Assessment of High-Sensitivity C-Reactive Protein Levels as Diagnostic Discriminator of Maturity-Onset Diabetes of the Young Due to *HNF1A* Mutations

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OBJECTIVE — Despite the clinical importance of an accurate diagnosis in individuals with monogenic forms of diabetes, restricted access to genetic testing leaves many patients with undiagnosed diabetes. Recently, common variation near the HNF1 homeobox A (*HNF1A*) gene was shown to influence C-reactive protein levels in healthy adults. We hypothesized that serum levels of high-sensitivity C-reactive protein (hs-CRP) could represent a clinically useful biomarker for the identification of *HNF1A* mutations causing maturity-onset diabetes of the young (MODY).

RESEARCH DESIGN AND METHODS — Serum hs-CRP was measured in subjects with HNF1A-MODY ($n = 31$), autoimmune diabetes ($n = 316$), type 2 diabetes ($n = 240$), and glucokinase (GCK) MODY ($n = 24$) and in nondiabetic individuals ($n = 198$). The discriminative accuracy of hs-CRP was evaluated through receiver operating characteristic (ROC) curve analysis, and performance was compared with standard diagnostic criteria. Our primary analyses excluded ~11% of subjects in whom the single available hs-CRP measurement was >10 mg/l.

RESULTS — Geometric mean (SD range) hs-CRP levels were significantly lower ($P \leq 0.009$) for HNF1A-MODY individuals, 0.20 (0.03–1.14) mg/l, than for any other group: autoimmune diabetes 0.58 (0.10–2.75) mg/l, type 2 diabetes 1.33 (0.28–6.14) mg/l, GCK-MODY 1.01 (0.19–5.33) mg/l, and nondiabetic 0.48 (0.10–2.42) mg/l. The ROC-derived C-statistic for discriminating HNF1A-MODY and type 2 diabetes was 0.8. Measurement of hs-CRP, either alone or in combination with current diagnostic criteria, was superior to current diagnostic criteria alone. Sensitivity and specificity for the combined criteria approached 80%.

CONCLUSIONS — Serum hs-CRP levels are markedly lower in HNF1A-MODY than in other forms of diabetes. hs-CRP has potential as a widely available, cost-effective screening test to support more precise targeting of MODY diagnostic testing.

Diabetes Care 33:1919–1924, 2010

Mutations in the hepatocyte nuclear factor 1-α (HNF1 homeobox A; HNF-1α; *HNF1A*) gene represent the most common cause of maturity-onset diabetes of the young (MODY) and are estimated to account for ~2% of all diabetes (1). Demonstrating that an *HNF1A* mutation is responsible for diabetes in a given individual has important clinical implications for both patients and their relatives: for example, in contrast to typical type 2 diabetes, low-dose sulfonylureas rather than metformin should be the first-line treatment (2,3). Despite the clinical value of an accurate molecular diagnosis, many individuals with HNF1A-MODY are never tested and are consequently misclassified as having type 1 or type 2 diabetes.

The principal barriers to the implementation of systematic diagnostics for monogenic forms of diabetes include the high cost and restricted availability of genetic testing. Currently, patients are typically selected for molecular testing on the basis of nonspecific clinical features such as age of onset, parental history of diabetes (4), and/or a clinical presentation that is otherwise atypical for the assumed etiology (such as an apparent absence of insulin resistance in an individual presumed to have type 2 diabetes [5]). However, the performance of these criteria is such that it is difficult to combine acceptable levels of specificity and sensitivity. Consequently, there would be considerable value in identifying additional screening tools, which, in conjunction with existing clinical and biochemical markers, would assist selection of individuals who merit further investigation, including *HNF1A* sequencing. Because a relative β-cell defect is common to all forms of diabetes, the best prospects for identifying such a marker may be the extrapancreatic manifestations of an *HNF1A* mutation.

HNF1A encodes the transcription factor HNF-1α, initially identified in the liver, where it is involved in regulation of a large number of genes (6) and also is expressed in the pancreas, gut, and kidney. There have been several previous attempts to identify HNF1A-MODY biomarkers, most based on candidates initially highlighted by studies on *Hnf1a* knockout mice (6,7). However, the candidates examined, including apolipoprotein M (8–10), aminoaciduria (11,12), complement components (13), and glycosuria (14–16), have either not demonstrated sufficient sensitivity and/or specificity to warrant further evaluation or have thus far not translated into a clinically useful biomarker.

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Received 14 February 2010 and accepted 17 June 2010.
DOI: 10.2337/dc10-0288

Recently, genome-wide association studies have revealed that common variants mapping near the *HNF1A* gene on chromosome 12q24 are associated with small alterations in serum C-reactive protein (CRP) levels in healthy adults (17,18). The presence of HNF-1α binding sites in the *CRP* promoter (19) suggests that the effect on CRP is mediated through altered regulation of *HNF1A* expression. Moreover, a loss of HNF1A binding has been shown to result in a loss of CRP expression (19). If so, it follows that the rare, but large-effect, loss of function mutations in *HNF1A* responsible for MODY might be expected to lead to more substantial reductions in serum CRP levels. This would be analogous to observations involving the glucokinase (*GCK*) gene: although a common *GCK* promoter variant is associated with a modest (~0.06 mmol/l) effect on fasting plasma glucose levels in healthy adults (20), rare pathogenic mutations in *GCK* are responsible for a far larger (~2 mmol/l) increase (21).

We aimed to test whether individuals with HNF1A-MODY have reduced serum CRP levels compared with those in individuals with other forms of diabetes and to establish whether CRP could be a useful diagnostic marker.

RESEARCH DESIGN AND METHODS

The subjects included in this study are more fully described in the supplementary data (available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/dc10-0288/DC1). In brief, we included 31 individuals with HNF1A-MODY, 24 with GCK-MODY, 275 with classic type 1 diabetes, 41 with latent autoimmune diabetes of adulthood, 240 with young-onset type 2 diabetes (diagnosed up to 45 years of age), and 198 nondiabetic individuals. The study was approved by the Oxfordshire Local Research Ethics Committee, and all subjects gave informed consent.

Serum high-sensitivity CRP (hs-CRP) levels were measured using a wide-range latex-enhanced immunoturbidimetric assay on an ADVIA 2400 analyzer (Siemens Healthcare Diagnostics, Frimley, U.K.), with a quoted method linearity of 0.03–160 mg/l. Imprecision, expressed as coefficients of variation, at concentrations >0.05 mg/l was <10% and at 23.5 mg/l was <1%. GAD antibodies were measured by a radioimmunoassay using a ^{35}S-labeled full-length GAD65, with results expressed in World Health Organization (WHO) units per milliliter derived from a standard curve calibrated from international reference material (National Institute for Biological Standards and Control code 97/550). Samples were considered positive if they had levels above 14 WHO units/ml (97.5th percentile of healthy school-aged children) (22).

Analysis

hs-CRP values for all subjects were inspected. Means ± SD for the two autoimmune groups (type 1 diabetes and latent autoimmune diabetes of adulthood) were very similar, so these were combined for further analyses. Supplementary Fig. 1 (available in an online appendix) documents the distribution of individual hs-CRP levels in all subjects. Median (interquartile range) values for hs-CRP are also reported in Table 1, along with other clinical characteristics of the groups. In line with previous studies (23,24), we considered that hs-CRP values >10 mg/l were likely in many instances to represent an acute inflammatory response and, prior to any analysis, had decided to exclude such values from our primary analyses. From the 809 subjects with hs-CRP values, 90 (11%) with hs-CRP >10 mg/l were removed: 3 (10%) with HNF1A-MODY, 22 (7%) with autoimmune diabetes, and 53 (22%) with type 2 diabetes and 12 (6%) of the nondiabetic subjects.

For subsequent analyses, all variables were log-transformed. Differences between the hs-CRP values in the HNF1A-MODY and other groups were evaluated using a *t* test, both before and after adjustment for BMI (because BMI was correlated with hs-CRP). Because certain drugs, particularly statins and aspirin, can lower CRP levels (25), we performed a further analysis after removing subjects treated with either. Other variables (sex, age at sampling, A1C, duration of diabetes, and ethnic origin) were not associated with hs-CRP levels in our data and were therefore not included as covariates. We also examined whether the hs-CRP level was affected by the type of mutation (nonsense versus missense) or the isoform of HNF1A affected by missense mutations. Finally, because some of the subjects with MODY were related to each other, we sought to account for nonindependence in two ways. First, we reanalyzed data by using only a single individual from each family (either the proband or if he or she was not available the youngest individual in the family) and, second, by including family membership as a covariate in the analysis.

The measurement of hs-CRP as a diagnostic test for HNF1A-MODY was examined by ROC curve analysis and compared with standard clinical criteria. Sensitivities and specificities were calculated from our data and then used to model the effect of using hs-CRP with or without clinical criteria as a screening test for *HNF1A* mutations in a patient group with apparent type 2 diabetes diagnosed up to age 45 years (supplementary Table 2, available in an online appendix). For this purpose, we assumed that 4% of the group were subjects with misclassified HNF1A-MODY, based on observations we have made in a large undifferentiated group of young-onset type 2 diabetic subjects (K.R.O., M.I.M., unpublished data).

All statistical analysis was performed with SPSS (version 16). $P < 0.05$ was considered significant.

RESULTS

The results confirm our hypothesis that hs-CRP levels are significantly lower in subjects with HNF1A-MODY compared with those for all other groups, including nondiabetic subjects (Table 1, Fig. 1*A*). The geometric mean (SD range) unadjusted hs-CRP value for subjects with HNF1A-MODY (after exclusion of those with hs-CRP >10 mg/l; see RESEARCH DESIGN AND METHODS) was 0.20 (0.03–1.14) mg/l, compared with 1.33 (0.28–6.14) mg/l for those with type 2 diabetes, 0.58 (0.10–2.75) mg/l for those with autoimmune diabetes, and 1.01 (0.19–5.33) mg/l for subjects with GCK-MODY, and 0.48 (0.10–2.42) mg/l for nondiabetic subjects ($P \leq 0.009$ for all pairwise comparisons with subjects with HNF1A-MODY). The value for the other diabetic groups combined was 0.81 (0.15–4.50) mg/l, significantly greater than that for the HNF1A-MODY group ($P = 0.00003$).

Use of statin and/or aspirin therapy had negligible effects on the comparisons with the HNF1A-MODY group (Table 1). In the type 2 diabetic group, the hs-CRP level was the same for the statin/aspirin users as for the nonusers ($P = 0.27$), whereas in the type 1 diabetic group those taking statins and/or aspirin had a higher hs-CRP than nonusers ($P = 0.004$). Thus, it seems unlikely that use of these drugs would lower hs-CRP toward the range seen in the HNF1A-MODY group. In contrast, adjustment for BMI had a large impact on the estimated means (Table 1) and, given the correlation between BMI and CRP ($r^2 = 0.28$, $P < 10^{-6}$ for control subjects), abolished much of the differ-

ence observed between the type 2 diabetic and other groups. However, the differences in hs-CRP levels between the HNF1A-MODY and other groups were preserved ($P \leq 0.01$). Estimated mean adjusted hs-CRP (95% CI) levels for the HNF1A-MODY group compared with the other diabetic groups combined were 0.28 (0.16–0.49) vs. 0.82 (0.71–0.94) mg/l ($P = 0.003$). Reanalysis with accounting for shared family membership (see RESEARCH DESIGN AND METHODS) did not alter the magnitude or significance of the most important between-group differences (e.g., using the probands only, HNF1A-MODY vs. type 2 diabetes $P = 7 \times 10^{-6}$ and HNF1A-MODY vs. autoimmune diabetes $P = 0.009$). There was no difference between the hs-CRP levels seen in families segregating missense mutations (11 families) compared with those featuring a premature stop codon (8 families, $P = 0.6$). Because all but two of the missense mutations were in exons represented in all three HNF1A isoforms, we could reach no conclusions concerning the relationship between CRP levels and the HNF1A isoform.

The differences in mean values between HNF1A-MODY and the other groups are striking but do not necessarily translate into clinical utility. We therefore evaluated the performance of hs-CRP levels as a potential diagnostic test for distinguishing HNF1A-MODY from other kinds of diabetes (supplementary Tables 1 and 2). Figure 1*B* displays the (cumulative) distribution of hs-CRP values for individuals in the different subject groups. In our data, a diagnostic threshold value of 0.4 mg/l (the vertical line on Fig. 1*B*) equates to a sensitivity of 71% and specificity of 77% for distinguishing HNF1A-MODY from type 2 diabetes. These figures are revised to 65 and 82%, respectively, if subjects with hs-CRP≥10 mg/l are not excluded. Inclusion of individuals with autoimmune diabetes and GCK-MODY reduces the specificity of the hs-CRP threshold of 0.4 mg/l to 63%. Use of an hs-CRP threshold <0.4 mg/l improves specificity (and would therefore reduce the number of individuals with type 2 diabetes who might undergo "unnecessary" *HNF1A* sequencing), but this comes at the expense of sensitivity (i.e., more individuals with HNF1A-MODY are missed). The consequences of using different hs-CRP criteria on these measures of sensitivity and sensitivity, in our data, are illustrated in supplementary Table 1.

The ROC-derived C-statistic (a mea-

Table 1—*Characteristics of subjects*

	HNF1A-MODY	Autoimmune diabetes	Type 2 diabetes	GCK-MODY	Nondiabetic	*P* value
n	31	316	240	24	198	
Sex (% male)	35.5	56.3	55.0	41.6	46.5	
Age of diagnosis (years)	21.4 (14.3–32.0)	21.5 (12.2–37.8)	34.4 (26.6–44.4)	21.3 (11.8–38.6)	43.4 (38.4–49.0)†	$<1 \times 10^{-6}$‡
Duration of diabetes (years)	13.8 (4.0–48.1)	11.7 (5.8–23.7)	8.5 (2.4–30.8)	13.1 (6.8–25.2)	—	0.001‡
BMI (kg/m^2)	25.6 (21.0–31.2)	25.9 (21.8–30.8)	32.7 (26.4–40.4)	27.3 (21.7–34.3)	26.2 (22.0–31.1)	$<1 \times 10^{-6}$‡
A1C (%)	7.1 (5.9–8.5)	NA	8.0 (6.5–9.9)	6.8 (5.6–8.3)	NA	5×10^{-5}‡
FPG (mmol/l)	7.9 (5.2–11.8)	NA	8.3 (5.6–12.1)	7.7 (5.8–10.3)	5.1 (4.7–5.7)	$<1 \times 10^{-6}$‡
% treated with aspirin	16.1	7.5	28.1	12.5	0	0.0028
% treated with statin	22.6	29.7	63.8	8.3	1.0	3×10^{-6}§
Median hs–CRP (mg/l) (includes all values)*	0.19 (1.54)	0.92 (3.07)	3.25 (8.86)	1.25 (3.59)	0.63 (2.14)	$<1 \times 10^{-6}$#
hs-CRP (mg/l) (hs-CRP<10 mg/l, unadjusted)	*n* = 28	*n* = 294	*n* = 187	*n* = 24	*n* = 186	$<1 \times 10^{-6}$‡
	0.20 (0.03–1.14)	0.58 (0.10–2.75)	1.33 (0.28–6.14)	1.01 (0.19–5.33)	0.48 (0.10–2.42)	
P value vs. HNF1A-MODY		0.001	$<1 \times 10^{-6}$	0.0004	0.009	
hs-CRP (mg/l) (hs-CRP<10 mg/l, users of aspirin and/or statin excluded)	*n* = 20	*n* = 186	*n* = 61	*n* = 20	*n* = 184	$<1 \times 10^{-6}$‡
	0.11 (0.02–0.50)	0.45 (0.07–2.75)	1.61 (0.30–8.59)	0.95 (0.16–5.42)	0.48 (0.09–2.45)	
P value vs. HNF1A-MODY		0.001	$<1 \times 10^{-6}$	0.001	0.001	
hs-CRP (mg/l) (hs-CRP<10 mg/l, adjusted for BMI)‖	*n* = 28	*n* = 294	*n* = 187	*n* = 24	*n* = 186	$<1 \times 10^{-6}$‡
	0.27 (0.15–0.46)	0.76 (0.64–0.91)	0.76 (0.60–0.96)	0.95 (0.47–1.89)	0.57 (0.46–0.71)	
P value vs. HNF1A-MODY		0.0004	0.001	0.005	0.01	

Data are geometric mean (SD range) unless otherwise indicated. NA, not available. *Median (interquartile range). ‖Estimated marginal means (95% CI). †Age of sampling for control subjects. *P* value compares all groups and was calculated by ‡ANOVA, §χ^2 test (diabetic groups only), or #Kruskal-Wallis test.

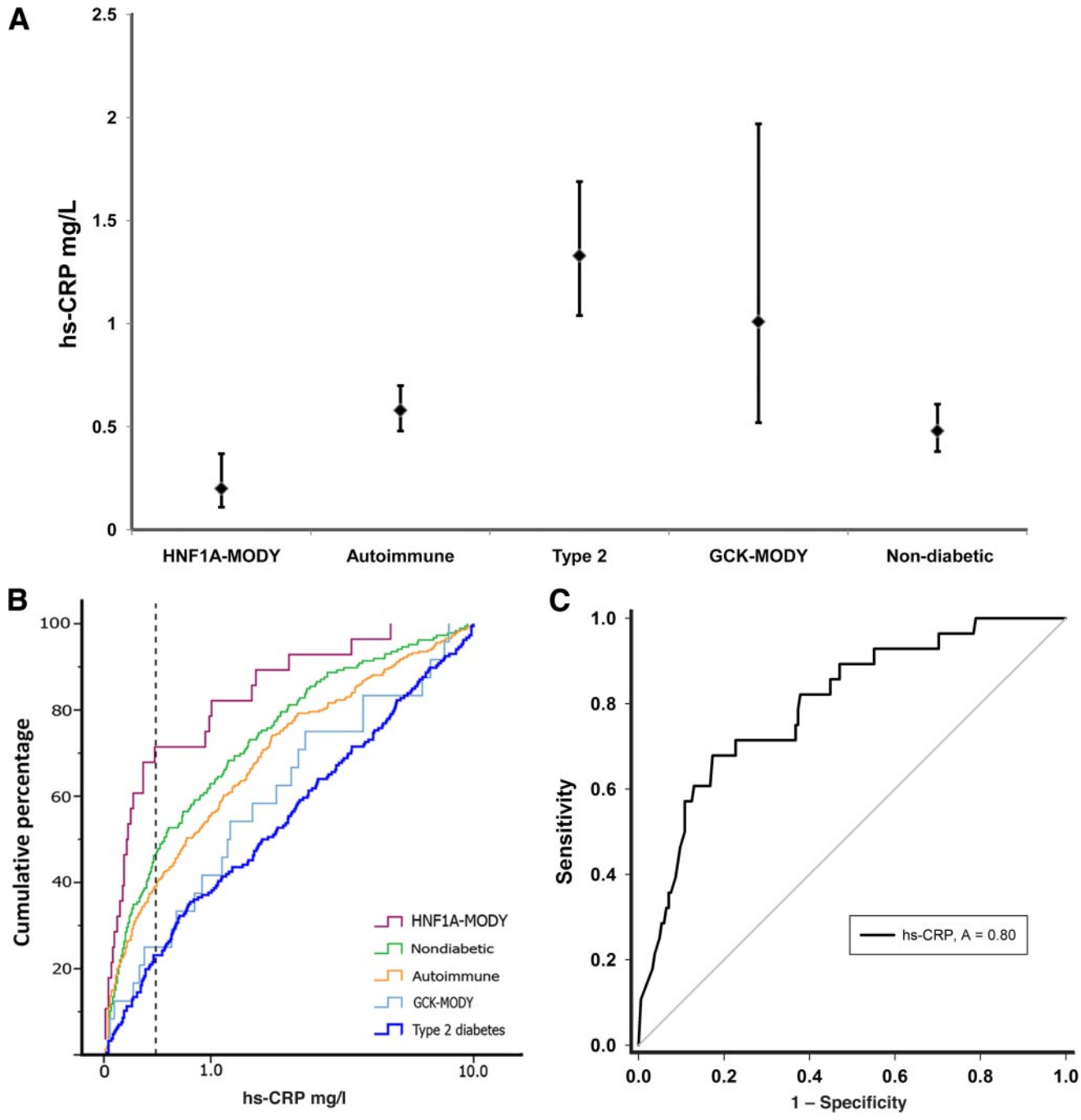

Figure 1—*The analyses included 28 subjects with HNF1A-MODY, 294 with autoimmune diabetes, 187 with type 2 diabetes, and 24 with GCK-MODY and 198 nondiabetic control subjects. Values of hs-CRP >10 mg/l are excluded.* A*: Geometric mean hs-CRP levels for the different groups; error bars show 95% CI.* B*: Cumulative percentage plot for hs-CRP levels in the different groups. The dotted reference line corresponds to a hs-CRP value of 0.4 mg/l. hs-CRP levels are plotted on a* $\log_{10}$ *scale.* C*: ROC curve illustrating the capacity of hs-CRP to distinguish between HNF1A-MODY and type 2 diabetes. The C-statistic (area under the curve) for this comparison is 0.8.*

sure of discriminative accuracy) for unadjusted hs-CRP levels was 0.80 for distinguishing HNF1A-MODY from type 2 diabetes (Fig. 1*C*) and 0.75 for distinguishing HNF1A-MODY from all other diabetes subtypes combined. We compared the performance of hs-CRP (again excluding those with hs-CRP>10 mg/l) to current diagnostic criteria including age of diabetes onset ≤25 years and a first-degree family history of diabetes. In our dataset, these existing criteria had lower sensitivity (58%) than hs-CRP testing, but they were highly specific for the discrimination of HNF1A-MODY and type 2 diabetes (only 6% of the latter met these criteria). The combination of existing diagnostic criteria or an hs-CRP level of ≤0.2 mg/l produced, in our dataset, a sensitivity of 79% and specificity of 83% for distinguishing HNF1A-MODY from type 2 diabetes, a considerable improvement in sensitivity ($P = 0.035$) over traditional criteria alone with little loss of

specificity (supplementary Table 1). Use of these criteria as a screening test for selection of individuals for further investigation would lead to sequencing of 20% of type 2 diabetic subjects diagnosed up to 45 years of age with a detection rate of *HNF1A* mutations of 16% of those sequenced (supplementary Table 2).

CONCLUSIONS — We have shown for the first time that subjects with HNF1A-MODY maintain substantially lower levels of serum hs-CRP levels than individuals with other forms of diabetes or nondiabetic control subjects. By demonstrating these effects of rare coding mutations in *HNF1A*, our findings therefore extend recent observations that common variants near *HNF1A* are associated with CRP levels. Thus, our study confirms that the common variant associations are almost certainly mediated through alterations in *HNF1A* transcription. However, the observation that some individuals with HNF1A-MODY had hs-CRP levels >10 mg/l (including one of 52 mg/l) demonstrates that *HNF1A* haploinsufficiency is not sufficient to prevent substantial elevation in CRP levels, presumably as part of an acute inflammatory response.

We have shown that as a standalone diagnostic test to select individuals for HNF1A-MODY sequencing, hs-CRP performed reasonably well in our dataset, with a C-statistic of 0.80 for differentiating HNF1A-MODY from young-onset type 2 diabetes. In clinical use, it seems likely that hs-CRP levels would be combined with other clinical and biochemical data within a broader diagnostic algorithm. We have shown that such a combination of clinical criteria and hs-CRP can achieve sensitivities and specificities of ~80% (supplementary Table 1). We are aware of the dangers of overfitting, and clearly these performance metrics need to be evaluated in independent datasets. We also note that to take full advantage of the sensitivity afforded by this combination, molecular diagnostic testing of ~20% of those with the diagnosis of apparent type 2 diabetes who were aged <45 years would be required (supplementary Table 2). It is worth emphasizing that in this study we focused on individuals with a relatively early diagnosis of type 2 diabetes on the basis that these individuals constitute the group in which the diagnostic differentiation from HNF1A-MODY is most pertinent.

One point for discussion is how values outside the normal range for the assay should be handled. By removing all individuals with hs-CRP >10 mg/l, we sought to avoid the loss of discriminatory accuracy associated with what we assume to be acutely elevated hs-CRP levels. Our current recommendation would be for individuals with elevated CRP levels to have a repeat measure some weeks later, allowing any acute inflammatory response to subside. Because our study did not include serial measurements of hs-CRP, future studies will be needed to explore this particular issue. It is entirely plausible that the low-grade chronic elevation of CRP, which is characteristic of type 2 diabetes, would enhance the discriminatory performance of repeated over single hs-CRP measures.

Although we included reasonably large collections of subjects with HNF1A-MODY and GCK-MODY, the very low prevalence of HNF4A-MODY means that subjects with this form of MODY were not available for comparison. HNF4A-MODY presents with a β-cell phenotype very similar to that of HNF1A-MODY, but many of the extrapancreatic manifestations are distinct (4). Because the *CRP* promoter lacks any HNF-4α binding sites, it seems probable that hs-CRP levels in subjects with HNF4A-MODY will not be reduced: if future studies confirm this theory, hs-CRP levels may provide useful diagnostic differentiation between HNF1A and HNF4A-MODY.

In summary, we have shown that subjects with pathogenic mutations in *HNF1A* have significantly lower serum levels of hs-CRP than those with other types of diabetes. Although these findings require validation in independent datasets and extension to other rarer subtypes of MODY, hs-CRP emerges from this study as the most promising diagnostic biomarker for HNF1A-MODY identified to date. Because hs-CRP estimation is already widely available at low cost in many routine pathology services, it should be relatively easy to incorporate this biomarker into diagnostic pathways. We expect this inclusion to lead to better targeting of molecular diagnostic testing for monogenic forms of diabetes and for consequent improvements in detection rates to result in more effective treatment of this currently underdiagnosed condition.

Acknowledgments — The study was funded by the National Institute for Health Research (NIHR) Biomedical Research Centre, Oxford, Diabetes UK, the European Community FP6 integrated project MolPAGE (LSHG-512066), the European Community FP7 programs CEED3 (HEALTH-F2-2008-223211) and ENGAGE (HEALTH-F4-2007-201413), and the Oxford Hospitals Charitable Fund. K.R.O. is an NIHR-funded clinician scientist. A.J.F. is supported by the NIHR School of Primary Care Research. A.L.G. is a Medical Research Council New Investigator (grant 81696).

No potential conflicts of interest relevant to this article were reported.

K.R.O. and A.L.G. contributed to the conception and design of study, acquired data, analyzed and interpreted data, wrote the manuscript, and reviewed/edited the manuscript. G.T. acquired data, analyzed and interpreted data, and reviewed/edited the manuscript. T.J.J., F.K., and A.J.F. acquired data and revised/edited the manuscript. M.I.M. contributed to the conception and design of study, analyzed and interpreted data, and reviewed/edited the manuscript.

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

We are grateful to our research nurses Mary Selwood, Christina Dudley, Jilly Grew, Beryl Barrow, and Vanessa Loach (all University of Oxford) and Rachel Craven-Todd and Julie Sutton (Thames Valley Diabetes Local Research Network) for recruitment of the diabetic subjects in the study. We thank the patients and families taking part and acknowledge the support of the NIHR Thames Valley Diabetes Local Research Network, part of the U.K. Clinical Research Network.

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