Urinary C-Peptide Creatinine Ratio Is a Practical Outpatient Tool for Identifying Hepatocyte Nuclear Factor 1-α/Hepatocyte Nuclear Factor 4-α Maturity-Onset Diabetes of the Young From Long-Duration Type 1 Diabetes

RACHEL E.J. BESSER, MBBS[1]
MAGGIE H. SHEPHERD, PHD[1]
TIMOTHY J. MCDONALD, MSC[1,2]
BEVERLEY M. SHIELDS, PHD[1]
BRIDGET A. KNIGHT, PHD[1]
SIAN ELLARD, PHD[1,3]
ANDREW T. HATTERSLEY, DM[1]

OBJECTIVE—Hepatocyte nuclear factor 1-α (HNF1A)/hepatocyte nuclear factor 4-α (HNF4A) maturity-onset diabetes of the young (MODY) is frequently misdiagnosed as type 1 diabetes, and patients are inappropriately treated with insulin. Blood C-peptide can aid in the diagnosis of MODY, but practical reasons limit its widespread use. Urinary C-peptide creatinine ratio (UCPCR), a stable measure of endogenous insulin secretion, is a noninvasive alternative. We aimed to compare stimulated UCPCR in adults with HNF1A/4A MODY, type 1 diabetes, and type 2 diabetes.

RESEARCH DESIGN AND METHODS—Adults with diabetes for ≥5years, without renal impairment, were studied (HNF1A MODY [*n* = 54], HNF4A MODY [*n* = 23], glucokinase MODY [*n* = 20], type 1 diabetes [*n* = 69], and type 2 diabetes [*n* = 54]). The UCPCR was collected in boric acid 120 min after the largest meal of the day and mailed for analysis. Receiver operating characteristic (ROC) curves were used to identify optimal UCPCR cutoffs to differentiate HNF1A/4A MODY from type 1 and type 2 diabetes.

RESULTS—UCPCR was lower in type 1 diabetes than HNF1A/4A MODY (median [interquartile range]) (<0.02 nmol/mmol [<0.02 to <0.02] vs. 1.72 nmol/mmol [0.98–2.90]; $P <$ 0.0001). ROC curves showed excellent discrimination (area under curve [AUC] 0.98) and identified a cutoff UCPCR of ≥0.2 nmol/mmol for differentiating HNF1A/4A MODY from type 1 diabetes (97% sensitivity, 96% specificity). UCPCR was lower in HNF1A/4A MODY than in type 2 diabetes (1.72 nmol/mmol [0.98–2.90] vs. 2.47 nmol/mmol [1.4–4.13]); $P = 0.007$). ROC curves showed a weak distinction between HNF1A/4A MODY and type 2 diabetes (AUC 0.64).

CONCLUSIONS—UCPCR is a noninvasive outpatient tool that can be used to discriminate HNF1A and HNF4A MODY from long-duration type 1 diabetes. To differentiate MODY from type 1 diabetes of >5 years' duration, UCPCR could be used to determine whether genetic testing is indicated.

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Maturity-onset diabetes of the young (MODY) describes dominantly inherited young-onset non–insulin-dependent diabetes (often <25 years' duration) with persistent endogenous insulin secretion (1,2). Heterozygous activating mutations in the transcription factor genes hepatocyte nuclear factor 1-α (HNF1A) and hepatocyte nuclear factor 4-α (HNF4A) are common causes of monogenic diabetes, accounting for 52 and 10% of MODY cases, respectively (3)

Recognition and genetic diagnosis of HNF1A/4A MODY is crucial for optimal management. HNF1A/4A MODY frequently is misdiagnosed as type 1 diabetes because patients are diagnosed young (mean age of diagnosis: HNF1A age 20 years and HNF4A age 23 years) and are nonobese (4–6). In our diagnostic genetics service in Exeter, of 405 referrals for HNF1A MODY, 140 (35.5%) initially were misdiagnosed with type 1 diabetes and inappropriately treated with insulin (R.E.J.B., S.E., A.T.H., unpublished data). Patients are extremely sulfonylurea sensitive and, once diagnosed, often are able to stop insulin treatment (6–8).

Genetic testing is needed to confirm HNF1A and HNF4A MODY, but it is too costly to be used to screen all patients with diabetes. An alternative cheap and practical biomarker to identify patients with a higher probability of HNF1A/4A MODY for genetic testing would improve diagnosis.

Various biomarkers have been proposed (apoliprotein M, complement 5 [C5], complement 8 [C8], transthyretin [TTR], and 1,5-anhydroglucitol). All have attempted to discriminate HNF1A or HNF4A MODY from type 2 diabetes rather than type 1 diabetes (9–13), but they were not sufficiently accurate for routine clinical use.

From the [1]Peninsula National Institute for Health Research Clinical Research Facility, Peninsula Medical School, University of Exeter, Exeter, U.K; the [2]Department of Clinical Biochemistry, Royal Devon and Exeter National Health Service Foundation Trust, Exeter, U.K.; and the [3]Department of Molecular Genetics, Royal Devon and Exeter National Health Service Foundation Trust, Exeter, U.K.
Corresponding author: Andrew T. Hattersley, andrew.hattersley@pms.ac.uk.
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C-peptide is a good candidate biomarker to differentiate patients with MODY from type 1 diabetes. C-peptide is a polypeptide cosecreted in equimolar amounts with insulin. Measurement of blood C-peptide is available in hospital and will identify patients who still are making their own insulin. In type 1 diabetes, autoimmune destruction of β-cells results in absolute insulin deficiency, usually within 5 years of diagnosis (14). In HNF1A and HNF4A MODY, β-cell function is maintained despite a reduction in insulin secretion and progressive hyperglycemia (15,16). Blood C-peptide outside the honeymoon period should alert the clinician to a diagnosis of MODY (17). Although blood C-peptide is available, it is not routinely measured because of practical limitations. The rapid degradation by proteases means that samples need to be separated and frozen prior to analysis, restricting testing to the hospital setting (18). C-peptide usually is measured after stimulation with a mixed meal or glucagon to detect β-cell reserve (19), and the need to discontinue short-acting insulin prior to testing may further limit its clinical use.

C-peptide is renally metabolized, and 5–10% of C-peptide is excreted unchanged in urine (20). Urinary C-peptide, creatinine ratio (UCPCR) is a useful alternative method to measuring C-peptide, and the use of a creatinine ratio accounts for the effects in urine concentration. UCPCR is stable for 3 days in boric acid preservative at room temperature, offering the potential to use postal samples in the community (21). No studies to date have used UCPCR to differentiate MODY from other types of diabetes.

We hypothesize that endogenous insulin secretion persists in HNF1A/4A MODY, and UCPCR will discriminate HNF1A/4A MODY from type 1 in long-standing diabetes. The aim of this study was to compare stimulated UCPCR in adults with HNF1A/4A MODY, type 1 diabetes, and type 2 diabetes.

RESEARCH DESIGN AND METHODS

—Adults with diabetes of ≥5 years' duration were identified from existing research databases at the Peninsula Medical School, Exeter, U.K., and from routine diabetes clinics. Because C-peptide is metabolized largely in the kidney, patients were excluded from taking part if they had known renal impairment (estimated glomerular filtration rate <60 ml/min per 1.73 m^2). A total of 97 patients with a genetic diagnosis of MODY were recruited (54 patients with mutations in the HNF1A gene, 23 with HNF4A mutations, and 20 with glucokinase [GCK] mutations). A total of 69 patients with type 1 diabetes were studied (defined as those who had an age of diagnosis of <30 years and on insulin since diagnosis) as well as 54 patients with type 2 diabetes (defined as those with an age of diagnosis of ≥35 years and not on insulin for the first year of diagnosis). Patients were contacted initially either by letter or by their health care professional. Follow-up contact was made by telephone by the research team, and the following information was documented: age of diagnosis, current medication, A1C, creatinine, weight, and height. Consent was gained to contact the patient's general practitioners for missing information.

Sample collection

Urine containers with boric acid preservative were mailed to participants. A urine sample was collected 120 min after completing the largest meal of the day, having voided prior to eating. Participants took their normal medication during the test. The sample was either mailed the same day or refrigerated overnight and mailed first class to the research laboratory the following morning. Samples were analyzed only if they reached the biochemistry laboratory within 72 h of the sample void.

Ethics

The study was approved by the Devon and Torbay Research and Ethics Committee. All subjects gave informed consent.

UCPCR analyses

Urinary C-peptide was measured by electrochemiluminescence immunoassay (intra-assay coefficient of variation <3.3%; interassay coefficient of variation <4.5%) on a Roche Diagnostics (Mannheim, Germany) E170 analyzer by the biochemistry department at the Royal Devon and Exeter National Health Service Foundation Trust, Exeter, U.K. Urinary creatinine was analyzed on the Roche P800 platform using creatinine Jaffé reagent (standardized against isotope dilution mass spectrometry) to UCPCR (nmol/mmol).

Statistical analysis

Results are presented as medians (interquartile range), unless otherwise stated. Patients with HNF1A and HNF4A MODY were combined for the purposes of the analyses because there was no significant difference in UCPCR values ($P = 0.87$), clinical characteristics, or treatment between the two groups (Supplementary Table 1).

Characteristics of patients in the HNF1A/4A MODY and type 1 diabetic (or type 2 diabetic) groups were compared using a χ^2 or generalized Fisher exact test for categorical data (sex and treatment) and Mann-Whitney *U* test for continuous variables not normally distributed (age of diagnosis, current age, diabetes duration, BMI, and UCPCR). Receiver operating characteristic (ROC) curves were used to identify cutoffs of UCPCR that provided the optimal sensitivity and specificity for discriminating HNF1A/4A MODY from type 1 diabetes (which we defined as absolute insulin deficiency) and HNF1A/4A MODY from type 2 diabetes. Analysis was repeated examining only those who were treated with insulin. Likelihood ratios were calculated to estimate the effect of a UCPCR value above the cutoff identified to discriminate between HNF1A/4A MODY and type 1 or type 2 diabetes to calculate a posttest probability.

Statistical analysis was performed using SPSS version 15, and $P < 0.05$ was assumed to be significant.

Assessment of patients with type 1 diabetes with persistent C-peptide

Type 1 diabetic patients who were identified as having UCPCR values greater than the identified cutoff for insulin deficiency (as defined by ROC analysis) provided a repeat urine sample and were contacted again for a mixed-meal tolerance test. Patients arrived having fasted and were given an Ensure high-protein milkshake (6 ml/kg, maximum 360 ml). A sample was taken at 90 min for measurement of stimulated serum C-peptide (19), with a value of 0.2 nmol/L indicating persistent endogenous insulin production (14,22). These patients also were tested for GAD and IA2 antibodies, and the HNF1A and HNF4A genes were sequenced. Pancreatic antibodies were considered positive if >99th percentile of 500 control samples.

RESULTS

—Patient characteristics are presented in Table 1.

HNF1A/4A MODY versus type 1 diabetes

Postprandial UCPCR was markedly lower in type 1 diabetes than HNF1A/4A MODY

Table 1—*Patient characteristics*

	HNF1A/4A MODY	Type 1 diabetes	*P* value (MODY vs. type 1 diabetes)	Type 2 diabetes	*P* value (MODY vs. type 2 diabetes)
n (% male)	77 (42.9)	69 (47.8)	0.55	54 (44.4)	0.86
Age of diagnosis (years)	22 (15–30)	13 (8–19)	<0.0001*	60 (50.8–65.3)	<0.0001*
Age (years)	46.6 (38.1–64.3)	45.4 (37–58.4)	0.21	72 (66.3–75.4)	<0.0001
Duration of diabetes (years)	23.1 (14.7–35.4)	33.5 (21.9–42.2)	0.002*	11.4 (8.7–15.3)	<0.0001*
BMI (kg/m^2)	24.3 (22.4–26.7)	26.3 (24.0–29.0)	0.004*	28.5 (26.1–32.7)	<0.0001*
Treatment (*n*)			<0.0001*		0.48
Diet alone	4	0		6	
OHA alone	42	0		29	
Insulin with or without OHA	31	69		19	
A1C (%)	7.4 (6.6–8.0)†	7.9 (7.3–8.9)	0.001*	7.6 (7.0–8.2)	0.223
Parent affected (*n* [%])	70 (91)	16 (23)			
Three or more consecutive generations affected	64 (83)	6 (9)			
UCPCR (nmol/mmol)	1.72 (0.98–2.9)	<0.02 (<0.02 to <0.02)	<0.0001*	2.47 (1.4–4.13)	0.007*

Data are median (interquartile range), unless otherwise stated. OHA, oral hypoglycemic agent. *Statistical significance. †Missing data for A1C; HNF1A/4A MODY (*n* = 20).

(median [interquartile range]; <0.02 nmol/mmol [<0.02 to <0.02] vs. 1.72 nmol/mmol [0.98–2.90]; $P < 0.0001$) (Fig. 1*A*). ROC curves showed excellent discrimination (area under curve [AUC] 0.98) and identified a cutoff UCPCR ≥0.2 nmol/mmol for discriminating HNF1A/4A MODY from type 1 diabetes with 97% sensitivity and 96% specificity (Fig. 1*B*). This translates to a likelihood ratio of 24 for identifying HNF1A/4A MODY using a UCPCR ≥0.2 nmol/mmol.

This relationship persisted when assessing the discrimination of insulin-treated HNF1A/HNF4A from type 1 subjects (AUC 0.96, 94% sensitivity, and 96% specificity) (Supplementary Fig. 1). We showed that diabetes duration did not interfere with the discriminatory ability of UCPCR by performing further analysis on 50 HNF1A/4A MODY and 50 type 1 diabetic patients, matched within ±2 years' diabetes duration, and a further subanalysis on patients with 5–25 years' diabetes duration (Supplementary results).

UCPCR-positive type 1 diabetes

We went on to study further the 3 of 69 (4%) type 1 diabetic patients with diabetes duration of ≥5 years who had a UCPCR ≥0.2 nmol/mmol. All three patients remained positive on repeat UCPCR testing. Two of three patients exceeded the serum C-peptide concentration that is used to define significant endogenous insulin

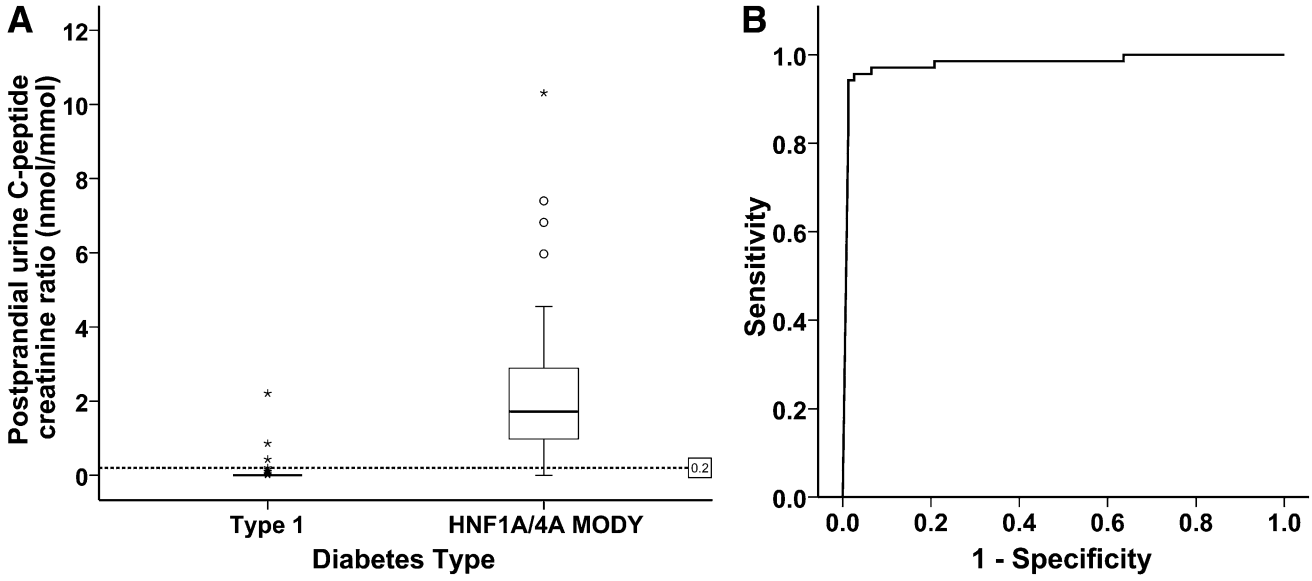

**Figure 1—*Box plot and ROC curve to identify HNF1A/4A MODY from type 1 diabetes.* A*: Box plot to show the UCPCR in HNF1A/4A MODY* (n = *77) and type 1 diabetes* (n = *69). Dotted line indicates a UCPCR cutoff of 0.2 nmol/mmol. Circles, outliers; stars, extreme outliers.* B*: The ROC curve identified a cutoff UCPCR ≥0.2 nmol/mmol for discriminating HNF1A/4A MODY from type 1 diabetes (AUC 0.98) with 97% sensitivity and 96% specificity.*

secretion (0.2 nmol/l) (22), despite diabetes duration of over 25 years (Supplementary Table 2). Two of three patients had either elevated GAD65 or IA2 antibodies (see supplementary material), and none of these patients had mutations in HNF1A and HNF4A genes.

HNF1A/4A MODY versus type 2 diabetes

Although postprandial UCPCR was lower in HNF1A/4A MODY than in type 2 diabetes (1.72 nmol/mmol [0.98–2.90] vs. 2.47 nmol/mmol [1.4–4.13]; P = 0.007), the ROC curve (Fig. 2) showed weak discrimination between HNF1A/4A MODY and type 2 diabetes (AUC 0.64). A UCPCR ≤3.1 nmol/mmol could discriminate HNF1A/4A MODY from type 2 diabetes with 81% sensitivity and 44% specificity. In patients treated with insulin, UCPCR was better, identifying a cutoff of 1.27 for discriminating type 2 diabetes (n = 19) from HNF1A/4A MODY (n = 31), (AUC 0.69, 74% sensitivity, and 68% specificity) (Supplementary Fig. 2). UCPCR results did not differentiate HNF1A/4A MODY from type 2 diabetes in patients treated with oral agents (AUC 0.54, P = 0.55) (Supplementary Fig. 3).

MODY subtypes (HNF1A, HNF4A, and GCK MODY)

There was no difference in UCPCR between HNF1A and HNF4A MODY (1.72 nmol/mmol [0.90–2.90] vs. 1.48 nmol/mmol [1.01–2.93]; P = 0.87), but combined values were significantly lower than GCK MODY (1.72 nmol/mmol [0.98–2.90] vs. 3.1 nmol/mmol [2.16–4.05]; P = 0.002) (Supplementary Table 1).

CONCLUSIONS—In this study, UCPCR showed excellent discrimination between HNF1A/HNF4A MODY and type 1 diabetes in patients >5 years after diabetes diagnosis, suggesting that this is a useful tool to detect patients with possible HNF1A or HNF4A MODY in clinical practice.

UCPCR compared with serum C-peptide

We have shown that a value of ≥0.2 nmol/mmol is highly specific (96%) and sensitive (97%) in discriminating HNF1A/HNF4A MODY from type 1 diabetes when patients are tested >5 years after diagnosis. This high level of discrimination remains even when the MODY patients are treated with insulin. The ability to discriminate whether an insulin-treated patient should be considered for genetic analysis is important for diagnosis and family screening. Finding a UCPCR >0.2 nmol/mmol would suggest that a genetic test may be appropriate. If a diagnosis of HNF1A/4A MODY is made in a patient treated with insulin from diagnosis, this may bring about treatment change (6–8). A diagnosis of monogenic diabetes also has implications for the genetic testing of other family members.

This test cannot be used close to diagnosis because persistent C-peptide is likely to still be present in patients with type 1 diabetes in the first few months and occasionally years after diagnosis (14). In cases where there is an affected parent, this will usually be of long duration at the time the child is first diagnosed. If the parents' duration is >5 years, UCPCR testing and, if appropriate, subsequent genetic testing could be performed in the parent.

This result is not surprising because persistent endogenous insulin secretion resulting in patients not being insulin dependent is a key feature of MODY (1,2). It already has been established that serum C-peptide is likely to be persistent in MODY (1,8,15,16), and this can be used to distinguish it from type 1 diabetes (23). Our results from patients who had both a serum C-peptide measured in a mixed-meal tolerance test and UCPCR show that the results are similar and both can detect relatively low levels of endogenous insulin secretion.

The major advantage of UCPCR over serum C-peptide is its practical utility. Serum C-peptide requires separating the serum by spinning rapidly and subsequent freezing of the sample. This effectively limits its use to hospital settings

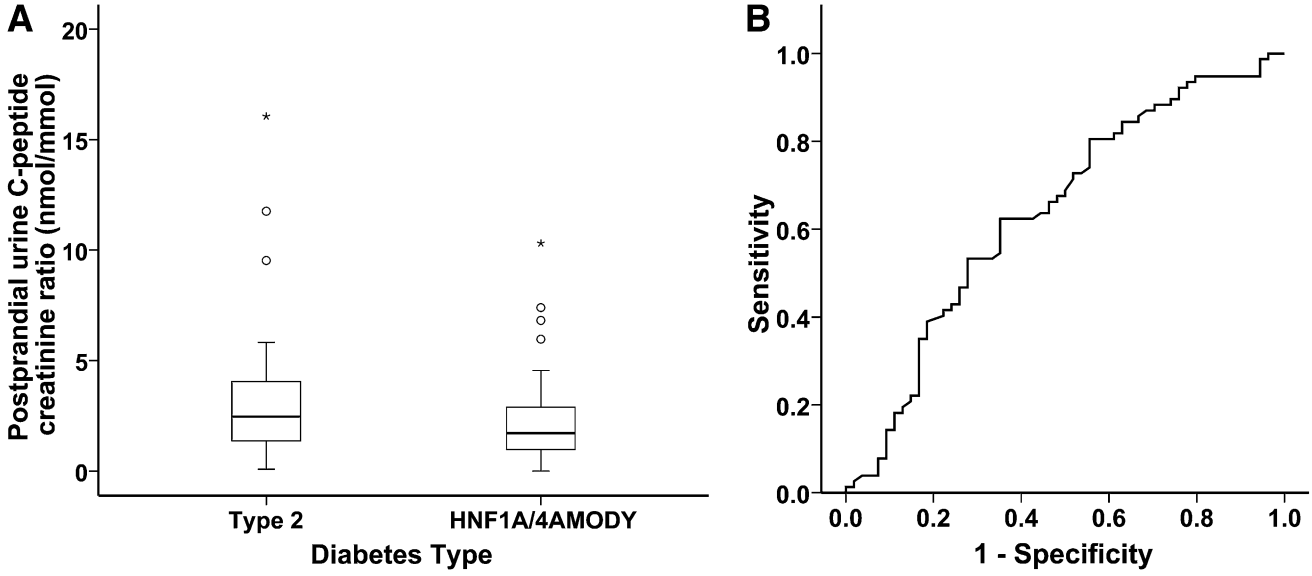

Figure 2—*Box plot and ROC curve to identify HNF1A/4A MODY from type 2 diabetes.* A*: Box plot to show the UCPCR in HNF1A/4A MODY* (n = *77) and type 2 diabetes* (n = *54). Circles, outliers; stars, extreme outliers.* B*: The ROC curve indicates that UCPCR was not able to easily discriminate HNF1A/4A MODY from type 2 diabetes (AUC 0.64).*

(18). In contrast, UCPCR is stable at room temperature for 3 days (21), and our results using mailed samples offer an approach that can be used in the patient's home or other settings away from laboratory equipment.

Likelihood ratios can be used to alter the pretest probabilities, or prevalence of a disease, to help the clinician decide who would benefit from diagnostic genetic testing. Based on our results, a UCPCR ≥0.2 nmol/mmol gave a likelihood ratio of 24, and conversely a UCPCR <0.2 nmol/mmol would give a likelihood ratio of 0.03. This means that assuming the prevalence of MODY is 0.7% of patients with young-onset diabetes (24), the posttest probability of a patient having HNF1A/4A MODY would be 0.0002% if UCPCR was <0.2 nmol/mmol, allowing genetic testing to be excluded on the basis of a simple urine test. If UCPCR is positive (≥0.2 nmol/mmol) the probability of a patient having HNF1A/4A MODY is 14%. Other nongenetic tests, such as those for diabetes autoantibodies, and clinical features, such as family history, also may be useful to further modify this probability.

UCPCR compared with other criteria to screen for MODY versus type 1 diabetes

Little work has been done on biomarkers to discriminate MODY from type 1 diabetes. The monosaccharide 1,5-anhydroglucitol was unable to discriminate HNF1A MODY from type 1 diabetes (10,13). An important biomarker would be pancreatic autoantibodies, and this is likely to be a better test than C-peptide close to diagnosis. Although 70–96% of patients with type 1 diabetes have autoantibodies, if multiple antibodies are tested at diagnosis, this positive rate falls off in the years after diagnosis (25). No studies have been done to systematically determine the autoantibody prevalence in MODY, but it is likely that autoantibody measurements will be better closer to diagnosis but less sensitive and specific 5 years after diagnosis than UCPCR measurement.

Although parental history has relatively good sensitivity (91%) in identifying MODY, it falls down on the specificity (77%) (Table 1), which would increase the number of patients who are falsely identified as having MODY. In contrast, three or more generations have good specificity (91%) but only modest sensitivity (83%) (Table 1), which would miss cases of MODY. Using parental history or multigenerational family history in isolation is likely to miss cases attributed to the selection bias for screening patients using traditional MODY criteria. The advantage of using UCPCR is that it has both strong sensitivity (97%) and specificity (96%) for discriminating HNF1A/4A MODY from type 1 diabetes.

UCPCR in discriminating type 2 diabetes from HNF1A/4A MODY

UCPCR was less robust at discriminating HNF1A/4A MODY from type 2 diabetes, compared with type 1 diabetes. A UCPCR value ≤3.1 nmol/mmol was 81% sensitive and 44% specific for HNF1A/4A MODY compared with type 2 diabetes. This will reflect that in MODY the predominant defect is insulin secretion rather than the combined defect seen in type 2 diabetes of insulin secretion and insulin resistance, resulting in higher C-peptide whether measured in serum or urine. This level of discrimination, although significant ($P = 0.07$), is hard to use in clinical practice in contrast to the discrimination against type 1 diabetes.

UCPCR compared with other biomarkers to screen for MODY versus type 2 diabetes

1.5-AG and the proteins apolipoprotein M, C5, C8, and TTR all have been identified as potential biomarkers for discriminating HNF1A/4A MODY from type 2 diabetes (9–12), but the utility of these in clinical practice is unclear. Although they could discriminate between HNF1A MODY and type 2 diabetes, the sensitivity and specificity were not sufficiently powered to be clinically useful (sensitivity/specificity [%]: TTR 80/2, C5 90/11, and C8 60/5). The sensitivity (81%) and specificity (44%) of UCPCR to identify HNF1A/4A MODY from type 2 diabetes is higher than seen for these other biomarkers but probably not useful as a diagnostic tool. UCPCR may, however, have a role in identifying type 2 diabetes in patients with high UCPCR levels, and in those who are insulin treated, but further investigation will be required to assess this in a clinical setting.

Limitations

This study includes patients with long-duration diabetes ≥5 years to ensure patients are outside the honeymoon period of type 1 diabetes. Additional studies are needed with a shorter duration to assess the role of UCPCR in patients closer to diagnosis. However, the value of a negative test within 5 years of diagnosis may be of use and is likely to be as strong if not stronger than outside the honeymoon period.

Our study is cross-sectional, and a prospective study with serial measurement would let the evolution of changes in UCPCR be assessed in all of the different subtypes of diabetes.

Our patients only were included if they had normal renal function because C-peptide metabolism largely occurs in the kidney (20). The utility of UCPCR in renal impairment remains to be established. These caveats prove that our results should not be extrapolated to patients within 5 years of diagnosis or with renal impairment until other studies are performed.

Persistent C-peptide in type 1 patients

It is important to appreciate that C-peptide may persist in type 1 diabetes. In our cohort, 3 of 69 (4%) type 1 diabetic patients had detectable C-peptide ≥5 years after diagnosis. They probably represent unusual type 1 diabetes or an unidentified subtype, because autoantibodies were detected in two of three patients. The DCCT found that in 5 years after diagnosis, 3–7% type 1 diabetic patients had stimulated C-peptide >200 pmol/L (14).

In conclusion, UCPCR is a useful, simple, noninvasive biomarker for discriminating HNF1A and HNF4A MODY from long-duration type 1 diabetes.

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R.E.J.B. designed the study, recruited patients, performed analysis, and wrote the manuscript. M.H.S. recruited patients and reviewed and edited the manuscript. T.J.M. analyzed samples, contributed to the analysis, and reviewed and edited the manuscript. B.M.S. performed analysis and reviewed and edited the manuscript. B.A.K. contributed to the study design and researched, reviewed, and edited the manuscript. S.E. performed genetic testing of MODY case subjects and reviewed and edited the manuscript. A.T.H.

had the original idea, designed the study, performed analysis, and reviewed and edited the manuscript.

Parts of this study were presented in poster form at the 70th Scientific Sessions of the American Diabetes Association, Orlando, Florida, 25–29 June 2010.

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