Table 1.

Antibodies	Newly diagnosed diabetic patients ($n = 31$)	Diabetic patients long-standing ($n = 37$)	EMA positive		[a]Abnormal biopsies	
			[b]ND	[c]LS	[b]ND	[c]LS
tTGA, GADA, IA-2A	3	5	1	5	1	5
tTGA, GADA	1	5	0	3	–	3[d]
tTGA, IA-2A	1	1	0	1	–	1
GADA-IA-2A	12	4	0	0	–	–
tTGA	1	1	1	1	1	1
GADA	4	2	0	0	–	–
IA2A	4	6	0	0	–	–
No antibodies	5	13	0	0	–	–

[a] Only EMA positive patients were biopsied
[b] ND: newly diagnosed
[c] LS: long-standing
[d] 2 with total villous atrophy and 1 with "latent" coeliac disease

toimmune reaction [5]. Moreover one study [6] found a greater persistence of GADA in the patients with long-standing diabetes with signs of other autoimmune diseases, such as high concentrations of thyroid autoantibodies [6].

It is known that Type I diabetes is frequently associated with coeliac disease [7]. Recently it has been suggested that tTGA could represent a sensitive test replacing EMA detection for diagnosis and screening of coeliac disease. Because multiple immunological abnormalities are frequently reported at the time of diabetes diagnosis [8], our results might suggest that the detection of tTG-IgA in newly diagnosed patients could reflect an abnormal immunological response in the early stage of diabetes, with increased production of antibodies by specific clones of B lymphocytes, instead of markers of coeliac disease.

Yours faithfully,
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References

1. Lampasona V, Bonfanti R, Bazzigaluppi E et al. (1999) Antibodies to tissue transglutaminase C in Type I diabetes. Diabetologia 42: 1195–1198
2. Dieterich W, Ehnis T, Bauer M et al. (1997) Identification of tissue transglutaminase as the autoantigen of coeliac disease. Nat Med 3: 797–801
3. Bazzigaluppi E, Lampasona V, Barera G et al. (1999) Comparison of tissue transglutaminase-specific antibody assays with established antibody measurements for coeliac disease. J Autoimmun 12: 51–56
4. Seissler J, Schott M, Boms S et al. (1999) Autoantibodies to human tissue transglutaminase identify silent coeliac disease in Type I diabetes. Diabetologia 42: 1440–1441
5. Hermitte L, Atlan-Gepner C, Mattei C et al. (1998) Diverging evolution of anti-GAD and anti-IA-2 antibodies in long-standing diabetes mellitus as a function of age at onset: no association with complications. Diabet Med 15: 586–591
6. Yokota I, Matsuda J, Naito E, Ito M, Shima K, Kuroda Y (1998) Comparison of GAD and ICA512/IA-2 antibodies at and after the onset of IDDM. Diabetes Care 21: 49–52
7. Lorini R, Scotta MS, Cortona L et al. (1996) Celiac disease and type 1 (insulin-dependent) diabetes mellitus in childhood: follow-up study. J Diabetes Complications 10: 154–159
8. Atkinson MA, Maclaren NK (1994) The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 331: 1428–1436

Nature or nurture: an insightful illustration from a Chinese family with hepatocyte nuclear factor-1α diabetes (MODY3)

Dear Sir,
Mutations in the gene encoding the transcription factor hepatocyte nuclear factor (HNF)-1α are one of the most common causes of maturity-onset diabetes of the young (MODY3) [1]. Here we report the clinical course of HNF-1α diabetes/MODY3 in a Chinese family with early-onset diabetes and severe complications (Fig. 1) [2]. This family highlights the importance of early diagnosis and prompt treatment in the improvement of clinical outcomes even in genetically susceptible subjects.

Three family members had severe diabetic complications when they were referred to our hospital in 1987. The proband (III-5), 19 years of age, had severe proliferative retinopathy, heavy proteinuria (1.4 g protein a day) and necrobiosis lipoidica. She had been diagnosed with Type II (non-insulin-dependent) diabetes mellitus 3 months earlier and was treated with glibenclamide. We initiated retinal photocoagulation treatment and started her on insulin and an ACE inhibitor. She subsequently developed hypertension and progressed to end-stage renal disease by the age of 30 years, requiring dialysis. Her mean HbA_{1c} was 8.0 % over the years. She is currently receiving 42 units of insulin.

Her older sister (III-2) had a vitreous haemorrhage and had been treated with insulin since diagnosis at the age of 24 years. She became blind and had nephropathy (0.8 g protein a day) 2 years later. She is currently treated with insulin (16 units) and an ACE inhibitor, with a mean HbA_{1c} of 6.4 %.

The subject's mother (II-3) had glycosuria complicating pregnancy when she was 33 years old. She was diagnosed to have Type II diabetes at the age of 38 years and was then treated with glibenclamide for 10 years. She came to our attention through the index case, with proliferative retinopathy, nephropathy, peripheral neuropathy, necrobiosis lipoidica, hypertension and cataracts. We commenced insulin treatment (20 units) and her HbA_{1c} was reduced from 17.2 % to 9.2 % within 8 months. Two months later, she had a myocardial infarction followed by progressive deterioration of cardiac and renal functions. She died of pulmonary oedema and septicaemia with a gangrenous foot at the age of 52 years.

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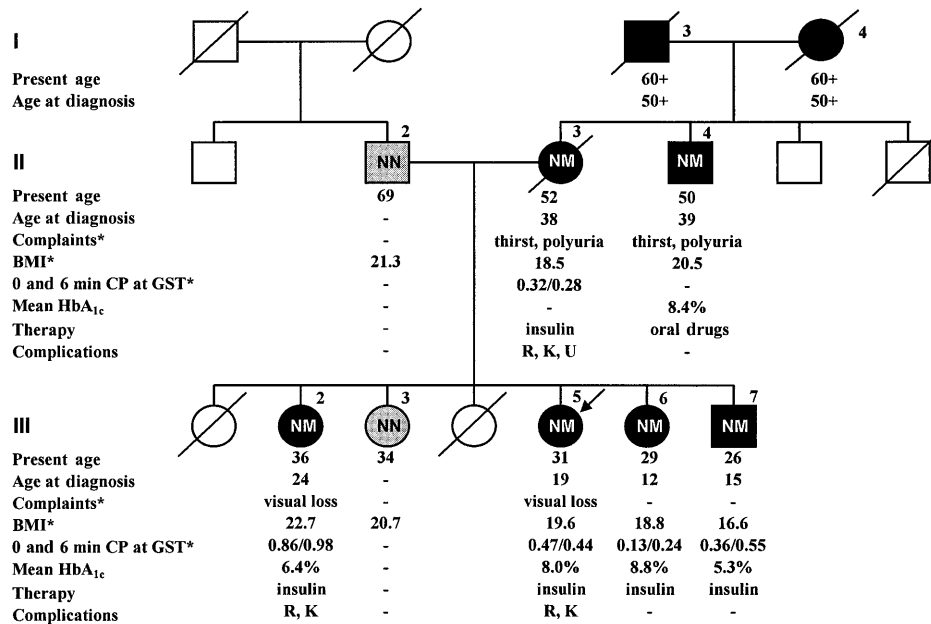

Fig. 1. Pedigree of a Chinese family with HNF-1α IVS2nt-1G→A mutation. Subjects with diabetes are represented by black symbols, subjects with IGT by grey symbols and untested ones by open symbols. The genotype of family members is indicated: N, normal allele; and M, mutant allele. The proband is indicated by an arrow. CP, C-peptide; GST, glucagon stimulation test; Complications: R, retinopathy; K, nephropathy; U, neuropathy. *Data in 1987 [2]

The fourth daughter (III-6) had been treated with insulin since her incidental diagnosis of diabetes at the age of 12 years after a nasal polypectomy. She is currently receiving 68 units of insulin, with a mean HbA_{1c} of 8.8 %.

Two other family members underwent screening by OGTT in 1987. The second daughter (III-3) fluctuated between having normal glucose tolerance and IGT over the last 11 years. A brother (III-7) had overt diabetes on screening with an initial HbA_{1c} of 10.5 %. Insulin was started after 3 months of dietary treatment. He is currently receiving 26 units of insulin, with a mean HbA_{1c} of 5.3 %.

One maternal uncle (II-4) was diagnosed with diabetes and hyperlipidaemia with thirst and polyuria at the age of 39 years. He has been treated with oral drugs since diagnosis, with a mean HbA_{1c} of 8.4 %. His children were not available for detailed genetic testing and clinical assessment. The affected members II-4, III-6 and III-7 have remained free of complications despite all having had diabetes for more than 10 years.

The father was diagnosed with IGT in 1998. He was non-obese and had hyperlipidaemia.

Sequencing of the *HNF-1α* gene in this family showed a novel splice acceptor site mutation (AG→AA) in intron 2 (IVS2nt-1G→A) which cosegregated with diabetes (Fig. 1) [3]. This mutation is expected to produce a non-functional mRNA. All the diabetic members, including the maternal uncle, (II-4, III-2, III-5, III-6 and III-7) were heterozygous for this mutation but the father (II-2) and the daughter (III-3) with IGT did not have the mutation. Thus it was very likely that the mother (II-3) for whom no DNA sample was available also carried this mutation. As with other subjects with HNF-1α diabetes [4], most affected family members exhibited defective pancreatic beta-cell function as assessed by the glucagon stimulation test. The mother and all the affected siblings, except subject III-2, were insulin deficient based on a definition of post-glucagon (1 mg intravenously) stimulated plasma C peptide at 6 min of less than 0.6 nmol/l (0.24–0.55 nmol/l respectively) [5]. The brother, III-7, who was diagnosed with diabetes by OGTT was also insulin deficient. All the *HNF-1α* mutant carriers, except II-4, required insulin treatment for glycaemic control.

Although all affected family members carried the same *HNF-1α* gene mutation, their clinical courses have varied tremendously. Severe complications were present in those family members whose diagnosis was delayed and who were presumed to have poor glycaemic control before diagnosis (II-3, III-2 and III-5). They were, however, absent in the uncle (II-4) and the younger siblings (III-6 and III-7) who were promptly diagnosed and had appropriate treatment despite having had diabetes for more than 10 years (Fig. 1). This is in accordance with a recent report suggesting poor glycaemic control is associated with a twofold to threefold increased risk among MODY3 patients of developing microalbuminuria and retinopathy, respectively [6].

It is noteworthy that both maternal grandparents (I-3 and I-4) had diabetes diagnosed in their late 50s. The effect of this bilineality on the natural course of HNF-1α diabetes in this family is uncertain. It is, however, possible the non-MODY maternal grandparent transmitted a modifier gene affecting the age at onset or severity of the diabetes in carriers with the *HNF-1α* mutation. The age at diagnosis of diabetes in this family was increasingly younger with successive generations despite all carriers being relatively non-obese. This earlier diagnosis could be due to ascertainment bias or, as we prefer, an epiphenomenon due to increasing westernisation of the Hong Kong lifestyle with increased intake of high fat food and decreased physical activity [7]. This highlights the important influence of environment interacting with genetics in the natural course of HNF-1α diabetes. In conclusion, this report emphasises the need for early diagnosis by glucose tolerance testing or genetic screening, and appropriate treatment in patients who have a strong family history of diabetes, especially those with early onset disease and insulin deficiency.

Yours sincerely,

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References

1. Frayling TM, Bulman MP, Ellard S et al. (1997) Mutations in the hepatocyte nuclear factor-1α gene are a common cause of maturity-onset diabetes of the young in the U.K. Diabetes 46: 720–725
2. Chan JC, Hawkins BR, Cockram CS (1990) A Chinese family with non-insulin-dependent diabetes of early onset and severe diabetic complications. Diab Med 7: 211–214
3. Ng MC, Cockburn BN, Lindner TH et al. (1999) Molecular genetics of diabetes mellitus in Chinese: identification of mutations in glucokinase and hepatocyte nuclear factor-1α genes in patients with early-onset type 2 diabetes mellitus/MODY. Diab Med 16: 956–963
4. Byrne MM, Sturis J, Menzel S et al. (1996) Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY3 on chromosome 12. Diabetes 45: 1503–1510
5. Service FJ, Rizza RA, Zimmerman BR, Dyck PJ, O'Brien PC, Melton LJ (1997) The classification of diabetes by clinical and C-peptide criteria. Diabetes Care 20: 198–201
6. Isomaa R, Henricsson M, Lehto M et al. (1998) Chronic diabetic complications in patients with MODY3 diabetes. Diabetologia 41: 467–473
7. Chan JC, Cockram CS (1997) Diabetes mellitus in Chinese and its implications on health care. Diabetes Care 20: 1785–1790

A simple test for the high-frequency P291fsinsC mutation in the HNF1α/MODY3 gene

Dear Sir,

Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous form of diabetes characterised by autosomal dominant inheritance, onset usually before the age of 25 and beta-cell dysfunction. The most common subtype in many populations seems to be MODY3 which is caused by mutations in the gene encoding hepatocyte nuclear factor (HNF)-1α. A mutation in exon 4 of *HNF1*α, namely P291fsinsC, is particularly prevalent among Caucasian patients. The nature of this MODY3 hot spot mutation is an insertion of an extra cytosine into a polycytidine tract consisting of eight residues, and it leads to a truncated protein which can act in a dominant-negative manner [1]. In materials of suspected MODY families, 30–70 % have been reported to possess mutations in the *HNF1*α gene [2–4]. P291fsinsC has been observed to occur in as many as 50 % of identified MODY3 families [4] but a proportion of 20–30 % seems more likely [1–3]. In a study of 60 MODY probands defined by strict criteria, eight P291fsinsC-positive subjects were found [5]. Thus, 10–15 % of all suspected MODY patients might bear this particular mutation.

When starting an investigation on the genetic epidemiology of MODY in Norway, we therefore found it reasonable first to screen suspected MODY families for P291fsinsC. The only published test specifically aiming at identifying P291fsinsC [5] is based on measuring size of a fluorescently labelled PCR product on an automated DNA sequencer. We decided to design a restriction enzyme-based test that would be simpler in use.

The insertion of a cytosine in codon 291 neither deletes an existing restriction site nor creates a new one. When designing the test, we therefore introduced an "artificial" restriction enzyme site by a mismatch (underlined) in the forward primer, 5′-CATGGACACGTACAGCGG(G/C)CCCCC-CTC-3′; thereby creating a *Bsu*36I site only for non-mutated subjects. To take into account the G-to-C polymorphism in codon 288 [2], the forward primer was a 1:1 mixture of two primers with G or C as the 19th base. As reverse primer we selected 5′-CCTCCCCAACCCTCCCACCTTAGGACGTGTCC-CTTG-3′, where the mismatch (underlined) always introduces a *Bsu*36I site serving as an internal control for restriction enzyme function. The size of the PCR product from the normal allele is 164 base pairs (bp) and digestion with *Bsu*36I yields fragments of 117 bp, 25 bp and 22 bp. The product size from the P291fsinsC allele is 165 bp and *Bsu*36I digestion gives rise to fragments of 143 bp and 22 bp.

We first carried out PCR amplifications with a standard PCR enzyme (AmpliTaq DNA polymerase; PE Corporation, Norwalk, Conn., USA) under conditions recommended by the manufacturer. We observed, however, an unexpected result as the restriction enzyme digest identified all normal subjects as heterozygotes for the mutation. It is well known that there is a tendency for DNA polymerases to introduce single-base deletions or additions when replicating stretches of iterated nucleotides. The rate of frame-shifting increases with the number of repeated nucleotides, and the postulated mechanism is misalignment of the growing DNA strand during replication (DNA slippage) [6]. This phenomenon could itself be the molecular basis for the relatively high frequency of P291fsinsC.

We therefore assumed that the AmpliTaq polymerase encountered fidelity problems when it amplified the hot spot area, resulting in frame-shifting and a partial loss of the *Bsu*36I site also in the PCR product from normal subjects. Of four other commercially available DNA polymerases that were tested, a modified version of *Taq* polymerase (SuperTaq; HT Biotechnology, Cambridge, UK) had the best ability to discern between normal and P291fsinsC alleles. The PCR amplification was done in a Perkin Elmer 2400 thermoycler using 0.15 U of SuperTaq and 200 ng genomic DNA per 50 μl reaction. The reaction mix consisted of 1 μmol/l of each primer, 200 μmol/l of each deoxyribonucleoside triphosphate (dNTP), 10 mmol/l TRIS-HCl (pH 9.0), 1.5 mmol/$MgCl_2$, 50 mmol/KCl and 0.1 % Triton X-100. An initial denaturation step at 94 °C for 2 min was followed by 35 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 1 min, and a final incubation at 72 °C for 7 min.

The P291fsinsC mutation is the disease-causing mutation of N1, the first registered MODY family in Norway [7, 8]. It was originally found by sequencing and we re-examined DNA samples from family N1 with the restriction enzyme-based test. A part of the N1 pedigree with the corresponding restriction pattern is shown in Figure 1. The test was further evaluated by screening DNA from twelve unrelated MODY3 patients with or without the hot spot mutation and with or without the G-to-C polymorphism in codon 288. Ten samples were from the University of Chicago and two samples were kindly provid-

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