Population-Based Assessment of a Biomarker-Based Screening Pathway to Aid Diagnosis of Monogenic Diabetes in Young-Onset Patients

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OBJECTIVE

Monogenic diabetes, a young-onset form of diabetes, is often misdiagnosed as type 1 diabetes, resulting in unnecessary treatment with insulin. A screening approach for monogenic diabetes is needed to accurately select suitable patients for expensive diagnostic genetic testing. We used C-peptide and islet autoantibodies, highly sensitive and specific biomarkers for discriminating type 1 from non–type 1 diabetes, in a biomarker screening pathway for monogenic diabetes.

RESEARCH DESIGN AND METHODS

We studied patients diagnosed at age 30 years or younger, currently younger than 50 years, in two U.K. regions with existing high detection of monogenic diabetes. The biomarker screening pathway comprised three stages: *1*) assessment of endogenous insulin secretion using urinary C-peptide/creatinine ratio (UCPCR); *2*) if UCPCR was ≥0.2 nmol/mmol, measurement of GAD and IA2 islet autoantibodies; and *3*) if negative for both autoantibodies, molecular genetic diagnostic testing for 35 monogenic diabetes subtypes.

RESULTS

A total of 1,407 patients participated (1,365 with no known genetic cause, 34 with monogenic diabetes, and 8 with cystic fibrosis–related diabetes). A total of 386 out of 1,365 (28%) patients had a UCPCR ≥0.2 nmol/mmol, and 216 out of 386 (56%) were negative for GAD and IA2 and underwent molecular genetic testing. Seventeen new cases of monogenic diabetes were diagnosed (8 common Maturity Onset Diabetes of the Young [Sanger sequencing] and 9 rarer causes [next-generation sequencing]) in addition to the 34 known cases (estimated prevalence of 3.6% [51/1,407] [95% CI 2.7–4.7%]). The positive predictive value was 20%, suggesting a 1-in-5 detection rate for the pathway. The negative predictive value was 99.9%.

CONCLUSIONS

The biomarker screening pathway for monogenic diabetes is an effective, cheap, and easily implemented approach to systematically screening all young-onset patients. The minimum prevalence of monogenic diabetes is 3.6% of patients diagnosed at age 30 years or younger.

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Correct classification of a patient's diabetes is important to ensure he or she receives the most appropriate treatment and ongoing management. The most common form of diabetes in children and young adults is type 1 diabetes, accounting for >90% of cases (1,2). Other forms of diabetes in this age group, such as monogenic diabetes (including Maturity Onset Diabetes of the Young [MODY]), or young-onset type 2, are not often considered. It is estimated that at least 80% of patients with MODY are misdiagnosed (3), and other rarer forms of monogenic diabetes often go unrecognized because of lack of awareness (4). Patients with MODY or type 2 diabetes misclassified as type 1 diabetes will be treated with insulin, whereas noninsulin therapy would be more appropriate. Diet and metformin are the treatment of choice in young type 2 diabetes (5). Patients with MODY because of mutations in the *HNF1A* or *HNF4A* genes respond well to low-dose sulphonylureas (6,7), and those with MODY because of mutations in the *GCK* gene require no pharmacological treatment (8). Getting a correct diagnosis for all forms of monogenic diabetes has important implications for management of an individual's diabetes, a prognosis, and recognition of associated clinical features; it also allows appropriate counseling of other family members regarding likely inheritance (4).

Identifying patients with monogenic diabetes, particularly MODY, can be challenging. Monogenic diabetes is confirmed by molecular genetic testing, but this is expensive, so testing all patients is not feasible. An approach that could be used to enrich for monogenic diabetes, increasing the proportion identified in those who undergo genetic testing, would be helpful. Clinical features can aid identification of those who may have an alternative diagnosis, and a probability calculator has been developed to help determine which patients are likely to have the most common forms of MODY (9). However, this will not pick up other forms of monogenic diabetes, and its performance is weaker for detecting MODY in insulin-treated patients compared with non–insulin-treated patients.

An alternative approach to enrich for monogenic diabetes is to use biomarkers that have been shown to discriminate well between type 1 and other forms of young-onset diabetes. Type 1 diabetes is characterized by autoimmune destruction of the β-cells in the pancreas, leading to absolute insulin deficiency, so two tests that could be used to diagnose type 1 diabetes are islet autoantibodies (markers of the autoimmune process) and C-peptide (a marker of insulin deficiency). C-peptide has been shown to be a highly sensitive and specific biomarker for discriminating between type 1 and type 2 diabetes and MODY 3–5 years after diagnosis (10,11). Urine C-peptide/creatinine ratio (UCPCR) can be used to remove the need for blood samples, which may be of particular concern in the pediatric population, and means that the sample can easily be taken at home and posted to the laboratory (12). GAD and IA2 islet autoantibodies also discriminate well between type 1 and MODY, with cross-sectional studies showing they are present in 80% of patients with type 1 diabetes and in <1% of patients with MODY (13). These biomarkers have been used to screen for MODY in other studies (14,15), but have been limited to pediatric cases only. Given that the median age at diagnosis for MODY is 20 years (from U.K. referrals data [3]), and there is on average a delay of 13 years from diabetes diagnosis to a confirmed genetic diagnosis (16), it is crucial to study adults as well. Furthermore, the combined diagnostic performance of the two biomarkers as a screening pathway has not been formally assessed.

By excluding those with type 1 diabetes using these two biomarkers, we can obtain a smaller percentage of patients in whom diagnostic molecular testing for monogenic diabetes could be performed. We tested a screening pathway using both C-peptide and islet autoantibodies to exclude type 1 diabetes in two populations with previously high pickup rates of MODY (3) and performed genetic testing on all patients with significant endogenous insulin and absence of islet autoantibodies. This allowed us to determine the prevalence of all monogenic diabetes subtypes in those diagnosed at 30 years or younger and to calculate the positive predictive values (PPVs) and negative predictive values (NPVs) for the pathway.

RESEARCH DESIGN AND METHODS

Subjects

Patients diagnosed at age 30 years or younger, and currently aged younger than 50 years, in the catchment areas of the Royal Devon and Exeter NHS Foundation Trust (Exeter, U.K.) and Ninewells Hospital (Dundee, U.K.) were invited to

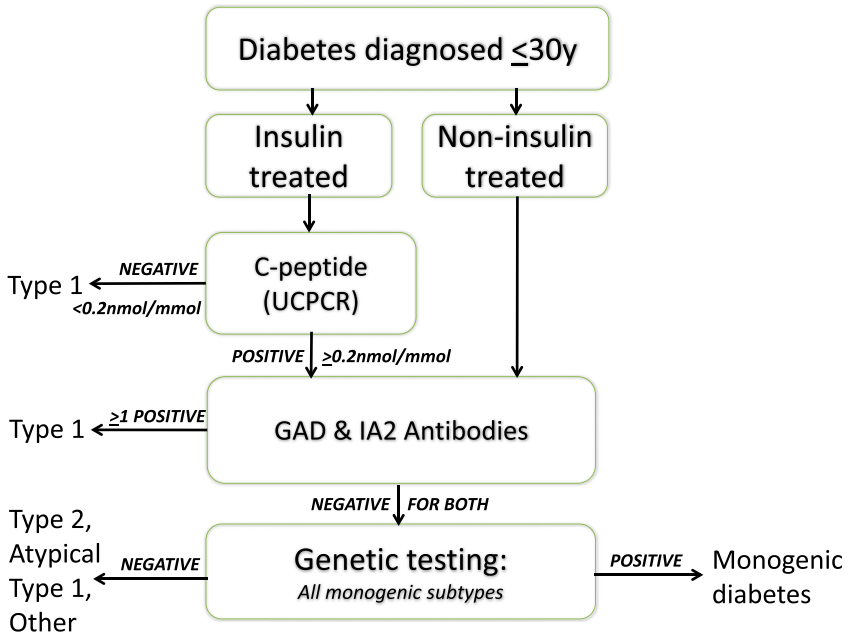

Figure 1—The UNITED biomarker screening pathway to investigate etiology of diabetes in patients diagnosed at age 30 years or younger. Genetic testing is carried out on all patients who have endogenous insulin (UCPCR ≥0.2 nmol/mmol) and do not have either GAD or IA2 islet autoantibodies. Patients without endogenous insulin or with GAD and/or IA2 islet autoantibodies are classed as having type 1 diabetes.

take part in the study via the doctors looking after their medical care. All patients with diabetes in this age group were eligible regardless of cause. Both regions had existing high pickup rates for MODY prior to the study because of research interests (3). Patients who consented provided samples as part of the biomarkers screening pathway (the Using pharmacogeNetics to Improve Treatment in Early-onset Diabetes [UNITED] study; clinicaltrials.gov NCT01238380).

Biomarker Screening Pathway

All recruited patients followed the biomarker screening pathway (Fig. 1).

Assessment of Endogenous Insulin, in Insulin-Treated Patients, Using UCPCR

A key determinant of requirement for insulin treatment is lack of endogenous insulin secretion, and UCPCR is an easy screening test that can be done at home. UCPCR was used to rule out the majority of patients with type 1 diabetes in the first stage of screening, with minimal patient burden.

Insulin-treated patients were asked to collect a urine sample 2 h after the largest carbohydrate-containing meal of the day and to post this direct to the laboratory in a pack provided to allow analysis within 72 h of sample collection, in line with assay stability (12).

Urinary C-peptide was measured by an electrochemiluminescence immunoassay (intra-assay coefficient of variation, 3.3%; and interassay coefficient of variation, 4.5%) on an E170 analyzer (Roche Diagnostics, Mannheim, Germany) (12). The lower limit of the C-peptide assay was 0.03 nmol/L. Urinary creatinine was analyzed on the Roche P800 platform using creatinine Jaffé reagent (standardized against isotope dilution mass spectrometry) and used to calculate UCPCR (nmol/mmol). Patients with UCPCR $\geq$0.2 nmol/mmol were considered to have significant endogenous insulin secretion (10).

Islet Autoantibody Measurement in Patients With Significant Endogenous Insulin

Islet autoantibodies (GAD and IA2) were measured in patients who tested positive for UCPCR ($\geq$0.2 nmol/mmol) or who were noninsulin treated. In order to minimize taking blood samples, particularly in children, the local pathology databases were checked for previous GAD and IA2 results, and these were used if available. Patients with no previous islet autoantibody results were invited to attend an appointment with the study's research nurse to provide blood samples for islet autoantibody testing and DNA.

GAD and IA2 antibody analysis was performed using commercial ELISA assays (RSR Ltd., Cardiff, U.K.) and a Dynex DSX automated ELISA system (Launch Diagnostics, Longfield, U.K.) (13). Both methods are highly specific and sensitive (GAD antibodies, 98 and 84%, and IA-2 antibodies, 99 and 74%, respectively). The laboratory participates in the Diabetes Autoantibody Standardization Program. Patients were considered positive for antibodies if their results were >99th centile (64 World Health Organization units/mL for GAD and 15 World Health Organization units/mL for IA2) (13).

Diagnostic Molecular Genetic Testing for Monogenic Diabetes in Patients With Significant Endogenous Insulin and Negative Antibody Results

Sequencing of Three MODY Genes, the Most Common Forms of Monogenic Diabetes. For all patients who were negative for both GAD and IA2 antibodies with significant endogenous insulin, DNA sequencing of *HNF1A*, *HNF4A*, and *GCK* was performed by PCR amplification of purified genomic DNA, followed by Sanger DNA sequencing of each gene's exons and flanking intronic regions. Dosage analysis of *HNF1A*, *HNF4A*, and *GCK* for partial- and whole-gene deletions was also performed by multiplex ligation-dependent probe amplification using the MRC-Holland MODY multiplex ligation-dependent probe amplification kit (P241-B1).

Targeted Next-Generation Sequencing for 35 Genes in Which Mutations Are Known to Cause Monogenic Diabetes. If no pathogenic mutation was identified in *HNF1A*, *HNF4A*, or *GCK*, further targeted next-generation sequencing was performed for mutations in 35 monogenic diabetes genes (all genes in which mutations are known to cause MODY, neonatal diabetes, and other genetic diabetic syndromes) using a custom SureSelect exon-capture assay (Agilent Technologies, Santa Clara, CA) (17) (see Supplementary Data and Supplementary Table 1 for methodology, sensitivity, and details of genes tested).

Statistical Analysis

For comparing new cases diagnosed through the screening pathway to known cases of monogenic diabetes and for comparing the biomarker screening pathway with an approach using clinical features (including the MODY probability calculator [9]) to detect monogenic diabetes, variables were categorical, and therefore, χ^2 and Fisher exact tests were used.

Prevalence of MODY

The prevalence of MODY in this population was determined as the proportion of positive cases (including both known MODY who were recruited and those identified through the study) out of the total recruited.

To determine whether there was any potential bias in recruitment of MODY patients that may affect our prevalence estimate, we also obtained summary data on the number of patients with previously confirmed monogenic diabetes in each study area who had not been recruited into this study.

Positive and Negative Predictive Values of Pathway

Calculating the prevalence in this population allows us to determine the PPVs and NPVs for the pathway, the most important statistics for the clinician. PPVs and NPVs were calculated as posttest odds = pretest odds $\times$ positive likelihood ratio, in which pretest odds is prevalence/(1 $-$ prevalence), and positive likelihood ratio is sensitivity/(1 $-$ specificity). PPV (equivalent to posttest probability) is posttest odds/(1 + posttest odds). NPV was calculated similarly, but using a negative likelihood ratio (1 $-$ sensitivity/specificity), with negative posttest probability equal to 1 $-$ NPV. Number needed to test was calculated as 1/PPV.

Performance of the Pathway: Sensitivity and Specificity

The key question is: how well, if applied to a whole population, do the biomarkers perform in a pathway for identifying new cases? Screening literature emphasizes the difference between program sensitivity/specificity and test sensitivity/specificity, in which assessing the sensitivity/specificity of a screening program such as this necessarily requires approximation using multiple data sources (18). As this was a population-based study rather than a case-control study, formal assessments of sensitivity and specificity (as normally conducted using a 2 $\times$ 2 table) of the pathway were limited because of the rarity of monogenic diabetes (meaning a small sample size of true positive cases of monogenic diabetes) and

the expense of genetic testing (restricting confirmation of all the true negative non-monogenic cases).

Assessments of sensitivity of the components of the pathway for detecting monogenic diabetes have been carried out in larger case-control cohorts (n = 508 monogenic diabetes cases for islet autoantibodies [99% sensitivity] [13]; and n = 160 for UCPCR [99% sensitivity, both studies combined] [10,11]), so it is more appropriate to use these estimates. We assumed a 98% sensitivity for both combined, based on these larger studies (assuming 1% missed because of false-negative UCPCR and 1% because of false-positive islet autoantibodies). However, the detection rate in all true monogenic cases in this pathway will be calculated for comparison.

Calculation of the specificity is limited, as we have not performed genetic testing on all C-peptide–negative patients. Previous larger studies have shown <1% of patients are missed (10,11,13). However, specificity of the biomarkers in these studies was assessed using gold-standard type 1 diabetes as the comparison group, rather than all non-MODY patients in this age range, and thus likely overestimates the performance because of spectrum bias (19). We therefore calculated specificity based on one minus the false-positive rate of the pathway (i.e., proportion UCPCR-positive/antibody-negative, but not having a confirmed diagnosis of monogenic diabetes on subsequent genetic testing). This assumes that all patients negative according to the pathway are true negatives. As an additional test of this assumption, a subset of patients negative for islet autoantibodies received genetic testing for the three main MODY genes, and the proportion of MODY was calculated.

Health economic evaluation of the pathway is addressed in a separate project (20,21).

RESULTS

Subjects

The flow of subjects through the study is shown in Fig. 2. A total of 2,288 patients were eligible in area, and 1,418 subjects (62%) in total consented to the study and were recruited: 716 from the Exeter area and 702 from Dundee. A total of 11 patients dropped out (9 did not provide blood samples for antibody testing, and 2 did not provide samples for DNA testing).

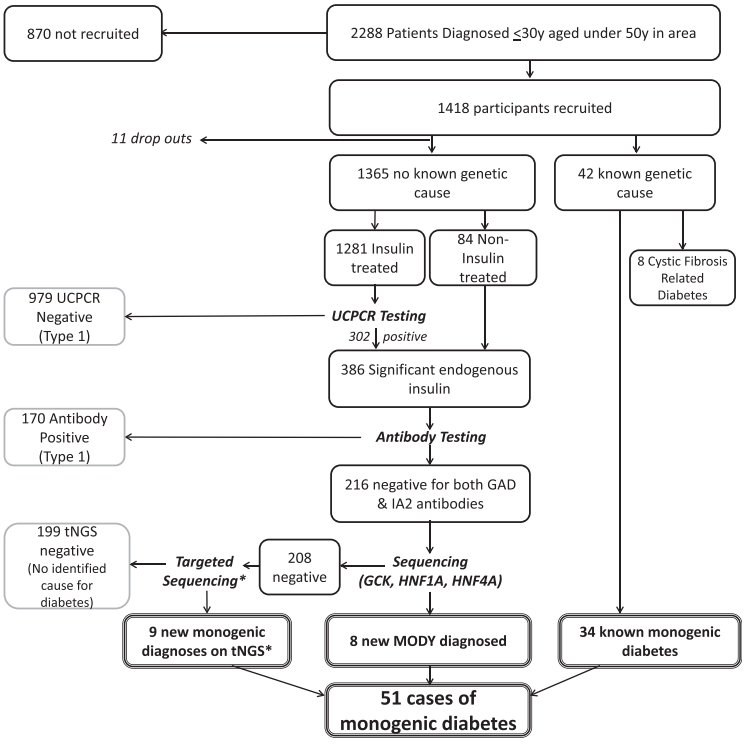

Figure 2—Flow chart of patients recruited as part of UNITED. Biomarker screening pathway in 1,376 patients with no known genetic cause for their diabetes in Exeter and Tayside. Eleven dropped out. Seventeen new cases of monogenic diabetes detected (*one case identified through exome sequencing).

Of the 1,407 remaining patients, 1,365 had no known genetic cause for their diabetes. Characteristics of these patients are shown in Supplementary Table 2, and subsequent results on the screening pathway are based on these patients. A total of 42 patients had a known genetic cause for their diabetes prior to participating in this pathway: 34 patients had confirmed monogenic diabetes (Table 1), and 8 patients had cystic fibrosis–related diabetes.

Biomarker Screening Pathway Identifies 17 New Cases of Monogenic Diabetes

Excluding dropouts, 1,281 (94%) of 1,365 patients with no known genetic cause for their diabetes were insulin treated and provided a sample for UCPCR testing. Two patients were anuric because of renal failure and therefore went straight on to antibody testing. A total of 979 of these patients (76%) had minimal endogenous insulin secretion (UCPCR <0.2 nmol/mmol), indicating a diagnosis of type 1 diabetes, and received no further testing.

Islet autoantibodies were tested in the 84 non–insulin-treated patients, 300 UCPCR-positive patients, and 2 anuric patients. A total of 170 out of 386 (44%) tested positive for GAD and/or IA2 antibodies, confirming islet autoimmunity and hence a diagnosis of type 1 diabetes. Therefore, these patients received no further testing.

Sanger sequencing for the three most common MODY genes was undertaken in 216 patients (16% of the whole cohort). Eight patients tested positive, confirming a diagnosis of MODY: five HNF1A, two HNF4A, and one GCK (Fig. 2 and Table 1).

Of the 208 who tested negative for the common MODY genes, additional testing by targeted next-generation sequencing identified mutations in genes associated with monogenic diabetes in a further 8 patients, and 1 patient had a mutation in *POLD1* identified through exome sequencing (Fig. 2 and Table 1).

New Cases of Monogenic Diabetes Identified Were More Likely to Be Rarer Causes and Atypical

More of the new cases of monogenic diabetes identified had mutations in genes other than the three most common forms of MODY (25 out of 34 [74%] of those diagnosed prior to the study had mutations in *HNF1A*, *HNF4A*, or *GCK* compared with 8 out of 17 [47%] identified from

Table 1—Characteristics of patients diagnosed with monogenic diabetes and details of mutations found for those recruited but diagnosed with monogenic diabetes prior to the study and those diagnosed as a result of the biomarker pathway

	Genetic characteristics				Clinical characteristics						
ID	Gene	Method	Zygosity	DNA level description	Age at diagnosis (years)	Treatment	Parent with diabetes	BMI	HbA_{1c}	Age at recruitment (years)	Additional clinical features
Recruited but diagnosed prior to the UNITED study											
211	GCK	Sanger	Het	c.97_117dup	3	Diet	Yes	34.0	48	16	
523	GCK	Sanger	Het	c.97_117dup	27	Diet	No	51.7	55	43	
537	GCK	Sanger	Het	c.683C>T	11	Diet	Yes			13	
538	GCK	Sanger	Het	c.683C>T	9	Diet	Yes			11	
542	GCK	Sanger	Het	c.184G>A	29	Diet	Yes	38.9	48	39	
543	GCK	Sanger	Het	c.184G>A	4	Diet	Yes			4	
544	GCK	Sanger	Het	c.184G>A	3	Diet	Yes			5	
1155	GCK	Sanger	Het	c.1343G>T	25	Diet	Yes	19.3	50	25	
82095	GCK	Sanger	Het	c.1019G>T	9	Diet	Yes	21.9	45	14	
535	HNF1A	Sanger	Het	c.379_381del	24	OHA	Yes	25.9	51	47	
547	HNF1A	Sanger	Het	c.1748G>A	22	Diet	Yes	24.4	40	30	Low renal threshold
554	HNF1A	Sanger	Het	c.872dup	18	OHA	Yes	30	86	39	
566	HNF1A	Sanger	Het	c.872dup	17	OHA	Yes	29.2	51	42	Sulphonylurea sensitivity, low renal threshold
603	HNF1A	Sanger	Het	c.1420C>T	20	OHA	Yes	26.5	56	42	Low renal threshold
617	HNF1A	Sanger	Het	c.779C>T	25	Diet	Yes	25.4	44	26	
892	HNF1A	Sanger	Het	c.476G>A	14	Insulin	Yes	30.0	63	40	
1370	HNF1A	Sanger	Het	c.872dup	21	Diet	Yes	36.1	83	21	
1409	HNF1A	Sanger	Het	c.872dup	21	OHA+Ins	Yes	32.8	95	42	Sulphonylurea sensitivity
80480	HNF1A	Sanger	Het	c.1093_1107+6del	19	OHA		22.9	73	40	
82261	HNF1A	Sanger	Het	c.185del	12	OHA+Ins	Yes	23.7	73	25	Low renal threshold
82276	HNF1A	Sanger	Het	c.434C>T	13	Insulin	Yes	23.8	60	27	
82301	HNF1A	Sanger	Het	c.1340C>T	20	OHA	Yes	27.4	91	37	
82310	HNF1A	Sanger	Het	c.185del	18	OHA	Yes	24.4	48	45	Low renal threshold
82374	HNF1A	Sanger	Het	c.1093_1107+6del	19	OHA	Yes	23.8	83	20	Sulphonylurea sensitivity
82258	HNF4A	Sanger	Het	c.322G>A	28	Insulin	Yes	20.9	60	31	
600	HNF1B	Sanger	Het	c.982_986del	20	Insulin	No	23.4	122	35	Renal cysts
82033	HNF1B	Sanger	Het	c.466A>G	17	Insulin	No	25.3	54	35	Genital tract malformations, renal hypoplasia
82006	KCNJ11	Sanger	Het	c.601C>T	0	OHA	No	26.6	33	35	Diagnosed at 12 weeks of age
539	LMNA	Sanger	Het	c.1930C>T	17	OHA+Ins	Yes	24.2	114	49	Lipodystrophy
595	LMNA	Sanger	Het	c.1444C>T	21	OHA+Ins	Yes	25.1	62	34	
604	3243		Hp	m.3243A>G	27	Insulin	Yes	26.9	54	36	
80541	3243		Hp	m.3243A>G	28	Insulin	Yes	26.4	83	48	
82399	3243		Hp	m.3243A>G	29	Insulin	Yes	26.4	56	41	Deafness
540	NEUROD1	Sanger	Het	c.616dup	21	OHA+Ins	Yes	49.8	83	36	Lipodystrophy and necrobiosis
Identified as part of the biomarker pathway											
82372	*GCK*	Sanger	Het	c.1340G>A	18	Diet	No	25.5	46	19	
82316	*HNF4A*	Sanger	Het	c.1064–5_1070del	14	Diet	Yes	32.3	38	33	
377	*HNF4A*	Sanger	Het	c.-12G>A	11	Insulin	Yes	28.4	104	14	
80089	*HNF1A*	Sanger	Het	c.1349dup	30	Insulin	Yes	31.0	72	48	
80170	*HNF1A*	Sanger	Het	c.391C>T	21	Insulin	No	23.5	52	35	Low renal threshold
80173	*HNF1A*	Sanger	Het	c.495G>C	17	Insulin	Yes	24.5	56	46	
82003	*HNF1A*	Sanger	Het	c.28A>C	26	Diet	Yes	29.8	73	26	
82352	*HNF1A*	Sanger	Het	c.814C>T	13	Insulin	Yes	32.3	91	45	
82013	*HNF1A*	tNGS	Het	c.-258A>G	24	OHA	Yes	39.6	75	43	
307	*HNF1B*	tNGS	Het	c.1-?_1674+?del	29	Insulin	No	22.7	62	31	Asperger syndrome, renal cysts, low fecal elastase, low magnesium
82014	*NEUROD1*	tNGS	Het	c.616dup	21	OHA	No	35.3	88	31	
183	*NEUROD1*	tNGS	Het	c.616dup	29	Insulin	No	27.1	55	46	
82010	*3243*	tNGS	Hp	m.3243A>G	27	OHA+Ins	Yes	28.6	91	46	

Continued on p. 1022

Table 1—Continued

	Genetic characteristics				Clinical characteristics						
ID	Gene	Method	Zygosity	DNA level description	Age at diagnosis (years)	Treatment	Parent with diabetes	BMI	HbA_{1c}	Age at recruitment (years)	Additional clinical features
82038	*PPARG*	tNGS	Het	c.1154G>A	22	OHA	No	26.6	53	36	Lipodystrophy, acanthosis
80925	*TRMT10A*	tNGS	Hom	c.79G>T	23	OHA+Ins	No	33.0	69	28	Microcephaly, learning difficulties, epilepsy
17	*WFS1*	tNGS	C/Het	c.874C>T & c.877del	20	Insulin	n/k	21.8	42	24	Bilateral optic atrophy, neurogenic bladder, diet treatment, muscle pain on exercise
175	*POLD1*	Exome	Het	c.1812–1814del	14	OHA	No	18.6	30	21	Total lipodystrophy, sensorineural deafness, mandibular hypoplasia, hypogonadism, undescended testes, severe insulin resistance

References for the genes and further details of the mutations are in Supplementary Table 3. C/Het, compound heterozygous; Het, heterozygous; Hom, homozygous; Hp, Hp gene deletion; Ins, insulin; OHA, oral hypoglycemic agent; tNGS, targeted next-generation sequencing.

Sanger sequencing as part of the biomarker screening pathway; $P = 0.06$) (Supplementary Fig. 1). Those diagnosed with monogenic diabetes as part of the study were less likely to have a parent known to be affected than those with a previous known monogenic diagnosis (8 out of 17 [47%] vs. 29 out of 34 [85%]; $P = 0.007$).

Minimum Prevalence of Monogenic Diabetes of 3.6% in Those Diagnosed at 30 Years or Younger, Currently Younger Than 50 Years

We found 51 cases of monogenic diabetes (which represents a further 50% [$n = 17$] in addition to the 34 previously diagnosed) out of 1,407 recruited patients, providing a prevalence of 3.6% (95% CI 2.7% to 4.7%) in patients diagnosed at 30 years or younger and currently younger than 50 years.

From the database of U.K. referrals, we identified 26 patients with a diagnosis of monogenic diabetes in the Exeter and Tayside regions who met study inclusion criteria but were not recruited to the UNITED study. Therefore, the proportion of known monogenic diabetes prior to the study in the recruited population (34 out of 1,407 [2.4%]) was similar to the proportion in the nonrecruited population (26 out of 870 [3.0%]) ($P = 0.4$), suggesting no overall bias in recruitment. More of the nonrecruited cases had MODY caused by mutations in the *GCK* gene, but this was not significant given the small numbers (46 vs. 26%; $P = 0.1$). There was no difference in terms of age at diagnosis (mean 18 vs. 19 years; $P = 0.5$), age at time of recruitment (using 2011 for nonrecruited patients) (32 vs. 32 years; $P = 0.98$), or sex (35 female vs. 45% male; $P = 0.4$).

Performance of the Pathway

In line with what was expected given larger studies of the diagnostic accuracy of UCPCR and islet autoantibodies and the known pathophysiology of monogenic diabetes, all case subjects with previously diagnosed monogenic diabetes who provided all samples for the pathway ($n = 21$) were UCPCR positive and antibody negative. Similarly, all antibody-positive patients with DNA available ($n = 47$) tested negative for the three main MODY genes, so no additional MODY cases were picked up in this group.

A total of 199 out of 1,348 (15%) patients were put forward for genetic testing who were not found to have monogenic diabetes (i.e., 15% false-positive rate, so 85% specificity). Assuming a 98% sensitivity and 85% specificity, the PPV for the pathway is 20%, suggesting a 1-in-5 pickup rate for monogenic diabetes, a 5.6-fold increase in probability over the background prevalence alone (Table 2). The NPV was 99.9%, indicating that the probability of having monogenic diabetes if a patient is UCPCR negative or islet autoantibody positive is 0.1% (1 in 1,000) (Table 2).

Comparison of Biomarker Screening Pathway With Clinical Features

If genetic testing had been limited to the standard clinical criteria for MODY (age at diagnosis younger than 25 years, non–insulin-requiring, and a parent known to be affected with diabetes), fewer patients would have required testing ($n = 33$), leading to a higher pickup rate and PPV (57.6%) than the biomarker pathway, but the majority of monogenic cases would have been missed (63% compared with 0% for the biomarker pathway) (Table 2). The MODY probability calculator also had a higher PPV (40.4%), but missed more cases (55%) compared with the biomarker pathway.

CONCLUSIONS

The biomarker screening pathway for monogenic diabetes is a systematic, cheap (U.K. UCPCR cost of £10.80 and antibodies cost of £20), and easily implemented approach to screening all patients with young-onset diabetes in a clinic or population that helps identify suitable patients for molecular diagnostic genetic testing. The pathway picked up new cases of monogenic diabetes, even in areas of existing high detection because of research interests in the regions. We found that 3.6% of patients diagnosed at younger than 30 years of age

Table 2—PPV and NPV values for the biomarker pathway, traditional MODY criteria (age at diagnosis younger than 25 years, non–insulin-treated, and parent affected with diabetes), and the MODY probability calculator (using a probability >25%, the pickup rate for the diagnostic laboratory)

	N	Prevalence of monogenic diabetes	PPV (%)	NPV (%)	Percentage of monogenic cases missed	Number needed to test
Biomarker pathway	1,407	3.6% (51/1,407)	20.0	99.91	0	5
Traditional MODY criteria	1,362	3.6% (49/1,362)	57.6	97.7	63	2
MODY probability calculator	1,347	3.3% (45/1,347)	40.4	98.3	55	3

Prevalence is the proportion of diagnosed monogenic diabetes, percentage of monogenic cases missed is the proportion of monogenic cases not picked up by the approach, and number needed to test is 1/PPV.

have monogenic diabetes. In areas in which no cases have been identified, we estimate that 1 in 5 patients referred for genetic testing because of the pathway will have monogenic diabetes, which is a 5.6-fold higher detection rate than if all patients in this age range received genetic testing. The high NPV of 99.9% indicates it is an extremely effective approach for ruling out monogenic diabetes.

There have been relatively few studies that have systematically screened whole populations for monogenic diabetes. The majority of studies have been in pediatric populations only (14,15,22–26), with only two studies that have screened adults (27,28). No other study has systematically screened a whole population of both adults and children together. Only 8 out of 51 (16%) of patients with a genetic diagnosis of monogenic diabetes in our cohort were in the pediatric age range (younger than 20 years) at the time of recruitment, highlighting the importance of looking for monogenic diabetes in adult diabetes clinics. This may explain why the prevalence we find is higher than in any of the previous pediatric studies.

The strength of our pathway is the integration of two biomarkers (C-peptide and islet autoantibodies [both GAD and IA2]), rather than relying on clinical features. This offers a simple approach that does not require specific clinician interpretation or complex algorithms of different combinations of features. We showed that by using clinical features alone, over half of the cases of monogenic diabetes would be missed. By combining the two biomarkers, we increase the discriminatory ability and allow the clinician to pick up even atypical cases and rarer forms of monogenic diabetes, which traditional criteria may miss. The use of clinical features, however, results in fewer cases being sent for genetic testing that are negative, which clearly has cost implications. The most cost-effective approach is likely to involve a combination of biomarkers and clinical features. Further studies are needed to determine whether the pickup rate could be further improved by integrating the pathway with clinical features, such as the MODY calculator, or whether this would result in more missed patients because of reduced testing.

In this study, we also systematically tested all known genes for monogenic diabetes, rather than just the most common MODY genes (*GCK*, *HNF1A*, and *HNF4A*). Nine out of 17 (53%) of the cases identified as part of our cohort had mutations identified through additional testing on the targeted capture, and 17 out of 51 (33%) of all of the monogenic diabetes cases found in total had mutations in other genes, highlighting the advantage of further testing using targeted next-generation sequencing.

Health economic evaluation of the pathway for detecting the common forms of MODY (GCK, HNF1A, and HNF4A) has been carried out as a separate project, which has shown the pathway to be cost-saving (20,21). The cost-effectiveness of additional testing for other forms of monogenic diabetes has not been assessed. Because of the rarity of other monogenic diabetes, there are few data available to inform such analyses. Treatment change from insulin to sulphonylureas is still possible in cases diagnosed with *ABCC8* and *KCNJ11* (29,30), and for other genes for which treatment change is not an option, a confirmed diagnosis can still help with management, prognosis, and advice on risk to other family members (4). The decision whether to pay for the more expensive, but more comprehensive, next-generation sequencing, rather than Sanger sequencing for MODY genes only, would depend on assessing the tradeoffs of additional costs with long-term benefits to the patient. The presence of additional clinical features (e.g., renal cysts associated with *HNF1B*) may also point to specific monogenic diagnoses and increase the likelihood of a positive genetic test result.

A limitation of our study was that we had small numbers of patients with monogenic diabetes on which to evaluate the sensitivity of the pathway. Considerably larger studies have shown the biomarkers individually to be highly sensitive for monogenic diabetes (99% for UCPCR [10,11] and >99% for islet autoantibodies [13]), and by using both of these markers in a pathway, the number of missed cases should be minimal at a population level (2% of 3.6% = 0.07%, reflected in the NPV of 99.9%). Although there have been reports of MODY patients who are positive for islet autoantibodies (reviewed in Ref. 13), these are rare and likely to be cases with coincidental type 1 diabetes. Previous studies reporting high prevalence of positive autoantibodies in their cohort have included clinically defined, rather than genetically confirmed, MODY (31) or use low cutoffs for antibody positivity, which can be inappropriate (32), and are likely to represent an overestimate. There is also the potential for missed cases based on UCPCR, but again, the number of these patients will be small, and as they have insulin levels suggestive of type 1 diabetes (33), they are unlikely to be able to transfer off insulin even if a genetic diagnosis is made.

A further limitation is that despite screening using C-peptide and antibody testing, the PPV is still fairly low at 20%, indicating that four out of five screened will not have a monogenic cause identified on diagnostic molecular genetic testing. However, the aim of our screening pathway is that it is used purely as a tool to narrow down those individuals who would be more appropriate for genetic testing. This approach is a vast improvement over no screening (which

would represent a PPV at the background prevalence rate of 3.6%), misses fewer cases than using clinical features alone, and is at a level that has been shown to be cost-effective (20,21). Furthermore, the screening pathway still provides useful test results for this age group that offer additional information to support patient care. Patients with severe insulin deficiency, as determined by very low C-peptide values, will not respond to noninsulin therapy (33). Positive C-peptide and negative antibody results are important clinically to highlight atypical cases of type 1 diabetes or in which other forms of diabetes, such as young-onset type 2 diabetes, should be considered. Patients with very high endogenous insulin without islet autoantibodies and no mutations in monogenic diabetes genes are likely to have type 2 diabetes and may be able to manage on noninsulin treatment.

Finally, this study comprised a 98% white population and assesses patients at a median of 14 years after diagnosis. Assessment of the pathway in other racial groups and in patients close to diagnosis is needed.

In conclusion, we have demonstrated a simple, cheap, effective screening pathway that could be implemented at a population level to help correctly diagnose patients with monogenic diabetes.

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Author Contributions. B.M.S. carried out all analyses and drafted the manuscript. M.S. collected data and reviewed and edited the manuscript. M.H. coordinated the UNITED study and reviewed and edited the manuscript. T.J.M. coordinated the C-peptide and islet autoantibody testing and reviewed and edited the manuscript. K.C. coordinated the genetic testing and reviewed and edited the manuscript. J.P. provided input on analysis and reviewed and edited the manuscript. B.K. wrote the protocol and ethics application and reviewed and edited the manuscript. C.H. provided input on study design and analysis and reviewed and edited the manuscript. S.E. led the genetic testing and reviewed and edited the manuscript. E.R.P. designed the study, led the Tayside arm of the project, and reviewed and edited the manuscript. A.T.H. designed the study, led the Exeter arm of the project, and reviewed and edited the manuscript. B.M.S. 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.

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