ORIGINAL ARTICLE

Can Biomarkers Help Target Maturity-Onset Diabetes of the Young Genetic Testing in Antibody-Negative Diabetes?

Shideh Majidi, MD,¹ Alexandra Fouts, BS,¹ Laura Pyle, PhD,² Christina Chambers, MD,² Taylor Armstrong, BS,¹ Zhenyuan Wang, PhD,³ Sat Dev Batish, PhD,³ Georgeanna Klingensmith, MD,¹ and Andrea K. Steck, MD¹

Abstract

Background: Maturity-onset diabetes of the young (MODY) is an antibody-negative, autosomal dominant form of diabetes. With the increasing prevalence of diabetes and the expense of MODY testing, markers to identify those who need further genetic testing would be beneficial. We investigated whether HLA genotypes, random C-peptide, and/or high-sensitivity C-reactive protein (hsCRP) levels could be helpful biomarkers for identifying MODY in antibody-negative diabetes.

Methods: Subjects ($N=97$) with diabetes onset $\leq$ age 25, measurable C-peptide (≥ 0.1 ng/mL), and negative for all four diabetes autoantibodies were enrolled at a large academic center and tested for MODY 1–5 through Athena Diagnostics. A total of 22 subjects had a positive or very likely pathogenic mutation for MODY.

Results: Random C-peptide levels were significantly different between MODY-positive and MODY-negative subjects (0.16 nmol/L vs. 0.02 nmol/L; $P=0.02$). After adjusting for age and diabetes duration, hsCRP levels were significantly lower in MODY-positive subjects (0.37 mg/L vs. 0.87 mg/L; $P=0.02$). Random C-peptide level ≥ 0.15 nmol/L obtained at ≥ 6 months after diagnosis had 83% sensitivity for diagnosis of MODY with a negative predictive value of 96%. Receiver operating characteristic curves showed that area under the curve for random C-peptide (0.75) was significantly better than hsCRP (0.54), high-risk HLA DR3/4-DQB1*0302 (0.59), and high-risk HLA/random C-peptide combined (0.54; $P=0.03$).

Conclusions: Random C-peptide obtained at ≥ 6 months after diagnosis can be a useful biomarker to identify antibody-negative individuals who need further genetic testing for MODY, whereas hsCRP and HLA do not appear to improve this antibody/C-peptide-based approach.

Keywords: Maturity-onset diabetes of the young, *HNFI1A*, *GCK*, *HNFI4A*, Biomarkers.

Introduction

PATIENTS WITH ANTIBODY-negative diabetes are typically diagnosed with type 2 diabetes mellitus (T2DM), with the remainder primarily believed to be type 1 diabetes mellitus (T1DM).¹ About 1% of the pediatric diabetes population are identified as maturity-onset diabetes of the young (MODY), also commonly referred to as monogenetic diabetes,²

although this may be an underestimate as patients with MODY are often misdiagnosed as T1DM or T2DM.^{3,4}

MODY is a group of genetic alterations that together account for a spectrum of autosomal dominant inherited forms of diabetes mellitus often diagnosed before 25 years of age. These disorders result in beta cell dysfunction, but have variable clinical and biochemical features according to the various types of known MODY. The most common types of

¹Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, Aurora, Colorado.

²Department of Pediatrics, University of Colorado Denver, Aurora, Colorado.

³Athena Diagnostics, Marlborough, Massachusetts.

Data in this article have been previously presented at the American Diabetes Association Scientific Sessions in 2014.

MODY are an enzyme mutation in the *GCK* gene (GCK-MODY or MODY 2) and a transcription factor mutation in the *HNF1A* gene (HNF1A-MODY or MODY 3), which results in inadequate insulin production.^{5–7} *HNF4A*, another transcription factor mutation identified as HNF4A-MODY (or MODY 1), is less common and accounts for ~5% of MODY.⁸ At least 12 types of MODY have been described, and there are likely to be other forms that have not yet been identified.^{9–11}

Identification of patients with MODY is important as these subjects are often able to switch from insulin therapy to oral sulfonylurea treatment (HNF1A-MODY and HNF4A-MODY) or no drug therapy (GCK-MODY) depending on type of MODY.^{12–16} In addition, identifying MODY is important for genetic counseling. There have been guidelines and studies published with clinical features to help guide genetic testing.^{2,17} Currently, individuals who are thought to have MODY undergo genetic testing, which can be costly, particularly when the majority of antibody-negative diabetes patients still have T1DM or T2DM. Specific clinical features have been used to decrease the number of antibody-negative individuals who need further screening for MODY,¹⁸ but none of the clinical characteristics can specifically confirm or rule out MODY.¹⁹ Additional biomarkers could help identify those who should have genetic testing done.^{9,20} A SEARCH for Diabetes in Youth study found that C-peptide levels and high-sensitivity C-reactive protein (hsCRP) were not significantly different between MODY-positive and other antibody-negative individuals after adjusting for body mass index z-score and waist circumference.³ However, hsCRP has been found to be significantly lower in individuals specifically with HNF1A-MODY than with other forms of diabetes.^{21–23} This study investigates whether biomarkers such as random C-peptide, hsCRP, and HLA genotypes are useful markers for targeting MODY genetic testing in patients with antibody-negative diabetes.

Methods

Study population

Subjects were recruited from a large academic center, the Barbara Davis Center (BDC). Diabetes autoimmune antibodies are measured in all patients at the BDC at diagnosis or at their first visit to the BDC if they had been diagnosed elsewhere. C-peptide values were measured for this study, once a subject was determined to have negative autoantibodies. Subjects who were <25 years of age at diagnosis and had nonautoimmune diabetes (defined as negative autoantibodies to GAD, IA2, ZnT8, and, if performed within 14 days of onset, IAA) were eligible for the study. A random C-peptide was obtained as part of the study and those with a level ≥ 0.1 ng/mL (the lower limit of the assay at the beginning of the study) were included in the study and offered genetic testing. Of the initial 118 potential participants, 21 were excluded because of iatrogenic diabetes from medications or diabetes secondary to Cystic Fibrosis, Prader Willi, Fanconi's anemia, other syndrome, or refusal to participate. A total of 97 participants were, therefore, recruited into the study. Informed consent and assent, when applicable, were obtained from each study subject. This research study was approved by the Colorado Multiple Institutional Review Board.

Laboratory methods

Autoantibodies to insulin, GAD65, IA-2, and ZnT8 were measured in the Clinical Immunology Laboratory at the BDC using previously described radioimmunoassays.^{24–26} The radioassay cutoffs were set at the 99th percentile of 500 normal control samples for GADA and IA-2, 106 controls for mIAA, and 100 controls for ZnT8, respectively. In the 2015 IASP Workshop, sensitivities and specificities were 52% and 100%, respectively, for mIAA, 82% and 99%, respectively, for GADA, 72% and 100%, respectively, for IA-2A, and 70% and 97%, respectively, for ZnT8A.

HLA Class II genotyping was done in the BDC Clinical Immunology Laboratory using Luminex xMAP technology (Luminex). The specific HLA region to be typed was amplified labeling the amplicon with Biotin. Amplicon was applied to Sequence Specific Oligonucleotide-coupled microspheres. After incubation, Streptavidin-PE was added to bead/amplicon mixture. Microspheres were observed using Luminex 200 instrument. HLA genotypes were categorized as susceptible (having at least one HLA DR3 and/or one HLA DR4-DQB1*0302), protective (HLA DR2-DQB1*0602), or other; analysis with high-risk HLA DR3/4-DQB1*0302 alone was also performed.

Random serum C-peptide (ng/mL) was measured by ELISA immunoassay (Alpco) in the BDC Clinical Immunology Laboratory and HbA1c levels were obtained using a DCA Vantage Analyzer (Siemens). hsCRP was measured by nephelometric assay (Siemens) at the Children's Hospital Colorado CTSC Lab. hsCRP levels >10 ng/mL ($n=9$) were removed from analyses to avoid any confounding such as acute inflammation or infection.

Genetic testing for mutations in *HNF1A*, *HNF4A*, *GCK*, *PDX1*, and *HNF1B* was performed for all participants through Athena Diagnostics. DNA sequencing was performed by PCR amplification of purified genomic DNA, followed by Sanger DNA sequencing of the gene's coding region. In addition, at least 10 bases of intronic DNA on either side of each exon containing the highly conserved exon-intron splice junctions were also sequenced. All abnormal sequence variants were confirmed using bidirectional sequencing. Variants of unknown significance (VUS) were further analyzed for potential pathogenicity by the Molecular Genetics Laboratory at the Royal Devon & Exeter Hospital, United Kingdom as described previously.¹⁹ Variants were classified as benign, likely benign, uncertain, likely pathogenic, or pathogenic based on an assessment of all available evidence obtained.

Statistical analysis

GraphPad Prism Version 6.00 and SAS version 9.4 was used for statistical analysis. C-peptide values equal to zero were changed to 0.0001. All C-peptide values were log-transformed before analysis, and then back-transformed after analysis, so that geometric means are reported. C-peptide levels were compared between the groups both using unadjusted linear models and linear models adjusted for age and duration of diabetes at the time C-peptide was measured. Sensitivity and specificity for HLA genotypes, C-peptide, and hsCRP levels were calculated for MODY-positive versus MODY-negative groups. All hsCRP values were log-transformed before analysis, and back-transformed after analysis, so that

geometric means are reported. hsCRP measurements >10 mg/L were excluded from the analysis, leaving 68 MODY-negative subjects, 3 HNF4A-MODY subjects, 8 GCK-MODY subjects, and 9 HNF1A-MODY subjects for these analyses. hsCRP levels were compared between groups using both unadjusted linear models and linear models adjusted for age and duration of diabetes at the time hsCRP was measured. Logistic models were used to find the hsCRP cutoff that maximized sensitivity and specificity for each of the MODY subgroups versus MODY-negative groups. Receiver operating characteristic (ROC) curves were generated to compare the different biomarkers tested. Logistic regression models and ROC analyses were performed using untransformed C-peptide and hsCRP values, so that the cutoff reported is on the original scale.

Results

Twenty-two of the 97 subjects (22.7%) had positive mutations for MODY (MODY-positive), with 10 subjects positive for HNF1A-MODY (MODY 3), 8 subjects positive for GCK-MODY (MODY 2), and 4 subjects positive for HNF4A-MODY (MODY 1). VUS were determined to be likely pathogenic in 9 of the 22 MODY-positive subjects (19; Supplementary Table S1; Supplementary Data are available at <http://online.liebertpub.com/suppl/doi/10.1089/dia.2017.0317>). The demographic characteristics of study participants (gender, ethnicity, and diabetes duration) were similar for MODY-positive and MODY-negative subjects, except for age of diabetes onset and family history (Table 1). Enrollment in this study was not restricted based on diabetes

diagnosis type. Our MODY-negative patients had a low C-peptide level (0.02 with 95% CI 0.01–0.05 nmol/L), suggesting that this study has more T1DM subjects. At study enrollment visit, the MODY-positive subjects were more likely to have a clinical diagnosis of unspecified diabetes (50%) or MODY (18%), whereas the MODY-negative subjects were mainly diagnosed as T1DM (76%) or T2DM (15%). However, only 1 of the 22 MODY-positive subjects had the correct MODY subtype diagnosis (4.5%) (19; Table 1).

Random C-peptide levels were significantly different between MODY-positive and MODY-negative subjects (0.16 vs. 0.02 nmol/L, $P=0.02$; Fig. 1), and remained significantly different when adjusting for age and diabetes duration (Table 2). After adjusting for age and diabetes duration, hsCRP levels were also significantly different between the MODY-positive and MODY-negative subjects (0.37 vs. 0.87 mg/L respectively, $P=0.02$). The frequency of having at least one susceptible HLA allele for T1DM (i.e., having at least one HLA DR3 and/or one HLA DR4-DQB1*0302) was lower in MODY-positive versus MODY-negative subjects (36% vs. 64% respectively, $P=0.02$, Table 2). MODY-positive and MODY-negative groups did not differ significantly in the frequency of high-risk HLA DR3/4-DQB1*0302 genotype or protective HLA DR2-DQB1*0602 allele, although numbers are small.

ROC analysis of the different biomarkers showed that random C-peptide was significantly better than hsCRP, high-risk HLA, and high-risk HLA/random C-peptide combined ($P=0.03$; Fig. 2). The area under the curve C-peptide was 0.75 compared with 0.54 for hsCRP ($P=0.049$), 0.59 for high-risk HLA (0.06), and 0.54 for the combination of high-risk HLA and random C-peptide ($P=0.005$).

Sensitivity, specificity, positive, and negative predictive values for C-peptide, hsCRP, and HLA are shown in Table 3. Random C-peptide with a cutoff of 0.15 nmol/L at diabetes duration of 6 months or greater gave a sensitivity of 83% and specificity of 67% for MODY-positive subjects, and a negative predictive value of 96%. Similar results were found for C-peptide levels at 12 months or 24 months with sensitivity of 83% and specificity of 68% at 12 months and 80% sensitivity and 66% specificity at 24 months. An hsCRP cutoff of

TABLE 1. CHARACTERISTICS OF MATURITY-ONSET DIABETES OF THE YOUNG-POSITIVE VERSUS MATURITY-ONSET DIABETES OF THE YOUNG-NEGATIVE SUBJECTS

Characteristics at onset of diabetes	MODY-positive (N=22)	MODY-negative (N=75)	P
Female gender	67%	47%	0.09
Age at diabetes onset, years	13.8 ± 4.4	10.6 ± 5.1	0.008
Ethnicity, n (%)			0.16
White	59	61	
Hispanic	41	27	
Other	0	12	
Family history of diabetes	95%	35%	<0.0001
Duration since diagnosis, ^a years	4.2 ± 5.8	4.0 ± 3.5	0.20
Weight category, ^a n (%)			0.76
Underweight	5	1	
Normal weight	55	57	
Overweight	27	26	
Obese	14	16	
Diagnosis, ^{a,b} n (%)			<0.0001
T1DM	23	76	
T2DM	9	15	
Unspecified	50	9	
MODY	18	0	

^aAt time of study visit.

^bOnly one of the 22 (4.5%) MODY-positive subjects had the correct MODY subtype diagnosis.

MODY, maturity-onset diabetes of the young.

FIG. 1. Random C-peptide level by MODY subgroup. MODY-neg, MODY-negative. The horizontal lines on Figure 1 are geometric means. $P=0.02$ for MODY-positive versus MODY-negative groups. MODY, maturity-onset diabetes of the young.

TABLE 2. BIOMARKERS IN MATURITY-ONSET DIABETES OF THE YOUNG-POSITIVE AND MATURITY-ONSET DIABETES OF THE YOUNG-NEGATIVE SUBJECTS

	<i>MODY-positive</i> (N = 22, 22.7%)	<i>MODY-negative</i> (N = 75, 77.3%)	P
Susceptible ^a HLA, N (%)	8 (36.3)	48 (64.0)	0.02
High-risk ^a HLA, N (%)	2 (9.1)	17 (22.7)	0.23
Protective ^a HLA, N (%)	4 (18.2)	8 (10.7)	0.46
Random C-peptide (nmol/L) (95% CI) ^b	0.16 (0.04–0.58)	0.02 (0.01–0.05)	0.02
Adjusted random C-peptide (nmol/L) (95% CI) ^{b,c}	0.13 (0.03–0.63)	0.02 (0.01–0.05)	0.046
hsCRP (mg/L) (95% CI) ^b	0.47 (0.24–0.91)	0.80 (0.56–1.15)	0.15
Adjusted hsCRP ^{b,c}	0.37 (0.19–0.69)	0.87 (0.61–1.24)	0.02

^aSusceptible HLA: at least one HLA DR3 and/or one HLA DR4-DQB1*0302; high-risk HLA: HLA DR3/4-DQB1*0302; protective HLA: HLA DR2-DQB1*0602.

^bgeometric mean $\pm$ 95% CI.

^cAdjusted for age and diabetes duration.

hsCRP, high-sensitivity C-reactive protein.

0.43 mg/L had a sensitivity of 45% and a specificity of 40% for a diagnosis of MODY. High-risk HLA had a specificity of 77% and the combination of high-risk HLA and random C-peptide level >0.15 nmol/L at ≥ 6 months after diagnosis ($n=2$) had a specificity of 97% (Table 3).

Discussion

Identifying patients with MODY can lead to changes in their diabetes treatment, and is important for evaluation of risk in other family members and for genetic counseling.¹⁰ Approximately 1% of those with diabetes are thought to have

one of the most common forms of MODY.³ Genetic testing of antibody-negative patients for MODY is expensive, and less expensive initial testing would be beneficial to determine which patients should have further genetic testing. This study investigated whether less expensive markers would be useful in narrowing down who would benefit from further genetic testing for MODY. This study found that MODY-positive subjects are more likely to have higher C-peptide levels. MODY-positive subjects are also less likely to have susceptible HLA genotypes for T1DM. Sensitivity and negative predictive value were highest for random C-peptide levels 6 months or greater after diagnosis. ROC curves comparing the biomarkers in this study showed that C-peptide provided the best measure for screening compared with hsCRP and high-risk HLA, indicating C-peptide may be a useful biomarker to identify antibody-negative diabetes patients who need further genetic testing for MODY, whereas hsCRP and HLA do not appear to improve this antibody/C-peptide-based approach.

Some studies have shown that C-peptide may be a useful screening tool to differentiate antibody-negative patients, including MODY patients, from T1DM patients,^{27,28} although results have been inconsistent.³ Agladioglu et al. found that both fasting C-peptide and urine C-peptide collected 2 h after a meal had high sensitivity and specificity for MODY when obtained at least 2 years after diagnosis of T1DM.²⁹ A recent study by Shepard et al. presented a method for systematic population screening to identify patients with MODY using antibody testing and urine C-peptide levels and identified a 2.5% prevalence of MODY.³⁰ The study by Chambers et al. done at the BDC found a 21% incidence of pathogenic mutations associated with MODY in subjects with negative diabetes antibodies and preserved random serum C-peptide levels.¹⁹ This study found that C-peptide could be obtained randomly and as early as 6 months and could be used to indicate whether further genetic testing for MODY is warranted. Although this study found overlap between C-peptide levels in MODY-positive and MODY-negative groups, cutoff levels of random C-peptide that can help distinguish these groups were identified. A cutoff level of 0.15 nmol/L for random serum C-peptide in antibody-negative subjects obtained at 6 months or later after diabetes diagnosis had a high negative predictive value of 96%. This did not change substantially when using C-peptide levels at 12 months (95%) or 24 months (93%),

FIG. 2. ROC curves for biomarkers. ROC curves were generated to compare the different biomarkers. Random C-pep, random C-peptide; high-risk HLA, HLA DR3/4-DQB1*0302; ROC, receiver operating characteristic.

TABLE 3. SENSITIVITY, SPECIFICITY, POSITIVE, AND NEGATIVE PREDICTIVE VALUES (AND 95% CI) OF MARKERS FOR MATURITY-ONSET DIABETES OF THE YOUNG-POSITIVE VERSUS MATURITY-ONSET DIABETES OF THE YOUNG-NEGATIVE SUBJECTS

Group	Cutoff value	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Lack of susceptible HLA ^a	—	64% (44%–84%)	64% (53%–75%)	34% (20%–49%)	86% (77%–95%)
High-risk HLA ^a	—	9% (0%–21%)	77% (68%–87%)	11% (0%–24%)	74% (65%–84%)
Random C-peptide ^b	0.15 nmol/L ≥6 months after diagnosis	83% (62%–100%)	67% (55%–78%)	31% (15%–47%)	96% (90%–100%)
High-risk HLA and random C-peptide ^b	Random C-peptide 0.15 nmol/L ≥6 months after diagnosis	8% (0%–24%)	97% (93%–100%)	33% (0%–87%)	85% (77%–93%)
hsCRP ^{c,d}	≤0.43 mg/L	45% (23%–67%)	40% (28%–52%)	19% (8%–30%)	70% (56%–85%)

^aSusceptible HLA: at least one HLA DR3 and/or one HLA DR4-DQB1*0302; high-risk HLA: HLA DR3/4-DQB1*0302; protective HLA: HLA DR2-DQB1*0602.

^bTotal $n=78$, based on c-peptide levels obtained ≥6 months after diagnosis.

^cAdjusted for age and diabetes duration.

^dTotal $n=85$, excluding hsCRP levels >10 mg/L.

suggesting that random C-peptide levels could potentially be used as soon as 6 months after diagnosis to help determine whether to obtain genetic testing for MODY in antibody-negative patients. Within this population, this study indicates that it may be best and less costly to use C-peptide levels to help determine which patients need additional testing, particularly to determine antibody-negative T1DM versus MODY patients. Random C-peptide is easier to obtain and, therefore, more clinically relevant to measure than fasting C-peptide and mixed-meal tolerance test.^{27,31} In a study of 1093 patients, random C-peptide, fasting C-peptide, and C-peptide levels after a glucagon stimulation all had highly discriminative power, and the ROC curve for random C-peptide levels was superior.³² Thus, random C-peptide testing would be an inexpensive and simple initial test to obtain for screening for potential MODY in antibody-negative patients.

Recent studies have suggested that hsCRP may be useful to help identify the most common MODY (*HNF1A*) type from other forms of diabetes. This is particularly interesting given that genome-wide association studies have found that the CRP gene has *HNF1A* binding sites in its promoter and that common variants mapping near the *HNF1A* gene on 12q24 are associated with alterations in CRP levels.³³ The studies looking at hsCRP in MODY-positive subjects found that *HNF1A*-MODY had lower hsCRP levels than other types of diabetes, including T1DM, T2DM,³⁴ and GCK-MODY,²² as well as *HNF4A*-MODY.²³ This study found that hsCRP was lower in MODY-positive compared to MODY-negative subjects (0.37 vs. 0.87 ng/mL, $P=0.02$).

The findings of this study indicate that C-peptide levels may be inexpensive markers that could be used to differentiate those antibody-negative patients who should obtain further genetic testing for MODY. Individual MODY gene testing can cost >10 times the cost of C-peptide, whereas testing for a combination of some or all MODY genes can cost >30–50 times the cost of C-peptide. These expenses are based on cost estimates in the United States and may not be the same in other countries. In addition, it is likely that these costs will change in the future as costs of genetic testing are decreasing. Other biomarkers such as hsCRP and HLA-DR

and DQ typing are also much less expensive than MODY genetic testing. Cost-effectiveness of MODY genetic testing has been analyzed by Naylor et al., showing that genetic testing is cost-effective.³⁵ However, genetic testing is not always covered by health insurance.

This study adds to the paucity of literature looking at additional, less expensive markers to screen patients who need further MODY testing. This is important given that MODY is likely underdiagnosed, and treatment of different MODY subtypes differs compared with treatment of T1DM or T2DM. A T1DM-genetic risk score (T1DM-GRS) has recently been developed from T1DM-associated common genetic variants, providing a possible way to discriminate monogenic diabetes from T1DM with numerous single nucleotide polymorphisms (SNPs).³⁶ The T1DM-GRS requires genotyping for multiple SNPs, whereas our study found use for less costly biomarkers at 6 months or greater after diagnosis. Using biomarkers in those >6 months postdiagnosis can help to narrow down those who would need further genetic testing with either T1DM-GRS or specific MODY genetic testing.

Strengths of this study include a systematic, unbiased method of selecting subjects for genetic testing of MODY, rather than a referral system. Patients followed at a large diabetes center with diabetes onset earlier than age 25 were tested for all four diabetes autoantibodies (GADA, IA-2, ZnT8, and, if performed within 14 days of onset, IAA) and random C-peptide. The higher incidence of MODY in this study is likely reflective of the necessity of having three to four negative diabetes antibodies and a preserved C-peptide. Family history of diabetes was not a selection criterion. This study also found that random C-peptide obtained as early as 6 months after diagnosis can be useful in identifying whether further genetic testing for MODY should be done, although sample size in this study is small. In addition, all subjects had genetic testing for all current clinically available MODY subtypes (MODY 1–5), and VUS were further analyzed for potential pathogenicity. Limitations of this study include the small sample size of MODY-positive patients. Limitations of hsCRP include that levels can be raised if the patient has an inflammation or infection, which is why this study removed patient samples with hsCRP levels >10 mg/L. In clinical

practice, if a patient has an hsCRP level >10 mg/L, repeat testing should be obtained before using the level in determining whether further genetic testing is needed, given that the elevated level is likely transient and related to an acute inflammatory response.

Conclusions

In summary, identification of patients with MODY diabetes is important to ensure that the most appropriate therapy is provided to patients. Random C-peptide obtained as early as 6 months after diagnosis can help distinguish antibody-negative patients needing further genetic testing for MODY from those who do not, whereas hsCRP and HLA do not appear to improve this antibody/C-peptide-based approach. These findings should be confirmed in larger populations of MODY patients and antibody-negative diabetes patients.

Author Contributions

S.M. wrote the article and researched data. A.F. and T.A. collected and researched data. C.C. researched data and reviewed the article. L.P. analyzed the data. Z.W., S.D.B., and M.J. were involved with genetic testing and reviewed article. G.K. reviewed/edited the article. A.K.S. designed the study, researched data, and reviewed/edited the article. This research was funded by The Children's Diabetes Foundation at Denver. S.M. was supported by the Talofa Foundation and NIH Grant No. K12DK0942712. A.K.S. was supported by ADA Grant No. 1-14-CD-17.

Acknowledgments

We would like to thank the patients and families who participated in this study. We would like to thank Malgorzata Jaremko, PhD, for her contribution to the study while working at Athena Diagnostics.

Author Disclosure Statement

The authors have no relevant conflict of interest to disclose. Dr. S.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Dabelea D, Pihoker C, Talton JW, et al.: Etiological approach to characterization of diabetes type: the SEARCH for Diabetes in Youth Study. *Diabetes Care* 2011;34:1628–1633.
2. Rubio-Cabezas O, Hattersley AT, International Society for P, et al.: ISPAD clinical practice consensus guidelines 2014. The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes* 2014;15 Suppl 20:47–64.
3. Pihoker C, Gilliam LK, Ellard S, et al.: Prevalence, characteristics and clinical diagnosis of maturity onset diabetes of the young due to mutations in HNF1A, HNF4A, and glucokinase: results from the SEARCH for Diabetes in Youth. *J Clin Endocrinol Metab* 2013;98:4055–4062.
4. Schober E, Rami B, Grabert M, et al.: Phenotypical aspects of maturity-onset diabetes of the young (MODY diabetes) in comparison with Type 2 diabetes mellitus (T2DM) in children and adolescents: experience from a large multi-centre database. *Diabet Med* 2009;26:466–473.
5. Steele AM, Wensley KJ, Ellard S, et al.: Use of HbA1c in the identification of patients with hyperglycaemia caused by a glucokinase mutation: observational case control studies. *PLoS One* 2013;8:e65326.
6. Kyithar MP, Bacon S, Pannu KK, et al.: Identification of HNF1A-MODY and HNF4A-MODY in Irish families: phenotypic characteristics and therapeutic implications. *Diabetes Metab* 2011;37:512–519.
7. Hattersley AT: Maturity-onset diabetes of the young: clinical heterogeneity explained by genetic heterogeneity. *Diabet Med* 1998;15:15–24.
8. Ellard S, Bellanne-Chantelot C, Hattersley AT, et al.: Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. *Diabetologia* 2008;51:546–553.
9. Owen KR: Monogenic diabetes: old and new approaches to diagnosis. *Clin Med* 2013;13:278–281.
10. Steck AK, Winter WE: Review on monogenic diabetes. *Curr Opin Endocrinol Diabetes Obes* 2011;18:252–258.
11. Naylor R, Philipson LH: Who should have genetic testing for maturity-onset diabetes of the young? *Clin Endocrinol (Oxf)* 2011;75:422–426.
12. Pearson ER, Starkey BJ, Powell RJ, et al.: Genetic cause of hyperglycaemia and response to treatment in diabetes. *Lancet* 2003;362:1275–1281.
13. Pearson ER, Liddell WG, Shepherd M, et al.: Sensitivity to sulphonylureas in patients with hepatocyte nuclear factor-1alpha gene mutations: evidence for pharmacogenetics in diabetes. *Diabet Med* 2000;17:543–545.
14. Shepherd M, Pearson ER, Houghton J, et al.: No deterioration in glycemic control in HNF-1alpha maturity-onset diabetes of the young following transfer from long-term insulin to sulphonylureas. *Diabetes Care* 2003;26:3191–3192.
15. Shepherd M, Shields B, Ellard S, et al.: A genetic diagnosis of HNF1A diabetes alters treatment and improves glycaemic control in the majority of insulin-treated patients. *Diabet Med* 2009;26:437–441.
16. Ajjan RA, Owen KR: Glucokinase MODY and implications for treatment goals of common forms of diabetes. *Curr Diab Rep* 2014;14:559.
17. Bellanne-Chantelot C, Levy DJ, Carette C, et al.: Clinical characteristics and diagnostic criteria of maturity-onset diabetes of the young (MODY) due to molecular anomalies of the HNF1A gene. *J Clin Endocrinol Metab* 2011;96:E1346–E1351.
18. Gandica RG, Chung WK, Deng L, et al.: Identifying monogenic diabetes in a pediatric cohort with presumed type 1 diabetes. *Pediatr Diabetes* 2015;16:227–233.
19. Chambers C, Fouts A, Dong F, et al.: Characteristics of maturity onset diabetes of the young in a large diabetes center. *Pediatr Diabetes* 2016;17:360–367.
20. Owen KR, Skupien J, Malecki MT, Consortium C: The clinical application of non-genetic biomarkers for differential diagnosis of monogenic diabetes. *Diabetes Res Clin Pract* 2009;86 Suppl 1:S15–S21.
21. Owen KR: RD Lawrence lecture 2012: assessing aetiology in diabetes: how C-peptide, CRP and fucosylation came to the party! *Diabet Med* 2013;30:260–266.
22. Owen KR, Thanabalasingham G, James TJ, et al.: Assessment of high-sensitivity C-reactive protein levels as diagnostic discriminator of maturity-onset diabetes of the young due to HNF1A mutations. *Diabetes Care* 2010;33:1919–1924.

23. McDonald TJ, Shields BM, Lawry J, et al.: High-sensitivity CRP discriminates HNF1A-MODY from other subtypes of diabetes. *Diabetes Care* 2011;34:1860–1862.
24. Bonifacio E, Yu L, Williams AK, et al.: Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney disease consortia. *J Clin Endocrinol Metab* 2010;95:3360–3367.
25. Yu L, Rewers M, Gianani R, et al.: Antiislet autoantibodies usually develop sequentially rather than simultaneously. *J Clin Endocrinol Metab* 1996;81:4264–4267.
26. Verge CF, Gianani R, Kawasaki E, et al.: Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 1996;45:926–933.
27. Ludvigsson J, Carlsson A, Forsander G, et al.: C-peptide in the classification of diabetes in children and adolescents. *Pediatr Diabetes* 2012;13:45–50.
28. Jones AG, Hattersley AT: The clinical utility of C-peptide measurement in the care of patients with diabetes. *Diabet Med* 2013;30:803–817.
29. Agladioglu SY, Sagsak E, Aycan Z: Urinary C-Peptide/Creatinine ratio can distinguish maturity-onset diabetes of the young from type 1 diabetes in children and adolescents: a single-center experience. *Horm Res Paediatr* 2015;84:54–61.
30. Shepard M, Shields B, Hammersley S, et al.: Systematic population screening, using biomarkers and genetic testing, identifies 2.5% of the U.K. pediatric diabetes population with monogenic diabetes. *Diabetes Care* 2016;39:1879–1888.
31. Redondo MJ, Rodriguez LM, Escalante M, et al.: Types of pediatric diabetes mellitus defined by anti-islet autoimmunity and random C-peptide at diagnosis. *Pediatr Diabetes* 2013;14:333–340.
32. Berger B, Stenstrom G, Sundkvist G: Random C-peptide in the classification of diabetes. *Scand J Clin Lab Invest* 2000;60:687–693.
33. Reiner AP, Barber MJ, Guan Y, et al.: Polymorphisms of the HNF1A gene encoding hepatocyte nuclear factor-1 alpha are associated with C-reactive protein. *Am J Hum Genet* 2008;82:1193–1201.
34. Thanabalasingham G, Shah N, Vaxillaire M, et al.: A large multi-centre European study validates high-sensitivity C-reactive protein (hsCRP) as a clinical biomarker for the diagnosis of diabetes subtypes. *Diabetologia* 2011;54:2801–2810.
35. Naylor RN, John PM, Winn AN, et al.: Cost-effectiveness of MODY genetic testing: translating genomic advances into practical health applications. *Diabetes Care* 2014;37:202–209.
36. Patel KA, Oram RA, Flanagan SE, et al.: Type 1 Diabetes genetic risk score: a novel tool to discriminate monogenic and type 1 diabetes. *Diabetes* 2016;65:2094–2099.

Address correspondence to:

Shideh Majidi, MD

Barbara Davis Center for Childhood Diabetes

University of Colorado Denver

1775 Aurora Ct, A140,

Aurora, CO 80045

E-mail: shideh.majidi@ucdenver.edu