of diabetes characterised by an early age of onset (usually <25 years). We investigated the prevalence and trans-activating activity of hepatocyte nuclear factor (*HNF*)-1 α mutations in southern Chinese families with MODY.

Methods. We screened for mutations in the *HNF*-1 α gene in 50 unrelated southern Chinese families, which fulfilled the minimum criteria for MODY. Functional properties of the mutant proteins were investigated using site-directed mutagenesis and luciferase reporter assay.

Results. Five of the 50 (10%) families were found to have mutations in the coding region, including a new nonsense mutation Q176X and four reported mutations (frameshift mutation P379fsdelCT, nonsense

mutation R171X, missense mutations G20R and P112L). These mutations had decreased trans-activating activity on the human insulin gene promoter. We also detected a new intronic sequence variation IVS7nt-6 G→A, which co-segregated with diabetes. The intronic variation creates a potential splice acceptor site and might alter the splicing of the *HNF*-1 α mRNA.

Conclusion/interpretation. Mutations in the *HNF*-1 α gene seem to be an important cause of MODY in southern Chinese. The mutations could affect normal islet function by altering the expression of target genes. [Diabetologia (2002) 45:744–746]

Keywords Maturity-onset diabetes of the young, hepatocyte nuclear factor, prevalence, functional analysis, Chinese.

Maturity-onset diabetes of the young is characterised by an early age of onset (usually <25 years) and an autosomal dominant mode of inheritance. Mutations in the hepatocyte nuclear factor (*HNF*)-1 α gene have been shown to cause a common type of MODY, MODY3 in many populations [1, 2, 3, 4]. *HNF*-1 α mutations accounted for 5% of early-onset (age at diagnosis <40 years) Type II (non-insulin-dependent) diabetes mellitus in Chinese subjects with a positive

family history in a previous report from Hong Kong [5]. The prevalence of *HNF*-1 α mutations in Chinese MODY families is not known. *HNF*-1 α is a tissue specific transcription factor, composed of three functional domains: a N-terminal dimerisation domain (residues 1–32), a DNA-binding domain (residues 98–281) and a C-terminal transactivation domain (residues 281–631). Recent studies showed that *HNF*-1 α transactivates the human insulin gene [6]. The functional properties of a minority of the reported *HNF*-1 α mutations, including P379fsdelCT, have been studied and were found to have decreased trans-activating activity. We investigated the prevalence and functional activities of mutations in the *HNF*-1 α gene in southern Chinese families fulfilling the minimum clinical criteria for MODY: two generations of Type II diabetes with at least one member diagnosed under the age of 25.

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Abbreviations: HNF, Hepatocyte nuclear factor

Subjects and methods

Subjects. Fifty southern Chinese MODY families were recruited from the Diabetes Center, Queen Mary Hospital. The mean age at diagnosis of the probands was 19.56 ± 3.42 years (range, 11–24). The control group consisted of 100 unrelated southern Chinese subjects with NGT and no family history of diabetes (aged 63.23 ± 4.98). The control subjects were selected from a previous cohort study [7]. All subjects gave their informed consent and the protocol was approved by the Ethics Committee of the University of Hong Kong.

Identification of mutations. The proband from each family was screened for mutations in the *HNF-1 α* gene. The 10 exons, flanking introns and minimal promoter of the *HNF-1 α* gene were amplified by PCR. Bi-directional sequencing was done using an ABI PRISM dRhodamine Dye Terminator Cycle Sequencing Kit on an ABI Prism 377 DNA Sequencer (Applied Biosystems, Foster City, Calif., USA) as described previously [1]. The occurrence of putative mutations in the control group was analysed by single strand conformation polymorphism or, if the nucleotide substitution affected an enzyme recognition site, by RFLP.

Construction of plasmids and transient transfection. The pBJ5 expression vector harbouring the coding sequence for *HNF-1 α* was a gift of Dr. G. Crabtree, (Stanford, Calif., USA). The G20R, P112L, R171X and Q176X mutations were introduced by site-directed mutagenesis (Stratagene, La Jolla, Calif., USA). A 389-bp fragment of the human insulin gene promoter fragment containing A3 element was amplified by PCR and subcloned into pGL3-Basic (Promega, Madison, Wis., USA). HeLa cells were transfected with the wild type or mutant expression vectors together with insulin reporter gene and pCMV- β -Galactosidase (Gibco BRL, Rockville, Md., USA) as an internal control using Lipofectin Reagent (Gibco BRL).

Western blot analysis and luciferase reporter assay. Forty-eight hours after the start of transfection, whole cell lysates were prepared. Protein (20 μ g) was loaded on NuPAGE 4–12% Bis-Tris Gel (Invitrogen, Carlsbad, Calif., USA) and transferred to a nitrocellulose membrane. The membranes were blocked and incubated with C-terminal goat anti-*HNF-1 α* antibody (Santa Cruz, Calif., USA) and then with horseradish peroxidase-conjugated anti-goat IgG antibody. The membranes were finally visualised using ECL Western blotting detection reagents (Amersham Pharmacia Biotech, Buckinghamshire, UK). Luciferase activities were measured with Luciferase Reporter Assay System (Promega), and β -Galactosidase activities with β -Galactosidase Enzyme Assay System (Promega). Luciferase activities were divided by the respective β -galactosidase activity to correct for transfection efficiency. Each experiment was repeated three times. A *p* value of less than 0.05 was considered to be statistically significant.

Results

Identification of mutations. In 50 unrelated MODY families, five mutations (10%) were identified in the coding region (Table 1): two nonsense mutations (Q176X and R171X), one frameshift mutation P379fsdelCT, and two missense mutations (G20R and P112L). In addition, we detected a new sequence variation IVS7nt-6 G→A. These mutations and variation were not detected in 100 unrelated subjects with NGT. The mutations G20R, P379fsdelCT and IVS7nt-6 G→A co-segregated with diabetes within the families and were not present in the non-diabetic family members. For the other three mutations, affected family members were residing overseas and could not be tested.

Clinical characteristics of patients with detected mutations. The proband with Q176X mutation was put on insulin 2 years after diagnosis. The other five probands were controlled with oral hypoglycaemic agents 2 to 46 years (mean 14.8) from diagnosis. The proband with IVS7nt-6 G→A had severe proliferative retinopathy. The other five probands had not developed any diabetic complications. There was no apparent relation between genotype and phenotype of MODY3 in these six families.

Fig. 1. Transcriptional activity of human insulin reporter gene by mutant and WT *HNF-1 α* . 1 μ g of WT *HNF-1 α* , mutant *HNF-1 α* or empty PBJ5 vector DNA were transfected with 1 μ g of insulin reporter gene and 0.5 μ g of pCMV- β -Galactosidase as an internal control. Luciferase activity was normalised by β -galactosidase activity and expressed as percentage of WT. Each assay was done in triplicate wells and repeated three times. Means $\pm$ SD, **p*<0.05 ***p*<0.01 compared with WT, Δ *p*<0.01 compared with empty vector (unpaired Student's *t* test)

Table 1. Mutations in the *HNF-1 α* gene in Southern Chinese MODY

Family number	Location of mutation		Nucleotide change	Amino acid	Designation change	RFLP
	Exon	Codon				
9	2	176	CAG→TAG	Gln→Stop	Q176X	Loss of <i>TseI</i> site
22	1	20	GGG→AGG	Gly→Arg	G20R	–
26	2	171	CGA→TGA	Arg→Stop	R171X	–
37	6	379	Deletion of CT	Frameshift	P379fsdelCT	–
101	2	112	CCG→CTG	Pro→Leu	P112L	Loss of <i>NlaIV</i> site

Functional analysis of mutant HNF-1 α . The expression of the wild type and mutant proteins in HeLa cells were similar by western blot analysis, except for Q176X and R171X which could not be detected with the C-terminal antibody (data not shown). The transactivation activities of Q176X and R171X were 24.16% and 22.43% of that of the wild type, respectively, and were similar to that of vector alone, indicating that Q176X and R171X had lost transactivation function (Fig. 1). A similar result was obtained for G20R. The transactivation activity of P112L on human insulin promoter was 51.36% of that of the wild type.

Discussion

We detected five mutations in 50 Chinese MODY families. Q176X is a novel mutation. P379fsdelCT was found previously in Japanese and Caucasian MODY families [1, 2], R171X in French [3] and Dutch families, and P112L in one Norwegian family [8]. G20R was also detected in two unrelated Chinese subjects [5], implying that this region might be a mutational hot spot in Chinese subjects. Alternatively this could suggest a founder effect. As ascertained from the proband, she had no relatives with diabetes followed up in the other Hong Kong Center. Unfortunately the other two families were not accessible to us and haplotype analysis could not be carried out to better establish the origin of this mutation.

Q176X and R171X produce truncated proteins of 175 and 170 amino acids, respectively, and lack the entire transactivation domain. In our study Q176X and R171X had lost transactivation activity. Our result on R171X is consistent with that of a French group [9]. G20R is located in the dimerisation domain and retains the intact DNA binding and transactivation domain. An analysis of the crystal structure of the HNF-1 α dimerisation domain suggested that G20R might lead to a dimeric molten globule, perturbing the stability of the dimer [10]. The loss of transactivation activity observed in G20R might be due to the instability of the dimer and hence the impaired binding of HNF-1 α to insulin promoter. P112L is located in the DNA binding domain and was found to have reduced transactivation activity on the human insulin gene, compared with that of the wild type ($p < 0.01$). The frameshift mutation P379fsdelCT generates a truncated protein of 416 amino acids and loses about two thirds of transactivation domain, resulting in a dominant negative effect on transcriptional activity of the human insulin gene [6]. The intronic variation IVS7nt-6G $\rightarrow$ A creates a potential splice acceptor site (CGGCCAG/CCCTC $\rightarrow$ CAG/CCAGCCCTC). Whether this sequence variation alters the splicing of HNF-1 α mRNA and results in functional defects is not known.

In conclusion, we have identified five coding region mutations in the HNF-1 α gene in southern Chinese families that fulfilled the minimal criteria of MODY. All these mutations have functional defects in vitro and might perturb pancreatic beta-cell function in vivo, hence contributing to the development of diabetes. The prevalence of the HNF-1 α mutations in southern Chinese MODY families (10%) is higher than that in early-onset Type II diabetes (5%) in Chinese subjects of another Hong Kong study [5], but lower than that in German MODY families, which met the same criteria (36%) [4]. The differences in the genetic background of the two populations could explain the difference in these results.

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