

A Prevalent Amino Acid Polymorphism at Codon 98 (Ala98Val) of the Hepatocyte Nuclear Factor-1 α Is Associated With Maturity-Onset Diabetes of the Young and Younger Age at Onset of Type 2 Diabetes in Asian Indians

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OBJECTIVE — Among Europeans, mutations in the hepatocyte nuclear factor-1 α (HNF1 α) gene are associated with the most common form of maturity-onset diabetes of the young (MODY)3. In Asian Indians, type 2 diabetes occurs earlier and often overlaps with MODY, but the genetics of the latter are unknown. The aim of this study was to estimate the prevalence of Ala98Val polymorphism of the HNF1 α gene in different types of diabetes in Asian Indians.

RESEARCH DESIGN AND METHODS — Genotyping of Ala98Val was done by the PCR–restriction fragment–length polymorphism method in the following groups: 1) MODY, defined as non–insulin-dependent diabetes (age at onset <25 years) and vertical transmission of diabetes through at least three generations ($n = 122$); 2) very-early-onset type 2 diabetes (age at onset <25 years) without family history ($n = 23$); 3) early-onset type 2 diabetes (age at onset between 26 and 40 years, $n = 171$); 4) late-onset type 2 diabetes (age at onset >40 years, $n = 133$); 5) type 1 diabetes ($n = 150$); and 6) normal glucose tolerance ($n = 130$). The frequency of the Val genotypes was compared in the diabetic and control groups.

RESULTS — The frequency of the Val allele was significantly higher in MODY patients ($P = 0.0013$) compared with control groups. Furthermore, in the total group of patients with type 2–like diabetes (groups 1–4), the Val allele was associated with an earlier diagnosis of diabetes ($P = 0.0002$).

CONCLUSIONS — Among Asian Indians, the Ala98Val polymorphism of HNF1 α gene is associated with MODY and with earlier age at onset of type 2 diabetes.

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Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes characterized by autosomal dominant mode of inheritance, onset of diabetes <25 years of age, and a predominant β -cell defect (1). In 1985, the senior author of this paper (V.M.) first reported that type 2 diabetes occurs at a much younger age in Asian Indians compared with Europeans and that 4.8% of all patients with nonautoimmune diabetes seen at a tertiary diabetes center at Chennai (formerly Madras) in southern India had an age at diagnosis <25 years (2). About one-third of these patients had evidence of vertical transmission of diabetes through two or more generations and thus presumably had MODY on clinical grounds, although the genetics of MODY was unknown at that time. Due to the earlier onset of type 2 diabetes among Asian Indians, the conventional criteria of age of onset and family history might not differentiate MODY mutation carriers from early-onset type 2 diabetic patients in this age range (3,4). Therefore, examination at the molecular level gains importance.

To date, six MODY subtypes (5–10) have been described, of which MODY3 is the most prevalent type and is caused by mutations in the gene encoding hepatocyte nuclear factor 1 α (HNF1 α). Previous reports (11,12) in Danish Caucasians have shown that the Val carriers of the HNF1 α Ala98Val polymorphism have decreased serum C-peptide and insulin responses to an oral glucose load compared with the Ala homozygous individuals, suggesting that this amino acid replacement might influence β -cell function. Earlier studies in a Finnish population showed an association between Ala 98Val polymorphism and type 2 diabetes with a prevalence of 13.2% (13).

Our objective in carrying out this study was to examine the prevalence of

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Abbreviations: HNF1 α , hepatocyte nuclear factor-1 α ; MODY, maturity-onset diabetes of the young; NGT, normal glucose tolerance.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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this polymorphism in five groups of Asian Indian diabetic patients including MODY and a control group of glucose-tolerant patients and to evaluate its role in conferring risk of diabetes in Asian Indians. This is the first report on the genetics of MODY from India and also the first showing an association of this polymorphism with MODY and age at onset of type 2 diabetes.

RESEARCH DESIGN AND METHODS

The following study groups were selected: group 1, MODY patients of unknown etiology defined as those who have non-insulin-dependent diabetes with age of diagnosis <25 years and vertical transmission of diabetes in at least three generations suggestive of autosomal dominant inheritance ($n = 122$); group 2, very-early-onset type 2 diabetic patients with age of diagnosis <25 years but without a known family history of diabetes ($n = 23$); group 3, early-onset type 2 diabetic patients with age of diagnosis between 26 and 40 years, irrespective of family history ($n = 171$); group 4, late-onset type 2 diabetic patients with age of diagnosis >40 years, irrespective of family history ($n = 133$); group 5, type 1 diabetic patients ($n = 150$); and group 6, a control group of patients with normal glucose tolerance (NGT, $n = 130$).

Patients with MODY, very-early-onset type 2 diabetes, and type 1 diabetes were recruited from Dr. Mohans' M.V. Diabetes Specialities Centre, a large tertiary diabetes center in Chennai. All patients chosen for the study, including MODY, were unrelated probands. Normal control subjects and the other two type 2 diabetic groups were recruited from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiological study conducted on a representative sample of Chennai (formerly Madras) in southern India. The methodology of CURES has been published elsewhere (14,15).

MODY was diagnosed based on Tattersal and Fajans (1) criteria: age at onset of diabetes ≤ 25 years, control of hyperglycemia for a minimum period of 5 years without insulin, absence of ketonuria at any time, and evidence of autosomal dominant inheritance, including a vertical transmission of the disease through at least three generations.

Type 2 diabetes was diagnosed if there was unequivocal evidence of diabetes, i.e., fasting plasma glucose ≥ 126 mg/dl or 2-h postglucose value ≥ 200 mg/dl (16), absence of ketosis or ketoac-

idosis, and treatment with diet and/or oral hypoglycemic agents. The three groups of type 2 diabetic patients were distinguished based on age of onset, i.e., those <25 years, 26–40 years, and >40 years. In the latter two groups, family history of diabetes was included because there was no overlap with MODY. However, to distinguish very-early-onset type 2 diabetes from MODY, only those without a family history of diabetes were included in the former group.

Type 1 diabetes was diagnosed based on the following criteria: an abrupt onset of diabetes, requirement of insulin for control of hyperglycemia from the time of diagnosis of diabetes, susceptibility to ketosis in the basal state, or documented episodes of ketoacidosis. No age criteria was used for this group. In all, type 2 diabetes, MODY patients, and glutamic acid decarboxylase antibodies were absent. A total of 105 (70%) of the type 1 diabetic patients showed presence of GAD antibodies.

NGT was defined as fasting plasma glucose <100 mg/dl and 2-h postglucose value ≤ 140 mg/dl (16). NGT subjects were purposely selected at >50 years of age since younger individuals with NGT may eventually develop diabetes at a later age.

Measurement of clinical and biochemical variables

Anthropometric measurements including weight, height, and waist measurements were obtained using standardized techniques. BMI was calculated using the formula of weight in kilograms divided by the square of height in meters. Blood pressure was recorded in the sitting position in the right arm to the nearest 2 mmHg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus; IEAP, Pune, India). Two readings were taken 5 min apart, and the mean of the two was recorded as the blood pressure.

A fasting blood sample was taken for the estimation of glucose and lipids. All biochemical assays were done on a Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany) using kits supplied by Roche Diagnostics (Mannheim, Germany). LDL cholesterol was calculated using the Friedewald formula (17). HbA_{1c} (A1C) was estimated by high-performance liquid chromatography using the Variant machine (Bio-Rad, Hercules, CA; normal value <5.6%). Serum C-peptide concentration was estimated using Dako kits (Dako, Ely, U.K.) in the fasting state

and after stimulation by a standard breakfast as previously described (18). The intra- and interassay coefficients of variation for C-peptide assays were 0.04 and 0.088, respectively, and the lower detection limit was 0.02 pmol/ml.

The pancreatic β -cell secretory capacity was estimated in 67 patients using the homeostasis assessment model calculator with the values of fasting C-peptide and fasting plasma glucose (available from www.dtu.ox.ac.uk) (19).

Detection of Ala98Val polymorphism in the gene encoding HNF1 α

Genomic DNA was isolated from whole blood by digestion with proteinase K followed by the phenol-chloroform extraction method (20). The DNA segment containing the variants was amplified using the PCR in a volume of 50 μ l containing 1 μ l (~100 ng) DNA, 1.5 mmol/l MgCl₂, 1 mmol/l dNTPs, 5 pmol of each primer, and 1 unit of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). The PCR conditions were denaturation (95° for 30 s), annealing (65° for 30 s), extension (72° for 30 s) followed by 35 cycles, and a final extension (72° for 9 min). The sequences of the sense and antisense primers used were 5'-GAAGGCCCTGGACAAGG-3' and 5'-CCCTCTA GGCTCTCCTG GGA-3', respectively. Restriction fragment-length polymorphism was carried out with 3 units of the enzyme *Hae*III for 3 h. The digested products were visualized using ethidium bromide-stained 3% agarose gel electrophoresis. To assure that the genotyping was of adequate quality, we performed random duplicates in 10% of the samples. The assays were performed by a technician who was blind to the phenotype. No genotype errors were detected in the random duplicates. Furthermore, a few variants were confirmed using direct sequencing by an ABI 310 Genetic Analyzer. All genomic studies were carried out at the Madras Diabetes Research Foundation, Chennai, India. Informed consent was obtained from all the patients who participated in this study. Institutional ethical committee approval was obtained for the study.

Statistical analysis

All statistical analyses were done using SPSS PC Windows version 10.0 (SPSS, Chicago, IL). Equality of genotypes and allele frequencies was tested using Fisher's exact test, using *R* (21). Comparison of the age at onset for type 2 diabetic pa-

Table 1—Clinical and biochemical characteristics of the six study groups

	MODY	Very-early-onset type 2 diabetes	Early-onset type 2 diabetes	Late-onset type 2 diabetes	Type 1 diabetes	NGT control group
Age (years)	21 ± 4	20 ± 4	33 ± 5*†	52 ± 8*††	16 ± 9*‡§	58 ± 7*†‡§
BMI (kg/m ²)	24.7 ± 4.1	23.5 ± 5.2	25.4 ± 3.9	25.3 ± 4.7	18.8 ± 4.4*†‡§	22.1 ± 4.1*†§
Systolic blood pressure (mmHg)	122 ± 14	128 ± 32	122 ± 14	133 ± 18*‡	110 ± 17*†‡§	126 ± 19‡
Diastolic blood pressure (mmHg)	80 ± 7	80 ± 8	79 ± 10	80 ± 12*	73 ± 9*§	77 ± 10
Fasting plasma glucose (mmol/l)	11.0 ± 4.1	9.2 ± 3.3	9.5 ± 3.6	9.1 ± 3.8*	13.2 ± 6.1*†‡§	4.6 ± 0.4*†‡§
A1C (%)	9.8 ± 2.4	10.1 ± 2.2	9.2 ± 2.1	8.6 ± 1.9	11.3 ± 3.0‡§	5.7 ± 0.4
Serum total cholesterol (mmol/l)	4.8 ± 1.1	4.9 ± 0.9	5.1 ± 1.1	5.2 ± 1.1*	4.3 ± 0.8*‡§	4.8 ± 0.9
Serum triglycerides (mmol/l)	1.6 ± 1.0	1.6 ± 1.0	2.0 ± 1.3	2.2 ± 1.7*	1.1 ± 0.7*‡§	1.2 ± 0.5‡§
Serum HDL cholesterol (mmol/l)	1.1 ± 0.2	1.1 ± 0.3	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.3*‡§	1.2 ± 0.2‡
Serum LDL cholesterol (mmol/l)	3.0 ± 0.9	3.1 ± 0.8	3.1 ± 0.8	3.1 ± 0.9	2.5 ± 0.7*‡§	3.1 ± 0.9
Fasting serum C-peptide (nmol/l)	1.7 ± 0.7	1.6 ± 0.7	1.4 ± 0.8	1.4 ± 0.8	0.4 ± 0.7*†‡§	2.1 ± 0.9†‡§
Stimulated serum C-peptide (nmol/l)	4.4 ± 2.2	3.3 ± 1.5	3.7 ± 2.4	3.9 ± 1.7	0.8 ± 0.7*†‡§	5.7 ± 1.7*†‡§

Data are means ± SD. *P < 0.001 compared with MODY; †P < 0.001 compared with young-onset type 2 diabetes; ‡P < 0.001 compared with early-onset type 2 diabetes; §P < 0.001 compared with late-onset type 2 diabetes; ||P < 0.001 compared with type 1 diabetes.

tients between genotype groups was done by one-way ANOVA and by comparing the distributions graphically. One-way ANOVA was used to compare means, and χ^2 or Fisher's exact test was used as appropriate to compare proportions.

RESULTS— Table 1 represents the clinical and biochemical variables of the six study groups. Patients with type 1 diabetes were younger compared with the other study groups ($P < 0.001$). Systolic and diastolic blood pressure levels were higher among patients with late-onset type 2 diabetes compared with MODY and early-onset type 2 diabetes ($P < 0.001$). Type 1 diabetic patients had higher fasting plasma glucose and A1C levels compared with the other study groups. Fasting serum levels of total cholesterol and triglycerides were higher among late-onset type 2 diabetic patients. Type 1 diabetic patients had the lowest serum C-peptide levels.

The frequency of the Ala/Val and Val/Val genotypes was significantly higher in the MODY compared with the NGT patients (Table 2). The tests of Hardy Wein-

berg equilibrium also showed that there was significant difference in the genotypic frequencies in the MODY group ($P = 0.0213$ for Hardy Weinberg equilibrium). The frequency of minor allele (Val) was highest in the MODY group (16.8%), which was significantly different from the NGT group ($P = 0.0013$). With respect to type 2 diabetes, the frequency of the minor allele increased with decrease in age at diagnosis and was highest in the very-early-onset type 2 diabetic patients, but statistical significance was not reached, probably as a result of small numbers.

We stratified all the non-type 1 diabetic patients, i.e., MODY, very early-onset type 2 diabetic patients, early-onset type 2 diabetic patients, and late-onset type 2 diabetic patients, based on the Ala98Val genotypes and analyzed the age of diabetes diagnosis according to genotype (Fig. 1). The mean ages at onset of diabetes in Ala/Ala, Ala/Val, and Val/Val groups were 35.7, 29.9, and 24.8 years, respectively, with a significant difference between the groups ($P = 0.0002$)

MODY patients with X/Val genotype had significantly higher fasting plasma

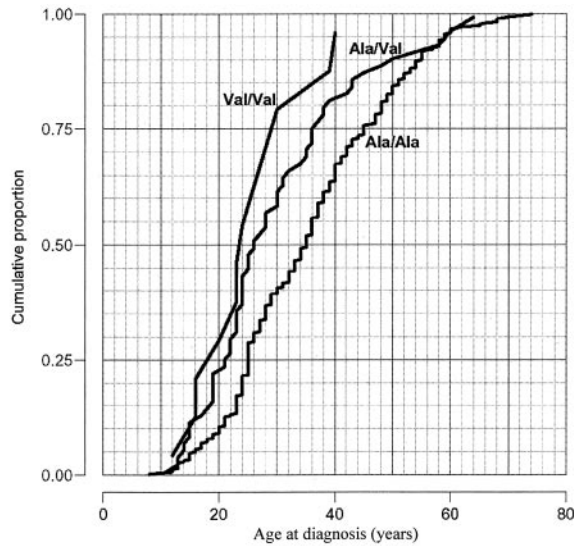
glucose ($P = 0.015$) and A1C levels ($P = 0.04$) compared with those with Ala/Ala genotype (Table 3). However, the significance disappeared when Bonferroni corrections were performed. This is probably because of the small sample size. The β -cell secretory capacity was compared in 19 patients with X/Val genotype and 48 patients with Ala/Ala genotype. Patients with X/Val had lower β -cell secretory capacity (63%) when compared with the Ala/Ala patients (91%), but this did not reach statistical significance. There was no significant difference in the clinical or biochemical variables between the patients with Ala/Ala genotype and X/Val genotypes in the other study groups (data not shown).

CONCLUSIONS— India already has the largest number of people with diabetes (22), and the age at onset of type 2 diabetes occurs at least a decade earlier (2). Hence, it is not clear whether the cut off <25 years proposed in the original Tattersal and Fajans (1) definition of MODY is appropriate for Indians. One of the aims of this study, therefore, was to

Table 2—Distribution of genotype and minor allele in the study groups

	n	Ala/Ala	Ala/Val	Val/Val	P for HWE	Val (%)	P vs. NGT
MODY	122	88 (72.1)	27 (22.1)	7 (5)	0.0213	16.8	0.0013
Very-early-onset type 2 diabetes	23	18 (80)	5 (20)	0	0.5586	10.9	0.3306
Early-onset type 2 Diabetes	171	144 (84.2)	22 (12.8)	5 (2.9)	0.0016	9.4	0.1682
Late-onset type 2 diabetes	133	121 (90.9)	12 (9)	0	0.5859	4.5	0.3291
Type 1 diabetes	150	138 (92)	12 (8)	0	0.6098	4.0	0.1746
NGT subjects	130	113 (87)	17 (13)	0	0.4251	6.5	

Data are n (%) or % unless otherwise indicated. HWE, Hardy Weinberg equilibrium.



Genotype	Age at diagnosis		
	Mean	Difference to Ala/Val (Years)	Difference to Val/Val (Years)
Ala/Ala	35.7 (34.4-37.1)	5.8 (2.3-9.3)	11.0 (3.4-18.6)
Ala/Val	29.9 (26.7-33.1)		5.2 (-3.0-13.3)
Val/Val	24.8 (17.3-32.2)		

Figure 1—Cumulative proportion of type 2 diabetic subjects with the three different genotypes plotted against age at diagnosis of diabetes. Data in parentheses are 95% CIs. $P = 0.0002$.

see whether a specific genetic marker for MODY could distinguish it from type 2 diabetes including its very-early-onset forms. This study shows that among Asian Indians, the Ala98Val polymorphism of HNF1 α is significantly associated with MODY and also with an earlier age of onset of type 2 diabetes and hence does not help distinguish these two forms of diabetes.

The mean age at onset of the Val/Val group was 5.2 years younger than that in the Ala/Val group, which in turn was 5.8 years younger than in the Ala/Ala group. This is clearly reflected in the frequency of the Val allele increasing with decreasing age at onset of type 2 diabetes (Fig. 1). Interestingly, the G319S variant of HNF-1 α , a major susceptibility gene for type 2 diabetes in the Oji-Cree Native Canadian population, is also associated with a significantly lower age at onset of diabetes (23). However, in Danish (11) and Swedish (13) populations, no significant difference was seen in the allele/genotype frequency of the Ala/Val polymorphism when comparing type 2 diabetic patients with glucose-tolerant patients (11,12) or when comparing patients with gestational diabetes with glucose-tolerant patients (24). An earlier study on South Indians

had shown an association of this polymorphism with type 2 diabetes (25), but it did not deal with MODY or with age at diagnosis of type 2 diabetes.

Because HNF1 α has a wide tissue dis-

tribution, defects in the HNF1 α gene might therefore affect glucose homeostasis at several organ levels, including small intestine, liver, and pancreatic β -cells (26). Earlier reports have shown that the Val allele is associated with decreased β -cell function (11,12). Indirect estimation of β -cell secretory capacity showed decreased serum C-peptide levels in X/Val compared with Ala/Ala MODY patients in our study. A previous report (27) showed that serum C-peptide responses to a glucose load were lower in Asian Indian MODY patients (diagnosed based on clinical grounds) compared with a matched group of control patients.

Carriers of the Val allele among MODY patients had significantly higher levels of fasting plasma glucose ($P = 0.015$) and A1C ($P = 0.04$) but lower C-peptide levels compared with Ala/Ala patients (Table 3). This suggests that the MODY patients with the Val allele probably have poorer β -cell function, and this may contribute to worse glycemic control.

The original definition of Tattersall and Fajans (1) was conceived 30 years ago when “control of hyperglycemia” had a different connotation than today, when much tighter glycemic control of diabetes is expected. The A1C levels of all patient groups included in this study, including the MODY patients, are undoubtedly high. However, ours is a tertiary referral center for diabetes, and physicians typi-

Table 3—Clinical and biochemical characteristics of MODY subjects

	Ala/Ala	X/Val	P value
n	88	34	
Age (years)	21 \pm 4	20 \pm 4	0.240
BMI (kg/m ²)	24.7 \pm 4.1	24.9 \pm 4.2	0.794
Systolic blood pressure (mmHg)	121 \pm 15	123 \pm 13	0.553
Diastolic blood pressure (mmHg)	80 \pm 7	80 \pm 7	0.866
Fasting plasma glucose (mmol/l)	10.3 \pm 4.1	12.3 \pm 3.6	0.015
A1C (%)	9.5 \pm 2.5	10.6 \pm 3.6	0.04
Serum total cholesterol (mmol/l)	4.8 \pm 1.1	5.0 \pm 0.8	0.429
Serum triglycerides (mmol/l)	1.6 \pm 1.1	1.6 \pm 0.8	0.949
Serum HDL cholesterol (mmol/l)	1.1 \pm 0.2	1.1 \pm 0.2	0.934
Serum LDL cholesterol (mmol/l)	2.9 \pm 1.0	3.1 \pm 0.7	0.331
Fasting serum C-peptide (nmol/ml)	1.8 \pm 0.6	1.5 \pm 0.6	0.744
Stimulated serum C-peptide (nmol/ml)	4.5 \pm 2.1	3.9 \pm 2.1	0.314
β -Cell secretory capacity (%)	91 \pm 74	63 \pm 40	0.121
Treatment			
Diet + exercise	2 (2.3)		0.53
OHA	63 (76.1)	24 (71)	
OHA + insulin	8 (9.5)	6 (18)	
Insulin	10 (12)	4 (12)	

Data are means \pm SD or n (%) unless otherwise indicated. OHA, oral hypoglycemic agent.

cally refer their cases to us when a patient's diabetes is very poorly controlled. The A1C levels are the values at the time of first registration at our center, and subsequently, they were brought down after optimal treatment of diabetes.

One of the potential concerns of a study such as this could be that the population studied may not be genetically homogeneous, thereby resulting in population stratification, which could affect the analyses. To address this issue, we did a cross-validation for the presence of population stratification using genomic controls (28). We performed a case-control study at five unlinked marker loci believed to be unrelated to the disease under study but known to have allelic diversity among different populations. These loci were: Alu Repeat TPA-25 (subfamily HS-2) on chromosome 8, Alu Repeat PV-92 (subfamily HS-1) on chromosome 16, Alu Repeat FXIIB (subfamily HS-1) on chromosome 1, Alu Repeat ACE (subfamily HS-1) on chromosome 17, and Alu Repeat D1 (subfamily HS-1) on chromosome 3, which have been found to have variable allele frequencies among different populations of South India (29). The allele frequency difference between diabetic and NGT patients was not statistically significant at any of the five loci, indicating that the findings in this study are not an artifact of population substructuring.

In summary, this study shows that in Asian Indians, the Ala98Val polymorphism of HNF1 α is associated with MODY and with earlier age at onset of type 2 diabetes. Further studies are needed on the functional significance of this modifier gene variant.

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This is the 13th paper from the CURES study. All genomic studies were carried out at Madras Diabetes Research Foundation, Chennai, India.

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