Mutations in HNF1A Result in Marked Alterations of Plasma Glycan Profile

Citation for published version:

Digital Object Identifier (DOI):
10.2337/db12-0880

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher’s PDF, also known as Version of record

Published In:
Diabetes

Publisher Rights Statement:
© 2013 by the American Diabetes Association.

Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
A recent genome-wide association study identified hepatocyte nuclear factor 1α (HNF1A) as a key regulator of fucosylation. We hypothesized that loss-of-function HNF1A mutations cause for maturity-onset diabetes of the young (MODY) would display altered fucosylation of N-linked glycans on plasma proteins and that glycobiosignatures could improve the efficiency of a diagnosis of HNF1A-MODY. In a pilot comparison of 33 subjects with HNF1A-MODY and 41 subjects with type 2 diabetes, 15 of 29 glycan measurements differed between the two groups. The DG9-glycan index, which is the ratio of fucosylated to nonfucosylated triantennary glycans, provided optimum discrimination in the pilot study and was examined further among additional subjects with HNF1A-MODY (n = 188), glucokinase (GCK)-MODY (n = 118), hepatocyte nuclear factor 4α (HNF4A)-MODY (n = 40), type 1 diabetes (n = 98), type 2 diabetes (n = 167), and nondiabetic controls (n = 98). The DG9-glycan index was markedly lower in HNF1A-MODY than in controls or other diabetes subtypes, offered good discrimination between HNF1A-MODY and both type 1 and type 2 diabetes (C statistic = 0.90), and enabled us to detect three previously undetected HNF1A mutations in patients with diabetes. In conclusion, glycan profiles are altered substantially in HNF1A-MODY, and the DG9-glycan index has potential clinical value as a diagnostic biomarker of HNF1A dysfunction. Diabetes 62:1329–1337, 2013

Genome-wide association studies are providing novel insights into the genetic architecture and biological basis of many diseases, but immediate translation into clinical practice has been limited. We recently performed a genome-wide association study of the human plasma N-glycome and found evidence of association involving common variants near the hepatocyte nuclear factor 1α (HNF1A) gene; follow-up functional experiments established HNF1A as a master regulator of plasma protein fucosylation (1). Fucosylation, a specific type of glycosylation, comprises the addition of fucose residues to glycans. Here we evaluate the hypothesis that mutations causing a more severe deficit in HNF1A function (resulting in the monogenic subtype of diabetes known as HNF1A maturity-onset diabetes of the youth [MODY, HNF1A-MODY]) are associated with marked alterations of plasma glycome composition, and we assess the value of glycan profiles as a diagnostic biomarker for HNF1A-MODY.

Most human proteins are posttranslationally modified by the addition of complex oligosaccharide structures (glycans) (2). Despite the impact on protein structure and function, the clinical consequences of changes in the human glycome remain largely unexplored, primarily because reliable analytical techniques have been developed only recently (3). In recent studies, HNF1A was shown to promote both the de novo and salvage pathways for the synthesis of guanosine diphosphate–fucose (1) and to regulate fucosyltransferase VI (1,4). HNF1A thereby controls the outer-arm (antennary) fucosylation of proteins with N-linked glycans through effects on both the supply of activated precursors and the incorporation of fucose (1,4).

Mutations disrupting HNF1A are responsible for the most common subtype of monogenic diabetes, HNF1A-MODY (5). Like other forms of MODY, HNF1A-MODY is characterized
by autosomal dominant inheritance and pancreatic β-cell dysfunction. This typically leads to diabetes diagnosed in the second to fourth decade of life in the absence of β-cell autoimmunity and insulin resistance (5,6). In clinical practice, diagnostic differentiation between HNF1A-MODY and other causes of early-onset diabetes (including type 1 and type 2 diabetes and other forms of MODY) is complicated by the overlap of phenotypic features. In most countries only a minority of cases of HNF1A-MODY are referred for definitive molecular testing (i.e., HNF1A sequencing), and many patients with HNF1A-MODY are misdiagnosed with type 1 or type 2 diabetes (7). Failures or delays in accurate molecular diagnosis can have clinical repercussions because, unlike other forms of diabetes, the optimal treatment for HNF1A-MODY is low-dose sulfonylureas (8). Patients with undiagnosed HNF1A-MODY may spend many years receiving inappropriate treatment (including exogenous insulin) and experiencing suboptimal glycemic control (9).

We aimed to test the hypothesis that inactivating mutations in HNF1A, such as those found in HNF1A-MODY, are associated with decreased antennary fucosylation of circulating proteins and to evaluate the clinical translational potential of measuring glycan profiles in diabetes.

**RESEARCH DESIGN AND METHODS**

**Subjects for initial and validation studies.** Full details of the subjects are available in Table 1 and the Supplementary Appendix. Subjects carrying mutations in HNF1A (n = 221), glucokinase (GCK; n = 115), and hepatocyte nuclear factor 4α (HNF4α; n = 40) were recruited from five European centers. Subjects with MODY had an established heterozygous loss-of-function mutation confirmed by sequencing in a certified diagnostic center. All MODY mutations were considered pathogenic if they met one or more of the following criteria: mentioned in previously published reports, presence of a truncating mutation, cosegregation of the mutation with a MODY phenotype within the family, and absence of the variant in normal chromosomes. In addition, we recruited 208 subjects with clinically labeled type 2 diabetes who were diagnosed at an age younger than 45 years, 98 subjects with clinically labeled type 1 diabetes, and 98 subjects who acted as nondiabetic controls. Most samples were collected when the subject was in a fasting state, although fasting status does not influence glycan levels (10).

**Glycan release, labeling, and analysis.** All samples were stored at −80°C before analysis. Glycan release, labeling, and analysis using hydrophilic interaction high-performance liquid chromatography and sialidase digestion was performed as previously reported (11,12). Chromatograms from fluorescently labeled glycans were separated into 16 glycan groups (GP series) and 13 desialylated glycan groups (DG series), composing a total of 29 peaks (Supplementary Table 1). The amounts of glycans present in each peak were expressed as the percentage of the total plasma glycan. Glycan analysis was performed in two centers: the National Institute for Bioprocessing Research and Training (Dublin, Ireland) and the Glycobiology laboratory of Genos Ltd. (Zagreb, Croatia). Both laboratories used the same columns and separation conditions and previously have demonstrated reproducibility of analytic results within and between laboratories (1,10).

**Study design and analysis.** All glycan traits were compared using Mann-Whitney U tests in an initial study of 33 subjects with HNF1A-MODY and 41 subjects with type 2 diabetes. Based on these results (Supplementary Table 1), the glycan ratio of DG0 to [DG0-DG30], hereafter referred to as the DG0-glycan index, was chosen for further follow-up.

The validation study was performed using 188 subjects with HNF1A-MODY, 118 subjects with GCK-MODY, 40 subjects with HNF4α-MODY, 167 subjects with type 2 diabetes, and 98 subjects with type 1 diabetes cases, plus 98 nondiabetic controls. There was no overlap between the initial and validation studies. We first sought evidence for important covariates through analysis of parameters including age, sex, BMI, HbA1c, triglycerides, sample origin, processing laboratory, and sample type (plasma vs. serum). An adjusted model incorporated significant covariates (age, BMI, sample origin, processing laboratory) as well as those already known to affect specific glycan traits (sex). An additional analysis was performed without covariates. The use of the DG0-glycan index as a discriminator of diabetes subtypes was analyzed using receiver operator characteristic (ROC) curves from which the C statistic was obtained.

**Table 1.** Clinical characteristics included in the initial and validation studies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Validation study</th>
<th>Initial study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td>HNF1A-MODY</td>
<td>HNF1A-MODY</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>HNF1A-MODY</td>
<td>HNF1A-MODY</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>HNF1A-MODY</td>
<td>HNF1A-MODY</td>
</tr>
<tr>
<td>Age at sampling</td>
<td>HNF1A-MODY</td>
<td>HNF1A-MODY</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>HNF1A-MODY</td>
<td>HNF1A-MODY</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>HNF1A-MODY</td>
<td>HNF1A-MODY</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>HNF1A-MODY</td>
<td>HNF1A-MODY</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>HNF1A-MODY</td>
<td>HNF1A-MODY</td>
</tr>
<tr>
<td>Normally distributed variables reported as mean ± SD; all others reported as median (25th–75th centiles). FPG, fasting plasma glucose; N/A, not available. Unavailable for subjects from Edinburgh.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Performance of the DG9-glycan index as a clinical tool was evaluated by calculating sensitivity and specificity and other measures for the detection of HNF1A-MODY at various thresholds. In particular, we estimated posttest diagnostic probabilities based on data from an etiological investigation of young adults with diabetes from the UK that indicated pretest probabilities of 4% for HNF1A-MODY in young-onset type 2 diabetes and 1% in type 1 diabetes (13).

The effect of the type of HNF1A mutation, the HNF1A isoform, and the mutated functional domain of HNF1A on DG9-glycan index levels was assessed. HNF1A mutations were classified as either protein-changing mutations (missense mutations resulting in a change of amino acid) or truncating mutations (which generate a premature stop codon). In addition, protein-changing mutations were grouped as exons 1-6 [affecting isoforms HNF1A (A), (B) and (C)], exon 7 [isoforms HNF1A (A) and (B)] and exons 8-10 [isoform HNF1A (A) only] (14,15). Protein-changing mutations also were grouped according to the affected functional domain: dimerization, DNA binding, or transcription activation (14,15).

We also assessed evidence for pathogenicity of the HNF1A missense mutations included in the validation study and examined whether the DG9-glycan index correlated with other indicators of pathogenicity. These included cosegregation within families, functional characterization of mutant proteins, and in silico prediction of the effect of the amino acid substitution on protein function.

**HNF1A sequencing.** In the subsequent case-finding study, we evaluated the value of the DG9-glycan index as a screening test for identifying HNF1A-MODY in a set of individuals with young-onset diabetes (diagnosed up to age 45 years) who had not previously been suspected of having an HNF1A mutation. We tested subjects with a DG9-glycan index <0.16 from the initial or validation studies with clinical labels of type 1 (n = 7) and type 2 diabetes (n = 41), as well as subjects with diabetes of any type diagnosed up to age 45 years from general population cohorts from Croatia (n = 6) and Scotland (n = 3) in whom glycan profiles had been measured previously (1). The 10 exons of HNF1A were amplified by PCR and bidirectional sequencing performed using M13 primers and a Big Dye Terminator Cycler Sequencing kit v1.1 (Applied Biosystems, Warrington, U.K.). Reactions were analyzed on an ABI 3730 capillary sequencer (Applied Biosystems), and results were compared with the reference sequence (NM_000545.3) using Mutation Surveyor v3.97 (SoftGenetics, Cambridge, U.K.). Mutation testing was undertaken in family members when available to establish cosegregation. In silico analysis of missense mutations was performed using the software program Condel (CONsensus DELeterious 19) and classifies missense single nucleotide polymorphisms (i.e., pathogenic) or probably “neutral” (i.e., benign).

All analyses were performed using SPSS version 17.0. The study was performed in accordance with the latest version of the Declaration of Helsinki.

**RESULTS**

**HNF1A-MODY and measures of antennary fucosylation.** In the initial study, we found marked differences in the plasma glycome profiles between 33 subjects with HNF1A-MODY and 41 subjects with early-onset type 2 diabetes. Fifteen of 29 glycan measures differed significantly between the two groups (P < 0.05) (Supplementary Table 1). Patterns were consistent with the known effects of HNF1A on fucosylation (1), in that subjects with loss-of-function mutations in HNF1A were characterized by an increase in the proportion of glycans without antennary fucose.

**Validation study.** For the validation study, we focused on DG9 and DG8 as measures of triantennary glycans with and without antennary fucose, respectively (Fig. 1). Therefore, the DG9-glycan index [DG9-to-(DG8+DG9) ratio] summarizes the proportion of triantennary glycans that are fucosylated. As well as consistency with the existing data on HNF1A effects on fucosylation (1) and strong evidence from the initial study (Supplementary Table 1), triantennary glycans are not affected by the removal of fibrinogen during coagulation (G. Lauc, unpublished observations), allowing our validation studies to include both serum and plasma samples.

The distributions of DG9-glycan index measures for the 709 individuals in the validation study differed significantly between the subject groups (Fig. 2; Supplementary Table 2). Median (interquartile range) DG9-glycan index levels were substantially lower in subjects with HNF1A-MODY [0.09 (0.06–0.13)] than in those with young-onset type 2 diabetes [0.24 (0.18–0.33); P = 1 × 10^{-30} vs. HNF1A-MODY], type 1 diabetes [0.28 (0.20–0.34); P = 1 × 10^{-34} vs. HNF1A-MODY], or GCK-MODY [0.25 (0.18–0.31); P = 5 × 10^{-32} vs. HNF1A-MODY]. DG9-glycan index levels in subjects with HNF1A-MODY were also lower when compared with controls [0.24 (0.19–0.29); P = 1 × 10^{-32} vs. HNF1A-MODY] and against all other diabetic patients combined [0.25 (0.18–0.31); P = 5 × 10^{-30} vs. HNF1A-MODY]. Consistent with evidence that HNF4A also regulates fucosylation (1), cases of HNF4A-MODY showed DG9-glycan index levels between those in HNF1A-MODY and other forms of diabetes [0.18 (0.09–0.24); P = 2 × 10^{-7} vs. HNF1A-MODY].

Adjustment for significant covariates had no appreciable impact on the magnitude or significance of differences in DG9-glycan index values between groups (Supplementary Table 2).

**Receiver operating characteristic (ROC) curve analyses.** To test whether glycan profiling had potential as a clinically...
valid screening test, C statistic measures of discriminative accuracy were derived from ROC curve analyses (Fig. 3; Supplementary Table 3). The C statistic was 0.94 for HNF1A-MODY against type 1 diabetes and 0.91 for HNF1A-MODY against early-onset type 2 diabetes. Similar discrimination was observed for the comparison of HNF1A-MODY and GCK-MODY (C statistic 0.90), but the DG9-glycan index performed less well in differentiating HNF4A-MODY and HNF1A-MODY (C statistic 0.76). These measures were not affected by sample type (serum vs. plasma; Supplementary Table 3).

We have reported previously that high-sensitivity C-reactive protein (hs-CRP) is a sensitive and specific biomarker for HNF1A-MODY (21,22). In the validation dataset, the ability of the DG9-glycan index to discriminate between HNF1A-MODY and type 2 diabetes was comparable with hs-CRP (C statistic 0.91 and 0.94, respectively). However, the DG9-glycan index provided near perfect discrimination between HNF1A-MODY and type 1 diabetes (C statistic 0.94 vs. 0.83 for hs-CRP). Other glycan measures. As described earlier, our primary validation analyses focused on the DG9-glycan index. However, the availability of full glycome profiles for the validation samples allowed us to explore the relative performance of other measures highlighted in the initial study. Other glycan ratios, such as DG7 to (DG5+DG6), representing the proportion of biantennary fucosylated glycans, offered good discrimination between diabetes subtypes (Supplementary Table 3). The DG7-to-(DG5+DG6) ratio provided near perfect discrimination between HNF1A-MODY and type 2 diabetes (C statistic >0.99) in plasma samples, but it performed less well in analyses of serum samples (C statistic 0.78).

Correlates of the DG9-glycan index within the HNF1A-MODY group. Eighteen subjects within the HNF1A-MODY group did not have diabetes when glycans were sampled. DG9-glycan index levels were not different in the patients with HNF1A-MODY with and without diabetes (P = 0.45). This further confirms that these changes in antennary fucosylation are specific to loss-of-function HNF1A mutations and are unrelated to dysglycemia. DG9-glycan index levels were not correlated with the age at diabetes diagnosis (P = 0.38).

Analysis by mutation type showed median DG9-glycan index was lower in subjects with HNF1A-MODY with protein-changing mutations than those with truncating mutations (0.08 vs. 0.10; P = 0.003) (Table 2). This difference in DG9-glycan index levels did not remain significant when analysis was restricted to one individual per family (the proband), a maneuver that renders the observations independent at the expense of a reduced sample size (P = 0.18). Analysis by functional domain indicated lower median DG9-glycan index in subjects with HNF1A-MODY with missense mutations affecting the dimerization/DNA-binding domains compared with those with missense mutations in the transactivation domain (0.08 vs. 0.13; P = 0.04). The latter relationship was consistent whether we considered all subjects with HNF1A-MODY or only the probands (P = 0.04 and 0.002, respectively). The HNF1A isoform affected had no effect on DG9-glycan index levels (P = 0.30).

We also examined whether the DG9-glycan index could be used as a marker of HNF1A function to provide additional

![FIG. 2. Dot histograms illustrating the DG9-glycan index in different diabetes subtypes and nondiabetic control subjects. Subjects are represented by the following symbols: ● = HNF1A-MODY; ○ = type 2 diabetes; ▲ = type 1 diabetes; △ = GCK-MODY (GCK-MODY); ■ = HNF4A-MODY (HNF4A-MODY); □ = nondiabetic controls. P values are calculated by Mann-Whitney U tests in comparison with subjects with HNF1A-MODY. The median value of the DG9-glycan index for each diabetes subtype is highlighted adjacent to a black dashed line.](http://example.com/figure2.png)
evidence regarding pathogenicity. All missense HNF1A mutations in this study were classified as probably “deleterious” (i.e., pathogenic) or probably “neutral” (i.e., benign) using the software program Condel. Four missense HNF1A mutations (in five subjects) had Condel scores within the benign range: c.92G>A p.Gly31Asp, c.142G>A p.Glu48Lys, c.871C>A p.Pro291Thr, and c.1816G>A p.Gly606Ser. Family data for these mutations were generally less supportive of pathogenicity because of incomplete cosegregation, later age at diagnosis of diabetes, or unavailability of family

**TABLE 2**

DG9-glycan index levels in subjects with HNF1A-MODY according to type and position of HNF1A mutation

<table>
<thead>
<tr>
<th>Subjects with HNF1A-MODY (n)</th>
<th>DG9-glycan index*</th>
<th>P</th>
<th>Unrelated subjects with HNF1A-MODY (n)</th>
<th>DG9-glycan index*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Truncating mutations</td>
<td>83</td>
<td>0.10 (0.07–0.21)</td>
<td>0.003</td>
<td>39</td>
<td>0.10 (0.07–0.14)</td>
</tr>
<tr>
<td>Protein-changing mutations</td>
<td>105</td>
<td>0.08 (0.05–0.21)</td>
<td>0.003</td>
<td>44</td>
<td>0.08 (0.05–0.14)</td>
</tr>
<tr>
<td>Classified by isoform affected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exons 1–6 [isoforms HNF1A(A), (B) and (C)]</td>
<td>93</td>
<td>0.08 (0.05–0.21)</td>
<td>0.003</td>
<td>42</td>
<td>0.08 (0.05–0.11)</td>
</tr>
<tr>
<td>Exons 7 [isoforms HNF1A(A) and (B)]</td>
<td>11</td>
<td>0.09 (0.05–0.18)</td>
<td>0.003</td>
<td>1</td>
<td>0.13</td>
</tr>
<tr>
<td>Exons 8–10 [isoform HNF1A(A) only]</td>
<td>1</td>
<td>0.21</td>
<td>0.003</td>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>Classified by affected functional domain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimerization/DNA-binding domains¹</td>
<td>62</td>
<td>0.08 (0.05–0.17)</td>
<td>0.043</td>
<td>26</td>
<td>0.08 (0.05–0.11)</td>
</tr>
<tr>
<td>Transactivation domain²</td>
<td>17</td>
<td>0.13 (0.08–0.25)</td>
<td>0.043</td>
<td>4</td>
<td>0.21 (0.17–0.24)</td>
</tr>
</tbody>
</table>

P values calculated using Mann-Whitney U and Kruskal-Wallis tests. The Mann-Whitney U test was used to test truncating versus protein-changing mutations and dimerization/DNA-binding versus transactivation domains, while the Kruskal-Wallis test was used to compare the isoforms. ¹Data expressed as median (25th–75th centiles). ²Dimerization domain (amino acids 1–32) and DNA-binding domain (amino acids 91–281). ³Transactivation domain (amino acids 282–631).
members for testing. The median (interquartile range) DG9-glycan index levels in these five subjects were significantly higher compared with the remainder of the group with HNF1A missense mutations [0.16 (0.11–0.21) vs. 0.08 (0.05–0.11); P = 0.01].

**Clinical potential of the DG9-glycan index.** To examine the performance of the DG9-glycan index as a diagnostic screen in clinical practice, we based analyses on U.K. data showing that subjects with unrecognized HNF1A-MODY account for approximately 4% of young-onset type 2 diabetes (diagnosed ≤45 years) and 1% of type 1 diabetes (13). On the basis of the validation study, we estimate that a diagnostic threshold for the DG9-glycan index of 0.16 confers 88% sensitivity and 81% specificity for the discrimination of HNF1A-MODY from young-onset type 2 diabetes and 88% sensitivity and 88% specificity for equivalent comparisons with type 1 diabetes. In contrast, an age younger than 25 years at diagnosis of diabetes, which is the most widely used diagnostic feature for MODY, has lower sensitivity (64%) but higher specificity (99%) for the discrimination of HNF1A-MODY from young-onset type 2 diabetes. We calculate that a patient with diabetes who is diagnosed before or at age 45 years, who has an existing clinical label of type 2 diabetes, and who is found to have a DG9-glycan index ≤0.16 has a posttest probability of harboring an underlying HNF1A mutation of 16%, whereas the same patient with a DG9-glycan index >0.16 has a 1% posttest probability of having unrecognized HNF1A-MODY.

**HNF1A-MODY case-finding in diabetic subjects.** The results of the validation study indicated that a diagnostic threshold of 0.16 for the DG9-glycan index provided optimum discrimination from both type 1 and type 2 diabetes. HNF1A sequencing was performed in 57 subjects with young-onset diabetes and who were clinically labeled as having type 1 or type 2 diabetes, all of whom had a DG9-glycan index ≤0.16. These subjects were either from the initial or validation studies or recruited from general population cohorts in Croatia and Scotland. Three of these 57 individuals were found to have HNF1A mutations. The first proband (Supplementary Fig. 1A) was heterozygous for the missense mutation c.608G>A, p.Arg203His in exon 3, which previously has been shown to be causal for MODY (23). The phenotype is consistent with HNF1A-MODY with a two-generation history of young-adult onset diabetes, and, although treated with insulin and labeled with type 1 diabetes since diagnosis at age 31, this patient was found to have residual endogenous insulin secretion 17 years after diagnosis (C-peptide, 0.27 mmol/L). Two sisters with insulin-treated diabetes also carry the Arg203His mutation. One sister has stopped basal-bolus insulin successfully after the diagnosis of MODY and her diabetes is well controlled with gliclazide; the proband is considering a similar change in therapy. In the second proband (Supplementary Fig. 1B), a novel missense variant c.139G>C, p.Gly47Arg was identified in exon 1. This subject, now aged 53 years, was diagnosed with presumed type 2 diabetes at age 37. Her mother, diagnosed with type 2 diabetes at age 70, also carries the mutation. A normoglycemic son, currently 29 years old, does not carry the mutation. In the third proband (Supplementary Fig. 1C), a novel missense mutation c.751G>A, p.Ala251Thr was found in exon 4. This proband was diagnosed with diabetes at age 43 and is being managed with sulfonylurea (and metformin) therapy 25 years later. Sensitivity to sulfonylureas and maintenance of diabetes control for many years while taking these agents is typical of subjects with HNF1A-MODY. The proband’s mother and a sister had diabetes in old age.

To assess pathogenicity for the two novel variants (Gly47Arg and Ala251Thr), we first established that both variants were absent from 400 normal chromosomes and the October 2011 release of the consensus calls for the 1000 Genomes Project (24). The Ala251Thr variant is not reported in the National Heart, Lung, and Blood Institute’s Exome Sequencing Project (N = 6503; accessed June 2012 via the Exome Variant Server) (25), whereas the Gly47Arg variant is reported in a single European American individual. The phenotypic characteristics of this subject are not declared: because the Exome Sequencing Project includes cases of metabolic and cardiovascular disease, this finding does not exclude a pathogenic role for this variant. Second, we assessed whether the mutated residues were conserved across species. Both Gly47 and Ala251 are highly conserved: Ala251 is conserved in eight of the nine orthologs tested, including frog, chicken, and six mammalian orthologs, whereas Gly47 is conserved in seven of the nine orthologs tested, including *Xenopus*, chicken, and five mammalian orthologs. *In silico* prediction software was more ambiguous: Condel predicted that the variant Gly47Arg is “neutral” and that Ala251Thr is “deleterious.” Overall, we regard the clinical and bioinformatic data for these two variants as supportive, but not conclusive, evidence in favor of pathogenicity. The low DG9-glycan index levels associated with Gly47Arg and Ala251Thr (0.12 and 0.15, respectively) provide additional support for pathogenicity. Definitive evidence of a causal role would require more extensive clinical (including the detection of these mutations in additional MODY families) and functional studies.

**DISCUSSION**

The study confirms the hypothesis that the glycan profile of plasma proteins is altered substantially in those with HNF1A mutations. We also demonstrated that these differences could be exploited as biomarkers in diabetes diagnostics and showed that the DG9-glycan index can discriminate HNF1A-MODY from both type 1 and type 2 diabetes.

Recent efforts to improve diagnostic performance by identifying biochemical markers specific for MODY subtypes have met with varying success (21,26–32). We recently demonstrated that individuals with HNF1A-MODY have low levels of C-reactive protein and that hs-CRP assays can discriminate well between HNF1A-MODY and both type 2 diabetes and *HNF4A*-MODY (21,22,33). However, hs-CRP does not provide good discrimination between HNF1A-MODY and type 1 diabetes. Furthermore, hs-CRP is an acute inflammatory marker, and diagnostic discrimination can be disturbed by intercurrent infection. Potential advantages of the DG9-glycan index in this context include stability over time (10) and differentiation of HNF1A-MODY from both common types of diabetes. Although there is some indication that glycan profiles are affected by acute inflammation (34), all four subjects with HNF1A-MODY and elevated levels of hs-CRP (>10 mg/L) in the current study had DG9-glycan indices below 0.16. This suggests that the DG9-glycan index is less prone to spurious elevation from intercurrent infection than hs-CRP, although this will require confirmation in larger numbers.
The ability to discriminate between HNF1A-MODY and type 1 diabetes in subjects with recently diagnosed diabetes is particularly important because diagnostic misclassification can lead to the unwarranted decision to recommend lifelong therapy with exogenous insulin. Detectable C-peptide can indicate HNF1A-MODY rather than type 1 diabetes of long duration, but it is not helpful close to a diagnosis of diabetes because a substantial proportion of type 1 diabetes patients retain some production of endogenous insulin (35). In this study we did not explicitly examine type 1 diabetes during the honeymoon period; however, glycan profiles are stable within a individual over time, which suggests these measures will continue to provide useful discriminative power from type 1 diabetes close to diagnosis (36). In principle, therefore, the addition of the DG9-glycan index to existing biomarkers such as hs-CRP (21), 1,5-anhydroglucitol (29), pancreatic autoantibodies (37), and C-peptide (13,35) should improve the capacity for clinical discrimination of all major diabetes subtypes.

With respect to clinical utility, our study showed that it was possible to identify subjects with HNF1A mutations using the DG9-glycan index. Given the high sensitivity and specificity of the DG9-glycan index, the proportion of cases found was lower than might have been expected (3 of 57; 5%). There are several possible explanations. The first is that the estimate of discriminatory power based on the C statistics calculated during the validation study is inflated, perhaps because of overfitting (38). An alternative explanation is that discovery of novel cases of HNF1A-MODY might have been compromised in our study samples by the extent of existing clinical investigation (e.g., approximately 30% of the Oxford samples had undergone HNF1A sequencing). Another intriguing possibility is that a small proportion of individuals in large, population-based cohorts have low DG9-glycan index levels due to low-frequency alleles in genes other than HNF1A, which play an important role in protein fucosylation, such as those encoding the fucosyltransferases FUT6 and other genes (1). Further validation of the DG9-glycan index in unselected groups of subjects with young-onset diabetes will be required to assess performance in a more typical clinical scenario in which extensive prior screening for monogenic disease has not been undertaken.

The use of the DG9-glycan index in clinical practice currently is restricted by the cost and limited availability of accurate glycan profiling. Clinical translation is, therefore, dependent on the implementation of a focused assay for specific glycan moieties rather than the global chromatographic profiling used in this study. The DG9-glycan index seems to be the most promising candidate from this study, although the validation study suggests that other measures [such as the DG7-to-(DG5+DG6) ratio] might have superior performance when plasma (rather than serum) samples are available—a finding that requires further confirmation.

There is some evidence for a genotype-phenotype relationship of glycan levels within subjects with HNF1A-MODY, including that DG9-glycan index levels are lower in those with protein-changing rather than truncating HNF1A mutations. We reported a similar relationship in our study of HNF1A sequencing. Another intriguing possibility is that those with protein-changing rather than truncating HNF1A-MODY, including that DG9-glycan index levels are lower in subjects with young-onset diabetes will be required to assess performance in a more typical clinical scenario in which extensive prior screening for monogenic disease has not been undertaken.

In summary, the use of the DG9-glycan index both as a biomarker for HNF1A-MODY and as a promising biochemical measure of HNF1A function represents a compelling example of the potential for rapid clinical translation of a genetic discovery originating from a genome-wide association analysis.

**ACKNOWLEDGMENTS**

Recruitment in Oxford was supported by the National Institute for Health Research (NIHR) Thames Valley Diabetes Local Research Network, part of the U.K. Clinical Research Network. The study was funded by the Oxford (NIHR) Biomedical Research Centre, the European Community FP7 programs CEED3 (HEALTH-F2-2008-223211), ENGAGE (HEALTHF4-2007-201413), and the Medical Research Council (81696). Glycan analysis was supported by the Croatian Ministry of Science, Education and Sport (Grant 309-0061194-2023), the Croatian Science Foundation (Grant 04-47), and grants from the European Commission GlycoBioM (contract 259869) and HighGlycan (contract 278535). The Danish portion of the study was supported by grants from the Lundbeck Foundation Centre for Applied Medical Genomics in Personalized Disease Prediction, Prevention and Care (LuCAMP), the Danish Diabetes Association, and the Danish Research Council. The CROATIA-Vis study was supported by grants from the Medical Research Council U.K. and Ministry of Science, Education and Sport of the Republic of Croatia (108-1080315-0302) and the European Union framework program 6 European Special Populations Research Network project (contract LSHG-CT-2006-018947). ORCADES was supported by the Chief Scientist Office of the Scottish Government, the Royal Society, and the European Union framework program 6 European Special Populations Research Network project (contract LSHG-CT-2006-018947).

A.L.G. is a Wellcome Trust Senior Fellow in Basic Biomedical Science (095101/Z/10/Z), B.A. and I.Ru. received funding from the European Union FP7 EuroGlycoArrays ITN (contract no. 215536). I.KL. received grant support from ERDF (Transendogen/26340220051), VEGA 2/0151/11, and APVV-148-10. E.T. and P.R.N. received funding from the University of Bergen, Helse Vest, Innovest, and the Research Council of Norway. N.D.H. and A.F.W. received financial support from the U.K. Medical
Research Council. K.R.O. is a Clinician Scientist funded by the NIHR. H.C., J.K., G.L., I.Ru., P.M.R., and A.F.W. are listed as inventors on a patent application PCT/EP2011/067112, which covers the use of the analysis of antennary fucose for the diagnosis of HNF1A-MODY. No other potential conflicts of interest relevant to this article were reported.

No funding bodies played any role in the study design, data collection, and analysis or the preparation of or decision to publish the manuscript.


H.C., M.I.M., P.M.R., K.R.O., G.L., and A.F.W. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

This study was presented in abstract form at the 71st Scientific Sessions of the American Diabetes Association, San Diego, California, 24–28 June 2011, and the 47th Annual Meeting of the European Association for the Study of Diabetes, Lisbon, Portugal, 12–16 September 2011.

ORCADES acknowledges the invaluable contributions of Lorraine Anderson (Centre for Population Health Sciences, University of Edinburgh, Edinburgh, U.K.); the research nurses in Orkney; and the administrative team in Edinburgh. The CROATIA-Vis and CROATIA-Korcula studies acknowledge the invaluable contributions of the recruitment team (including those from the Institute of Anthropological Research in Zagreb) in Vis and Korcula, the administrative teams in Croatia and Edinburgh, and the people of Vis and Korcula. DNA extraction for ORCADES was performed by the Genetics Core Laboratory at the Wellcome Trust Clinical Research Facility, WGH, Edinburgh, Scotland. The Slovak investigators acknowledge the expert technical assistance of Dr. Miroslava Huckova (Diabgene, Slovak Academy of Sciences, Bratislava). M.S. acknowledges Jill Little, Royal Infirmary of Edinburgh, for her work in the MODY clinic. The authors thank the National Heart, Lung, and Blood Institute GO Exome Sequencing Project and its ongoing studies, which produced and provided exome variant calls for the sequencing. The authors thank the National Heart, Lung, and Blood Institute, NIHBI Exome Sequencing Project (ESP, Exome Variant Server [Internet]. Seattle, WA, NESPE. Available from http://evs.gs.washington.edu/EVS/). The authors thank the families participating in the study and are grateful for the help of the research nurses in subject recruitment.

REFERENCES

5. Thanabalasingham G, Owen KR. Diagnosis and management of maturity onset diabetes of the young (MODY). BMJ 2011;343:d6044
7. Shields BM, Hicks S, Shepherd MI, Colclough K, Hattersley AT, Ellard S. Maturity-onset diabetes of the young (MODY): how many cases are we missing? Diabetologia 2010;53:2504–2508
22. Ellard S, Colclough K. Mutations in the genes encoding hepatocyte nuclear factor 1 alpha (HNF1A) and 4 alpha (HNF4A) in maturity-onset diabetes of the young. Diabetologia 2009;52:1073–1081
28. Ellard S, Colclough K. Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1 alpha (HNF1A) and 4 alpha (HNF4A) in maturity-onset diabetes of the young. Hum Mutat 2006;27:834–849
29. 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. Nature 2010;467:1061–1073
38. Copas JB. Overestimation of the receiver operating characteristic curve for logistic regression. Biometrika 2002;89:315–331