Candidate gene studies in pedigrees with maturity-onset diabetes of the young not linked with glucokinase

Y. Zhang¹, M. Warren-Perry¹, P.J. Saker¹, A.T. Hattersley¹, A.D.R. Mackie², J.D. Baird³, R.H. Greenwood⁴, M. Stoffel⁵, G.I. Bell⁵, R.C. Turner¹

¹Diabetes Research Laboratories, Radcliffe Infirmary, Woodstock Road, Oxford, UK

²Department of Diabetes and Endocrinology, Addenbrookes Hospital, Cambridge, UK

³Metabolic Unit, University Department of Medicine, Western General Hospital, Edinburgh, UK

⁴Norfolk and Norwich Hospital, Norwich, UK

⁵Howard Hughes Medical Institute and Department of Medicine, The University of Chicago, Chicago, Illinois, USA

Summary Maturity-onset diabetes of the young (MODY) is a form of non-insulin-dependent diabetes mellitus characterised by an early age of onset and an autosomal dominant mode of inheritance. Only a proportion of cases are due to mutations in the glucokinase gene. We have studied five Caucasian MODY families, including the first MODY family to be described, with five candidate genes implicated in regulation of insulin secretion. The affected subjects showed more marked hyperglycaemia than that found in subjects with glucokinase mutations. We assessed polymorphic markers close to the genes for glucokinase, hexokinase II, adenosine deami-

nase, pituitary adenylate cyclase-activating polypeptide receptor, and glucagon-like peptide-1 receptor. Linkage analysis with diabetes gave cumulative log of the odds (LOD) scores of less than -3, implying that mutations in these genes are unlikely to provide a major genetic contribution to this form of MODY. [Diabetologia (1995) 38: 1055–1060]

Key words Maturity-onset diabetes of the young, glucokinase, adenosine deaminase, pituitary adenylate cyclase-activation polypeptide receptor, hexokinase II, glucagon-like peptide-1 receptor, polymerase chain reaction, linkage analysis.

Non-insulin-dependent diabetes mellitus (NIDDM) is likely to have a genetic basis in view of the different incidences between ethnic groups, the familial nature of the disease and the high concordance in identical twins [1–3]. The use of linkage analysis to study candidate genes in families with classic, late-onset NIDDM is problematic because family studies do not indicate simple Mendelian inheritance [4, 5] and the late age of onset and early mortality make it diffi-

cult to obtain large multigeneration families suitable for genetic studies [6]. NIDDM is likely to be heterogeneous, with mutations in several different genes being liable to induce hyperglycaemia [7, 8].

A subgroup of NIDDM, maturity-onset diabetes of the young (MODY) provides a specific phenotype, with presentation often before age 25 years, that is inherited as an autosomal dominant trait [9, 10]. Mutations in the glucokinase (GCK) gene were first found in pedigrees with MODY [11, 12] and may account for half of the MODY pedigrees [13]. The nature of the genetic lesion in the other families is unknown. The first gene implicated in the development of MODY was identified by exclusion mapping in the large RW pedigree, with the observation of linkage with markers close to the adenosine deaminase (ADA) gene [14]. The affected members have recently been shown to have beta-cell dysfunction [15]. Identification of other pedigrees linked with adenosine deaminase (ADA) would be helpful in the further localisation of the gene in chromosome 20q.

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Corresponding author: Dr. R.C. Turner, Diabetes Research Laboratories, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, UK

Abbreviations: MODY, Maturity-onset diabetes of the young; GCK, glucokinase; ADA, adenosine deaminase; PACAP-R, pituitary adenylate cyclase-activation polypeptide receptor; HK-2, hexokinase II; GLP-1 R, glucagon-like peptide-1 receptor; PCR, polymerase chain reaction; STRP, simple tandem repeat polymorphism; NIDDM, non-insulin-dependent diabetes mellitus.

Whereas NIDDM may result from defects in insulin action as well as insulin secretion [16, 17] the available data suggest that MODY is primarily due to beta-cell deficiency [12, 18]. Genes that could regulate insulin secretion are candidates for mutations that could lead to MODY, include glucokinase and hexokinase II (HK-2), since the latter is also expressed in beta-cells [19, 20]. Beta-cell sensitivity to glucose is regulated by several hormones, including acetylcholine, glucagon, and glucagon-like peptide-1 (GLP-1), and their receptors are potential candidate genes [21]. The recently described pituitary adenylylate cyclase activation polypeptide (PACAP), which is a member of the secretin/glucagon/vasoactive intestinal peptide family of peptides, is a particularly potent stimulator of insulin secretion from pancreatic islets, at 10^{-10} to 10^{-9} mol concentration [22].

We report the results of linkage analyses of these candidate genes for regulation of beta-cell secretion with MODY in five Caucasian families, including the first MODY pedigree described by Tattersall [9]. We report the phenotype in these families, which show more marked hyperglycaemia and more often require pharmaceutical therapy than in the milder disease associated with glucokinase mutations [23].

Subjects and methods

Subjects. Five Caucasian MODY families are shown in Figure 1. They live in London, Oxford, Leicester, Norwich and Edinburgh and were referred by diabetologists. Each family had three patients with NIDDM diagnosed before the age of 25 years and included sufficient members for linkage studies.

Phenotype. Table 1 shows the age of onset, initial therapy and current status of patients in the pedigrees studied. Pedigree 1 is the original MODY pedigree described as "M" [9].

Genetic studies. DNA was extracted from peripheral blood using either the standard phenol/chloroform method [24] or Nucleon II DNA extraction Kit (Scotlab, UK). Subjects were genotyped at simple tandem repeat DNA polymorphisms (STRPs) near ADA, GCK, HK2, PACAP-R, and GLP-1R (Table 2) using the polymerase chain reaction (PCR). The ADA STRP, D20S119, is different from that used by Bell et al. [14]. The data for GLP-1R have been published previously [25]. The PCR products were separated by electrophoresis on a non-denaturing polyacrylamide gel and bands were detected by ethidium bromide staining.

Linkage analysis. Each family was considered separately for linkage analysis performed with the software LINKAGE program (version 5.1) assuming dominant inheritance [26]. As candidate genes were being examined, LOD (logarithm of the odds for linkage vs non-linkage) scores were primarily calculated with the recombination fraction θ equal to zero. Values at other recombination fractions were determined to provide an exclusion map around those markers and are available from the authors. Liability classes were based on age-related penetrance, calculated from the incidence of diabetes in first-degree relatives of MODY patients [27] and prevalence

studies of diabetes in the general population. Five liability classes for MODY were defined on the basis of age (class 1, > 50 years; class 2, 41–50 years; class 3, 31–40 years; class 4, 21–30 years; class 5, < 20 years). The penetrance factors for heterozygous and homozygous genotypes used for MODY were 0.99, 0.95, 0.90, 0.80, 0.60 for heterozygosity and 1 for homozygosity at all ages. Since the clinical presentation in these families was similar to that for classic NIDDM, phenocopy rates for each liability class were those of the prevalence of NIDDM in the UK 0.03, 0.005, 0.001, 0.001, and 0.0001 respectively. For comparison, the characteristics of patients in two large MODY pedigrees BX and AX, due to a missense mutation of the glucokinase gene, are also shown [23].

Results

Table 1 shows that the patients were non-obese and presented at median age 17 years, when 50 % were initially treated by diet and 50 % by oral hypoglycaemic agents or insulin. When recently assessed, at median age 29 years, the subjects were not obese, median body mass index (BMI) $23 \text{ kg} \cdot \text{m}^{-2}$. One-third were treated by diet at median age 26 years and had median fasting blood glucose levels 7 mmol/l . One-third were treated by oral hypoglycaemic agents at median age 43 years and had median fasting blood glucose levels 10 mmol/l . One third were treated by insulin at median age 26 years and had median fasting blood glucose level 7 mmol/l .

Figure 1 shows family trees with the genotypes for the PACAP-R polymorphism [28] in the MODY pedigrees. Table 3 shows the LOD scores at a recombination fraction (θ) of 0.00 for each family for the markers studied. The cumulative LOD scores for each marker were less than -2.0 . The maximum positive LOD scores that could be achieved in these five pedigrees are 4.0, 2.3, 1.4, 3.3 and 8.4, respectively, with a cumulative maximum of 19.4.

Discussion

In only one [12] of the six large UK MODY pedigrees we investigated has a glucokinase mutation been identified. None of the five pedigrees reported in this study were linked to the glucokinase gene, the cumulative LOD scores for the two markers being -11.5 and -17.6 , respectively. This contrasts with the report from France that glucokinase mutations account for over 50 % of MODY pedigrees [13]. This difference

Fig. 1. The MODY pedigrees [1–5] showing the inheritance of PACAP-R alleles. Roman numerals on the left of the pedigrees indicate generation number, and the numbers above the symbols indicate the subject's number within each pedigree. The PACAP-R DNA polymorphism alleles are noted under the symbols. □, males; ○, females; ●, ■, diabetes; diagonal shading, glucose intolerance; open symbol, normoglycaemia; central dot, not tested; slash, deceased

(1)

(2)

(3)

(4)

(5)

Table 1. Clinical characteristics of MODY patients in the five families and in subjects in pedigrees BX with glucokinase missense mutation

Pedigrees without glucokinase mutations	Gender (male, female)	At diagnosis		Current status				
		Age (years) median (range)	Diet/OHA/ins	Age (years) median (range)	BMI (kg · m ⁻²) median (range)	Therapy diet/OHA/ins	HbA _{1c} (%) Medians diet/OHA/ins	Fasting glucose (mmol/l) Medians diet/OHA/ins
1	1/8	15 (8–28)	6/–/3	29 (8–60)	23 (21–26)	4/5/–	6/–/–	9/10/–
2	4/3	17 (6–37)	1/3/3	38 (19–56)	23 (22–29)	1/3/3	7/8/7	7/6/–
3	2/2	19 (15–20)	2/2/0	35 (25–46)	21 (21–22)	2/2/0	7/8/–	8/10/–
4	5/4	21 (11–50)	5/0/3	26 (21–76)	24 (23–27)	3/2/3	–/8/8	9/11/7
5	4/11	19 (14–48)	6/4/4	47 (29–60)	25 (22–33)	0/9/5	–/8/10	–/–/–
Total or Medians	10/28	19 (6–50)	20/9/13	35 (8–76)	23 (21–33)	10/21/11	8/8/9	9/10/7
Patients with glucokinase mutations	7/11	21 (14–63)	16/1/1	41 (18–81)	24 (18–39)	15/2/1	6/6/8	7/7/–

OHA, Oral hypoglycaemic agents; ins, insulin; HbA_{1c}, haemoglobin A_{1c}**Table 2.** List of STRPs used as candidate gene markers

Localisation	Marker name	Primer sequence	Annealing temperature (°C)	Concentration of polyacrylamide (%)	Alleles size range (base pair)	Number of alleles (heterozygosity)
20q12	ADA	TITCCAGATTTAGGGGTG-TATG CTGACACAGTTTCAG-TATCTCTATC	62	12	106–120	8 (0.93)
7p13	GCK 1	TTGGTCAGTGTAGGCT-GAACTCAT CCCACACCAAACACTGC-CTGTATTAG	55	8	195–201	3 (0.34)
	GCK 2	AACAGATACGCTTCATC-CTG TGTCTGCAACTTACTCT-TAC	55	8	129–141	4 (0.60)
2p13.1	HK II-1	AGTGTGCACTGTGTGTC AATGTGCAACCAG-CAGCCCT	60	12	218–230	6 (0.64)
	HK II-2	TTCTAAGACTTGGCAG-AACAT AGCTCTTTCAGGTGACT-TCA	57	10	194–202	5 (0.68)
7p15	PACAP-R	CCATCTTGGTGTGAGGGC AACAAAGGGCTTCTGCT-GAG	66	8	125–137	7 (0.94)
6p21	GLP-1 R	TCCTTGCTGCAGCCGG-GTGG TACCTGTCTGATGGG-TTTGG	65	8	134–148	8 (0.82)

may result from ascertainment bias. We have primarily sought large pedigrees suitable for linkage analysis. The pedigrees were referred by diabetologists caring for adult diabetic patients, and were noticed because several members of the pedigrees had presented with symptomatic NIDDM before age 25 years. The French MODY pedigrees were often referred by paediatricians when mild diabetes was detected in

the second decade and affected relatives were identified by screening asymptomatic relatives. This resulted in collection of a mild phenotype. Patients with glucokinase mutations frequently have impaired glucose tolerance rather than diabetes; most family members are asymptomatic and the sub-clinical diabetes rarely leads to complications [12, 23]. On the other hand, our more hyperglycaemic pedigrees with

Table 3. Pedigree LOD scores at $\theta = 0.00$ for the five candidate genes

Families order	ADA	GCK		HK II		PACAP-R	GLP-1 R
		1	2	1	2		
1	- 2.90	- 4.63	- 4.97	-0.76	- 5.23	- 5.03	-2.59
2	- 7.99	- 3.47	- 0.05	0.23	- 6.98	- 4.76	-3.89
3	- 1.75	- 2.35	- 2.44	-2.99	0.00	- 2.90	0.00
4	- 8.50	- 0.63	- 1.74	0.35	- 6.51	- 5.00	-1.02
5	- 6.18	- 0.47	- 8.46	-3.39	- 9.09	-11.29	0.00
Cumulative LOD scores	-27.31	-11.54	-17.55	-6.56	-27.81	-28.98	-8.50

diabetes as defined by the World Health Organisation often required oral hypoglycaemic or insulin therapy and developed diabetic retinopathy and other diabetic complications.

The largest reported MODY pedigree is the RW pedigree in which linkage has been identified with markers close to the ADA chromosome 20. They also often require oral hypoglycaemic or insulin therapy and have impaired beta-cell function [15]. The localisation of the gene linked with diabetes in the region of ADA would be greatly aided by additional pedigrees, similar to the RW pedigree, but none of the large pedigrees we have studied are linked with markers close to ADA. It seems that the RW type of diabetes is likely to account for a minority of MODY.

These large pedigrees were not linked to either the glucokinase or hexokinase II genes. Whilst hexokinase II is predominately expressed in muscle and adipocytes, it is also expressed in beta-cells [19, 20]. The remarkable increase of beta-cell and alpha-cell responsiveness to glucose by pharmacological infusions of GLP-1 in NIDDM [29] show the potential for defects in the response to the hormones that regulate beta-cell function via a cyclic-AMP-dependent process. PACAP is the most potent hormone of this class [22], but we found that neither the PACAP receptor nor the GLP-1 receptor [25] were linked to diabetes in the MODY families studied.

Linkage analysis in pedigrees is a powerful tool for examining the role of candidate genes in the aetiology of inherited diseases with a defined mode of transmission [30]. Families with MODY are particularly amenable as they have a clearly discernible autosomal dominant inheritance and the early age of onset allows ascertainment of affected members over three generations [6, 31, 32]. When doing linkage analysis, the parameters for age-related liability classes were chosen to take into account the prevalence of NIDDM in the general population and the apparent age-dependent penetrance in MODY pedigrees. The search for genes can be done by two complementary methods; by the candidate gene approach, as was successful with glucokinase, or with exclusion mapping, as in insulin-dependent diabetes mellitus [34].

We found no evidence of linkage with MODY for the five candidate genes that we studied in these fam-

ilies. The results were not dependent on the models chosen and were, for instance, not affected by doubling the phenocopy rate or by increasing or decreasing by 50 % the penetrance of the putative MODY gene. Diabetes in these pedigrees had some similarities to that in patients with classic, non-obese NIDDM, including similar beta-cell deficiency [12, 23, 34]. Identification of the genes relevant to beta-cell dysfunction and their exclusion in this form of MODY may be relevant to NIDDM as well as to MODY, but the candidate genes tested so far are not implicated in MODY [35].

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