Systematic Population Screening, Using Biomarkers and Genetic Testing, Identifies 2.5% of the U.K. Pediatric Diabetes Population With Monogenic Diabetes

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OBJECTIVE

Monogenic diabetes is rare but is an important diagnosis in pediatric diabetes clinics. These patients are often not identified as this relies on the recognition of key clinical features by an alert clinician. Biomarkers (islet autoantibodies and C-peptide) can assist in the exclusion of patients with type 1 diabetes and allow systematic testing that does not rely on clinical recognition. Our study aimed to establish the prevalence of monogenic diabetes in U.K. pediatric clinics using a systematic approach of biomarker screening and targeted genetic testing.

RESEARCH DESIGN AND METHODS

We studied 808 patients (79.5% of the eligible population) <20 years of age with diabetes who were attending six pediatric clinics in South West England and Tayside, Scotland. Endogenous insulin production was measured using the urinary C-peptide creatinine ratio (UCPCR). C-peptide–positive patients (UCPCR ≥0.2 nmol/mmol) underwent islet autoantibody (GAD and IA2) testing, with patients who were autoantibody negative undergoing genetic testing for all 29 identified causes of monogenic diabetes.

RESULTS

A total of 2.5% of patients (20 of 808 patients) (95% CI 1.6–3.9%) had monogenic diabetes (8 GCK, 5 HNF1A, 4 HNF4A, 1 HNF1B, 1 ABCC8, 1 INSR). The majority (17 of 20 patients) were managed without insulin treatment. A similar proportion of the population had type 2 diabetes (3.3%, 27 of 808 patients).

CONCLUSIONS

This large systematic study confirms a prevalence of 2.5% of patients with monogenic diabetes who were <20 years of age in six U.K. clinics. This figure suggests that ∼50% of the estimated 875 U.K. pediatric patients with monogenic diabetes have still not received a genetic diagnosis. This biomarker screening pathway is a practical approach that can be used to identify pediatric patients who are most appropriate for genetic testing.

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Monogenic diabetes is often not recognized in children or adolescents, and misdiagnosis as type 1 in these individuals is common (1–8). Making the correct diagnosis of monogenic diabetes is vitally important because the management of the most common subtypes (GCK, HNF1A, and HNF4A maturity-onset diabetes of the young [MODY]) is markedly different from that for type 1 diabetes (9,10). Molecular diagnosis improves clinical care by confirming the diagnosis, aiding prediction of the expected clinical course of the disease, and guiding appropriate management and family follow-up (10–12). Because of the predominance of type 1 diabetes in children, the potential significance of a parent with diabetes or possible noninsulin dependence may be overlooked. This leads to unnecessary insulin treatment with a mean delay from diabetes diagnosis to the correct genetic diagnosis of 9.3 years (K. Colclough, S. Ellard, unpublished observations) (based on 1,240 patients who were initially diagnosed with diabetes <20 years of age but subsequently received a genetic diagnosis of GCK, HNF1A, or HNF4A MODY).

The present approach to diagnosing monogenic diabetes requires clinical recognition by an alert health care professional and subsequent genetic testing. Because genetic testing for monogenic diabetes is now widely available worldwide, the major barrier is clinician recognition (although costs and lack of medical insurance coverage of genetic testing can also limit who is tested). Despite the availability of guidelines advising when a diagnosis of monogenic diabetes in children should be suspected (10), genetic testing is under-requested. We have shown that the underdiagnosis of MODY in some regions in the U.K. reflects reduced testing rather than inappropriate testing (13).

Biomarker tests can help to identify appropriate candidates for genetic testing for monogenic diabetes, avoiding reliance on clinical recognition. These biomarkers are most useful in enabling a firm diagnosis of type 1 diabetes to be made, obviating the consideration of genetic testing. The lack of significant endogenous insulin production (stimulated serum C-peptide level <200 pmol/L) is seen in type 1 diabetes outside the honeymoon period. Urinary C-peptide creatinine ratio (UCPCR) provides a simple, stable, reliable, noninvasive measure, which can be tested on a sample posted from home direct to a laboratory (14,15) and has been validated against the mixed-meal tolerance test (16). A UCPCR ≥0.2 nmol/mol indicates the presence of endogenous insulin and has been used to differentiate patients with MODY from those with type 1 diabetes >5 years after diagnosis (17). Islet autoantibodies are found in the majority of patients with type 1 diabetes, especially when measured close to diagnosis, and are an excellent discriminator between type 1 diabetes and MODY (18).

A large number of studies have tried to assess the prevalence of monogenic diabetes in the pediatric population (Table 1); however, the majority of these studies did not use a systematic approach or were limited to single clinic populations. A further limitation is that no studies to date have investigated all the causes of monogenic diabetes (Table 1). Only three studies have systematically screened large populations, as follows: *1*) a U.S. multicenter systematic study (SEARCH) identified a minimum prevalence of 1.2% with MODY (1) and a further 0.2% with neonatal diabetes (19); *2*) a Norwegian nationwide study identified a minimum prevalence of monogenic diabetes in children of 1.1% (2); and *3*) a Polish study identified a minimum prevalence of 3.1–4.2% (7). Other smaller studies (4,8,20–22) report screening or assessment of single pediatric clinic populations, and, although islet autoantibody negativity is often used to identify children who could benefit from genetic testing, the screening and testing strategies are variable, with estimates of prevalence up to 2.5%. Survey/questionnaire or epidemiological data relying on physician reporting and recognition of the clinical features of monogenic diabetes in pediatric populations state widely varying prevalences of 0.6–4.2% (7,23–27). However, these approaches do not involve systematic screening and, therefore, may be considered less accurate.

We report the first prevalence study of monogenic diabetes in the U.K. pediatric population using a systematic screening algorithm and genetic testing for all subtypes of monogenic diabetes.

The aim of this study was to identify the prevalence of monogenic diabetes in the U.K. pediatric diabetes population by systematic screening.

RESEARCH DESIGN AND METHODS

Study Eligibility

All patients with diabetes who were <20 years of age attending one of six pediatric/transition clinics across South West England and Tayside, Scotland, were eligible to take part. Ethical approval was granted by the National Research Ethics Service, Committee South West–Central Bristol. Participants under 16 years of age were asked to provide assent, and their parents provided consent.

The total number of potential recruits (n = 1,016) was ascertained by the local pediatric clinical teams from their clinic records (i.e., all their patients with diabetes who were <20 years of age were identified; 779 in South West England and 237 in Tayside). Informed consent was obtained by a member of the research team prior to data collection, and participants ≥16 years of age were asked to provide consent themselves, and if they lacked capacity, their parents were asked to provide consent. Time from diagnosis was not an exclusion criterion. Data collection included the following: sex, ethnic group, current age/age at diagnosis, initial/current treatment, time to insulin treatment, family history of diabetes, most recent/highest HbA_{1c} level, height/weight at diagnosis and time of recruitment, and the presence of learning difficulties or deafness. BMI was reported as age-adjusted percentiles to enable comparison across age groups (28).

Screening Method

The study comprised three potential stages that systematically identified those patients who were eligible for genetic testing (Fig. 1).

Stage 1 consisted of a urine sample for the measurement of UCPCR (14–16). Participants receiving insulin treatment were asked to mail a urine sample collected 2 h after the largest meal of the day that contained carbohydrate to a single laboratory at the Royal Devon and Exeter NHS Foundation Trust. Participants with endogenous insulin production ascertained by UCPCR of ≥0.2 nmol/mmol and those not receiving insulin treatment progressed to stage 2 of the study. Patients with a UCPCR <0.2 nmol/mmol, indicating insulin deficiency, were considered to have a diagnosis of type 1 diabetes (14,16).

Stage 2 comprised a blood sample that tested for the presence of islet

Table 1—Approaches used to identify monogenic diabetes in pediatric populations

Type of study	Country	Area	Initial cohort (*n*)	Cohort characteristics	Testing strategy (subgroup tested)	Genes tested	Prevalence in genetically tested	Minimal prevalence of monogenic diabetes	Reference
Systematic studies ordered by number in study									
Multicenter population based	U.S.	6 centers: California, Ohio, Hawaii, South Carolina, Washington	5,963	*1*) Diagnosed <20 years *2*) Diagnosed <6 months	*1*) AB-ve (×2), fasting C-peptide ≥0.8 ng/mL (*n* = 586) *2*) Diagnosed <6 months (*n* = 7)	*1*) HNF1A, HNF4A, GCK *2*) KCNJ11, INS, ABCC8	*1*) 8.4% (47/586) *2*) 71.4% (5/7)	1.2% 0.2% (total 1.4%)	1 19
Nationwide population based	Norway	Nationwide	2,756	Newly diagnosed, age 0–14 years	*1*) AB-ve (×2) and affected parent (*n* = 46) *2*) AB-ve, HbA_{1c} <7.5% (58 mmol/mol) and not receiving insulin (*n* = 10) *3*) Diagnosed <12 months (*n* = 24)	*1*) HNF1A, HNF4A, MIDD *2*) GCK *3*) KCNJ11, ABCC8, INS	*1*) 13.0% (6/46) *2*) 30.0% (3/10) *3*) 16.6% (4/24)	1.1%	2
Epidemiological data/ nationwide genetic test results	Poland	3 centers: Lodz, Katowice, Gdansk	2,568	Age 0–18 years	*1*) AB-ve, affected parent, noninsulin dependent *2*) HbA_{1c} <7.5% (58 mmol/mol) *3*) Diagnosed <6 months *4*) Syndromic diabetes	*1*) HNF1A, HNF4A, HNF1B *2*) GCK *3*) KCNJ11, ABCC8, INS *4*) WFS, Alstrom	32.1% (100/311)	3.1–4.2%	7
Single pediatric clinic population	U.S.	New York	939	Clinical diagnosis T1D Age 6 months to 20 years	AB-ve (×3) plus either HbA_{1c} ≤7% (53 mmol/mol) and ≤0.5 units insulin/kg/day >1 year postdiagnosis C-peptide positive or 3 gen. FH (*n* = 58)	GCK, HNF1A	8.6% (5/58)	0.5%*	4
Pediatric clinics in single city	Australia	Sydney	497	*1*) Clinical diagnosis T1D *2*) Diagnosed 6 months to 16 years	AB-ve (×4 on 2 occasions) (*n* = 19)	*1*) HNF1A, HNF4A *2*) INS, KCNJ11	5% (1/19)	1.2%*	20
Single pediatric clinic population	Spain	Madrid	252	*1*) Clinical diagnosis T1D *2*) Diagnosed 6 months to 17 years	AB-ve (×5) (*n* = 25)	*1*) HNF1A, HNF4A *2*) KCNJ11, INS	8.0% (2/25)	0.8%*	8
Pediatric clinic: case histories	New Zealand	South Island	160	Pediatric diabetes <18 years	AB-ve (×2?) (*n* = 4)	GCK, HNF1B, HNF1A	2.5% (4/160)	2.5%	21

Continued on p. 1882

Table 1—Continued

Type of study	Country	Area	Initial cohort (*n*)	Cohort characteristics	Testing strategy (subgroup tested)	Genes tested	Prevalence in genetically tested	Minimal prevalence of monogenic diabetes	Reference
Nationwide	Japan	Centers throughout Japan	N/K	Age 6 months to 20 years	*1*) AB-ve (×2), BMI <25 kg/mm^2, dominant family history or *2*) Renal cysts (*n* = 80)	*1*) HNF1A, GCK, HNF4A, MIDD *2*) HNF1B	47.5% (38/80)		34
Single pediatric clinic population	U.S.	Colorado	N/K	Diabetes <25 years	C-peptide ≥0.1 ng/mL, AB-ve (×3) (*n* = 97)	HNF1A, HNF4A, GCK, PDX1, HNF1B	22.7% (22/97)	N/K	35

Type of study	Country	Area	Initial cohort of patients with diabetes and the population taken from (*n*)	Cohort characteristic	How monogenic diabetes was defined	Monogenic diabetes diagnosis, *n* (% all diabetes)	Prevalence per 100,000 population		Reference
Nonsystematic studies relying on clinical recognition and clinical testing									
Postal questionnaire survey	U.K.	Nationwide	15,255 (59 million population)	Diabetes <16 years non-T1D	Confirmed by genetic test	20 (0.13%)	0.17		23
Questionnaire and telephone survey	Germany	State of Baden-Württemberg	2,640 (2.6 million) population	0–20 years	Clinician diagnosis (45% genetically confirmed)	58 (2.1%)	2.3		24
Assessment of Childhood Diabetes Registry	Germany	Saxony (34 pediatric clinics)	865 new cases Prevalence cases not stated (4.8 million population)	Newly diagnosed, age 0–15 years	Confirmed by genetic test	21 (2.4%) prevalence in incident cases	Cannot be calculated		26
Surveillance questionnaire (physician reporting)	Canada	National	Not stated (35 million population Canada)	Newly diagnosed, non-T1D <18 years	Clinical diagnosis genetically confirmed in ~50%	31 (% cannot be calculated)	0.32		25#
Observational investigation of database	Austria/ Germany	262 pediatric clinics	40,567 population	Age <20 years, diagnosis for <18 years	Clinician diagnosis MODY usually confirmed by genetic test (polymorphisms not excluded#)	339 all cases (0.8%), 263 (0.65%) genetic positive#	Cannot be calculated		27

AB-ve, autoantibody negative; 3 gen. FH, three-generation family history; N/K, not known; T1D, type 1 diabetes. *Only patients with a clinical diagnosis of type 1 diabetes were included, so the prevalence is likely to be underestimated. #Subsequent study (30) indicated 38% of reported HNF1A patients were polymorphisms not mutations.

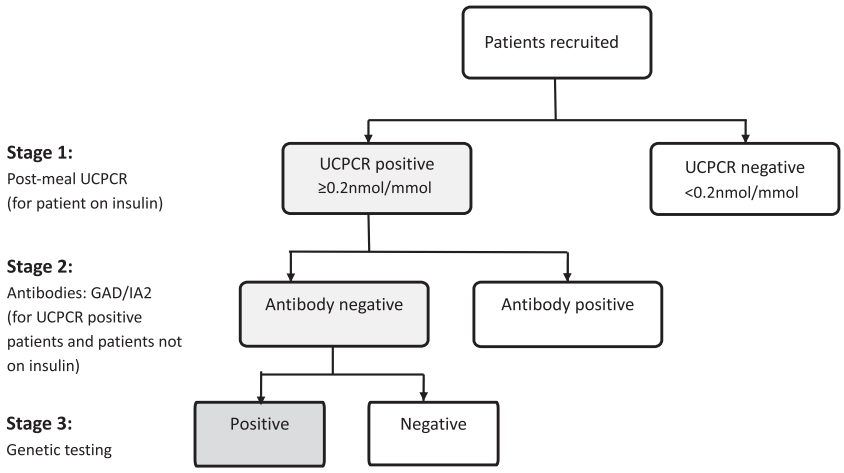

Figure 1—Pathway of testing.

autoantibodies (GAD and IA2) to identify those with autoimmune diabetes. This test was performed in all participants with significant endogenous insulin levels (either a UCPCR ≥0.2 nmol/mmol while receiving insulin treatment or not receiving insulin treatment). If islet autoantibody results were available from previous testing, these were used; otherwise, a blood sample was taken for antibody testing. Patients with GAD or IA2 levels >99th percentile were deemed islet autoantibody positive (18) and were considered to have a diagnosis of type 1 diabetes.

Stage 3 consisted of genetic testing in participants who were UCPCR positive and islet autoantibody negative. DNA was extracted, using standard methods, from a blood sample that was usually obtained at the same time as the sample for islet autoantibody testing. Sanger sequencing analysis of the *HNF1A* and *HNF4A* genes, and dosage analysis by multiplex ligation-dependent probe amplification to detect partial and whole gene deletions of *HNF1A*, *HNF4A*, *GCK*, and *HNF1B* was undertaken for all patients, with additional Sanger sequencing analysis of the *GCK* gene undertaken for patients with maximum HbA_{1c} levels of ≤7.6% (≤60 mmol/mol). This testing strategy was performed initially because these are the most common genes implicated in MODY, accounting for >95% of all MODY cases in the U.K. (13), and are amenable to treatment change. Patients with no pathogenic mutation identified by Sanger sequencing and multiplex ligation-dependent probe amplification then underwent targeted next-generation sequencing to look for mutations in 29 genes known to cause monogenic diabetes, and the mitochondrial mutation m.3243A>G causing maternally inherited diabetes and deafness using the assay published by Ellard et al. (29).

Statistical Analysis

Data were double entered onto a database and subsequently cleaned. Data are presented as proportions, and median (interquartile range [IQR]) where appropriate, because of the non-normality of data. Prevalence was calculated as the proportion of patients with monogenic diabetes out of the total number of patients studied. Data were analyzed using Stata version 13.1.

RESULTS

A total of 79.5% of the eligible population (*n* = 808 of 1,016) completed the study (Fig. 2). Fifteen of these participants had previously undergone genetic testing and were already known to have monogenic diabetes (Table 2).

Patient Characteristics

A total of 54% of participants were male (441 male, 376 female). The median age at study recruitment was 13 years (IQR = 10, 16), the median age at diagnosis was 8 years (IQR = 4, 11), and all individuals received a diagnosis of diabetes at >6 months of age. The median duration of diabetes was 4.3 years (IQR = 1.6, 7.9). The majority (788 participants [96%]) of the cohort were white, reflecting the population demographics in these areas. A total of 792 patients (97%) were receiving insulin treatment at the time of study recruitment, including 4 patients who were receiving treatment with insulin in addition to metformin. Twenty-five patients (3%) were noninsulin treated, with 11 patients receiving oral agents only and 14 were being treated with diet alone. The median HbA_{1c} level was 8.6% (IQR = 7.7, 9.7 [70 mmol/mol, IQR = 61, 83]), and the median BMI percentile was 79 (IQR = 56, 94).

Stage 1: UCPCR

A total of 547 of 817 patients (67%) were UCPCR negative (<0.2 nmol/mol), indicating insulin deficiency, and were therefore considered to have type 1 diabetes, and these individuals did not undergo further testing. In addition, 261 patients (32%) had significant endogenous insulin production (≥0.2 nmol/mol); this included 236 patients who were treated with insulin and 25 patients who were not treated with insulin.

Stage 2: Antibodies

The 253 patients with significant endogenous insulin levels underwent islet autoantibody testing, which included 236 patients who were treated with insulin and confirmed to be UCPCR positive through stage 1 of the study and 17 patients who were not treated with insulin. Eight of 15 patients who had previously received a diagnosis of monogenic diabetes did not undergo antibody testing (but none of these were treated with insulin), and 9 patients did not return their blood sample for antibody testing.

A total of 179 of 253 participants were islet autoantibody positive, confirming a diagnosis of type 1 diabetes. Forty-five of these participants were positive to both GAD and IA2, 28 were positive to GAD only, 21 were positive to GAD but IA2 was not tested for, and 85 were positive to IA2 only, indicating the importance of testing for both autoantibodies. The 74 participants who were antibody negative continued to stage 3 for genetic testing.

Stage 3: Genetic Testing

The prevalence of monogenic diabetes in this U.K. pediatric diabetes population that was <20 years of age was 2.5% (95% CI 1.5–3.9%). A total of 82 of 808 patients (10.1%) had undergone genetic testing, and 20 of these (24%, 1 in 4 patients) had monogenic diabetes (Table 2). Fifteen of 20 patients were previously known to have monogenic diabetes (7 GCK MODY, 5 HNF1A

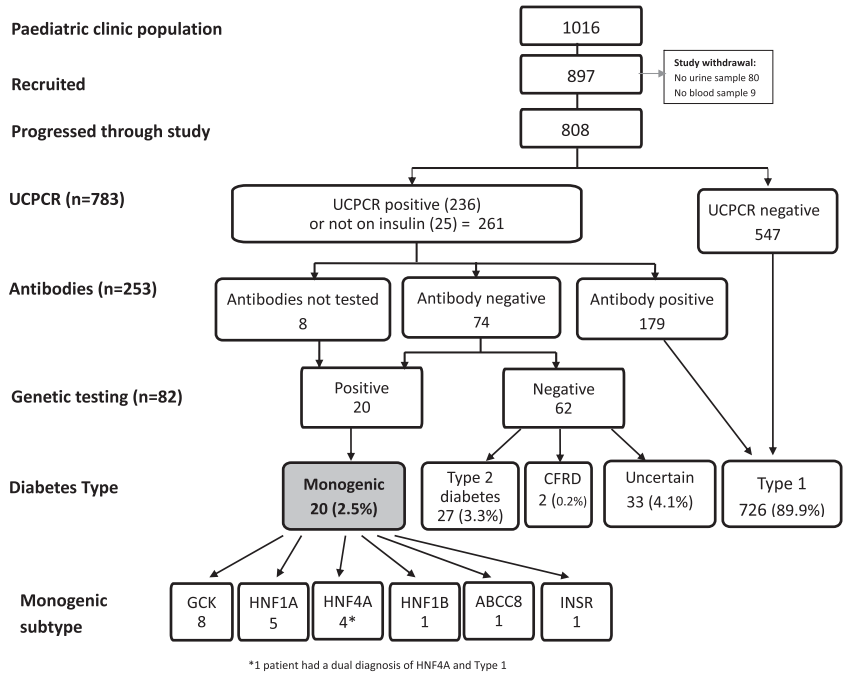

Figure 2—Patient progression through pathway.

MODY, 1 HNF4A MODY, 1 ABCC8 MODY, and 1 patient with type A insulin resistance due to a heterozygous *INSR* mutation), and 5 new cases of monogenic diabetes (3 HNF4A MODY, 1 HNF1B MODY, 1 GCK MODY) were identified during the study. One of these patients had a dual diagnosis of HNF4A MODY (heterozygous for the p.Arg114Trp mutation) and type 1 diabetes (GAD negative, as defined in this study as the <99th percentile, and therefore proceeded to genetic testing, but with a GAD titer of 25.9 >97.5th percentile and a UCPCR of 0.21 nmol/mol 2 years after diagnosis, and had received continuous insulin treatment from the time of diagnosis). Patients with monogenic diabetes were found in all six clinics, with a prevalence varying between 1.2% and 3.7%.

To assess whether we had missed cases of monogenic diabetes in those with islet autoantibodies, 65 of 179 patients with positive autoantibodies underwent Sanger sequencing analysis of the most common MODY genes (*GCK, HNF1A*, and *HNF4A*); no mutations were found.

Characteristics of Patients Negative on Genetic Testing

Diagnosis was not established using this testing pathway in 62 participants who were UCPCR positive, islet autoantibody negative, and negative for mutations in 29 genes known to cause monogenic diabetes. Secondary causes of diabetes were known in two individuals with a previously recorded diagnosis of cystic fibrosis–related diabetes. Twenty-seven of 62 of these patients (3.3% of the cohort) met the diagnostic criteria for type 2 diabetes (no monogenic or secondary cause, BMI ≥85th percentile, and antibody negative [http://web.ispad.org/sites/default/files/resources/files/idf-ispad_diabetes_in_childhood_and_adolescence_guidelines_2011_0.pdf]) but were not assessed for insulin resistance or other metabolic features.

Uncertainty over the diagnosis remained in 33 individuals (4% of the whole cohort). The most likely diagnosis in these individuals was islet autoantibody-negative type 1 diabetes because they were close to diagnosis (median duration 0.8 years [IQR = 0.4, 2.8]) and were not overweight (median BMI in the 51st percentile [IQR = 43, 67]). Twenty-six of 33 of these individuals had a diabetes duration of <3 years and so could be considered to be within the honeymoon phase; repeating testing for the UCPCR in these individuals over time could prove to be useful. However, 5 of 33 individuals had a median diabetes duration of 6.1 years (range 5–10 years), median BMI in the 53rd percentile (range 46th to 81st percentile) with a median UCPCR of 0.36 nmol/mmol (range 0.21–1.27 nmol/mmol); therefore, the diabetes in these individuals should be considered atypical and not fitting a clear diagnostic category.

Only 19.4% of the eligible patients within these pediatric diabetes populations (n = 198) did not take part in this study. This included 13 known patients with monogenic diabetes (10 patients had GCK MODY and were therefore not under the care of a diabetes team, 3 patients with HNF1A, and 1 patient with Wolfram syndrome). Therefore, this cohort was not biased to include all those patients with known monogenic diabetes. The prevalence of monogenic diabetes in those patients recruited was 2.5%, compared with 6.6% (P = 0.0038) in those patients not taking part in the study.

CONCLUSIONS

We found a prevalence of monogenic diabetes in patients diagnosed <20 years of age of 2.5% (95% CI 1.6–3.9%) by systematic testing using islet autoantibodies, C-peptide, and targeted next-generation sequencing of all monogenic diabetes genes. Using our approach of screening children/adolescents with diabetes using C-peptide followed by GAD and IA2 autoantibodies would identify a subpopulation of ~10%, in which genetic testing will have a pick up rate of ~1 in 4. Using the online probability calculator (http://www.diabetesgenes.org/content/mody-probability-calculator) could further aid in the identification of those individuals who were most likely to have MODY, because in our study 18 of 20 patients with monogenic diabetes were shown to have a 1 in 1.3 chance (or >75.5%) post-test probability of having MODY.

The 2.5% (95% CI 1.6–3.9%) prevalence of monogenic diabetes we identified is similar to the prevalence found in three other large systematic population studies (1,2,7,19), two from predominantly European white populations (Poland 3.1–4.2%, Norway 1.1%) and one from a multiethnic population from the U.S. (1.4%) (Table 1). The Polish study (7) used targeted case findings predominantly using clinical criteria supported by the lack of autoantibodies and measurable C-peptide levels. The Norwegian population-based study (2) predominantly used antibody negativity combined with a parental history of

Table 2—Characteristics of the 20 patients identified with monogenic diabetes

Study ID	Gene	Mutation#	Protein effect	Gender	Age at diagnosis (years)	Diabetes duration (years)*	Initial treatment	Current treatment	BMI percentile	Affected parent	UCPCR (nmol/mmol)	GAD	IA2	Notes
211	*GCK*	c.97_117dup	p.(Val33_Lys39dup)	M	3	13	Insulin	None	99th	Mother	3.57	N/A	N/A	Known MODY
537	*GCK*	c.683C>T	p.(Thr228Met)	M	11	2	Diet	None	N/A	Mother	1.94	N/A	N/A	Known MODY Sibling of 538
538	*GCK*	c.683C>T	p.(Thr228Met)	M	9	1	Diet	None	N/A	Mother	1.73	N/A	N/A	Known MODY Sibling of 537
543	*GCK*	c.184G>A	p.(Val62Met)	M	4	0.2	Diet	None	N/A	Mother	N/A	N/A	N/A	Known MODY Sibling of 544
544	*GCK*	c.184G>A	p.(Val62Met)	M	3	2	Diet	None	N/A	Mother	N/A	N/A	N/A	Known MODY Sibling of 543
1,396	*GCK*	c.1209del	p.(Ile404fs)	M	14	0.3	Diet	None	71st	Mother	N/A	N/A	N/A	Known MODY
8,002,095	*GCK*	c.1019G>T	p.(Ser340Ile)	M	9	5	Diet	None	88th	Father	0.79	Neg	N/A	Known MODY
8,002,372	*GCK*	c.1340G>A	p.(Arg447Gln)	M	18	0.6	Diet	None	90th	Neither	Not tested	Neg	Not tested	Newly identified MODY
599	*HNF1A*	c.608G>A	p.(Arg203His)	F	14	0.5	OHA	OHA	99th	Both parents	3.08	Neg	Neg	Known MODY
1,012	*HNF1A*	c.872del	p.(Pro291fs)	F	10	0.7	Diet	Diet	99th	Mother	5.6	Neg	Neg	Known MODY Sibling of 395
395	*HNF1A*	c.872del	p.(Pro291fs)	F	14	0.1	OHA	OHA	95th	Mother	5.8	Neg	Neg	Known MODY Sibling of 1012
455	*HNF1A*	c.872dup	p.(Gly292fs)	F	12	3	OHA	OHA	57th	Father	0.86	Neg	Neg	Known MODY
567	*HNF1A*	c.872dup	p.(Gly292fs)	M	8	2	Diet	OHA	94th	Mother	1.73	Neg	Neg	Known MODY
686	*HNF4A*	c.749T>C	p.(Leu250Pro)	M	16	0.7	Diet	Diet	99th	Father	4.74	N/A	N/A	Known MODY
1,348	*HNF4A*	c.340C>T	p.(Arg114Trp)	F	15	0.2	Insulin	OHA	86th	Father	3.00	Neg	Neg	Newly identified MODY
1,203	*HNF4A*	c.340C>T	p.(Arg114Trp)	M	7	2	Insulin	Insulin	39th	Neither	0.21	Neg■	Neg	Dual diagnosis: newly identified HNF4A/ known type 1 diabetes
377	*HNF4A*	c.-12G>A	p.(?)	F	11	2	Insulin	Insulin	99th	Mother	0.28	Neg	Neg	Newly identified MODY
854	*HNF1B*	c.1-?_*151 +?del	p.(0?) (whole gene deletion)	M	11	2	Insulin	Insulin	9th	Father	0.71	Neg	Neg	Newly identified MODY
555	*ABCC8*	c.4139G>A	p.(Arg1380His)	F	11	8	OHA	OHA	4th	Father	3.00	Neg	Neg	Known MODY
758	*INSR*	c.3706C>G	p.(Pro1236Ala)	F	12	3	OHA	Diet	55th	Mother	9.07	N/A	N/A	Known MODY

F, female; ID, identification; M, male; N/A, not applicable, genetic diagnosis made prior to study; Neg, negative; OHA, oral hypoglycemic agent.
■GAD negative, defined in this study as <99th percentile, but GAD 25.9 (97.5th percentile). #Mutations are described using the Human Genome Variation Society nomenclature guidelines according to the following reference sequences: *GCK* NM_000162.3; *HNF1A* NM_000545.6; *HNF4A* NM_175914.4; *ABCC8* NM_001287174.1; *INSR* NM_000208.2. *Diabetes duration at time of study.

diabetes or lack of insulin therapy and HbA_{1c} levels of <7.5% (58 mmol/mol). The lower prevalence (1.1%) probably reflected that they studied children 0–14 years of age rather than 0–20 years of age (mean age at diagnosis in our study 10.6 years) and only 10 patients were tested for GCK. The U.S. study (1,19), like our study, used systematic biomarker screening, with genetic testing performed in all patients who had measurable C-peptide levels and did not have GAD and IA2 autoantibodies. The lower prevalence in their cohort probably results from non-MODY patients having more "type 2 features," suggesting a greater proportion of patients with young-onset type 2 diabetes, because the combined prevalence of MODY in minority individuals was very similar to the prevalence of MODY in non-Hispanic white individuals (1). There are many other less comprehensive studies (25,27,30) of the prevalence of monogenic diabetes (Table 1): these are limited by studying a single clinic, using a nonsystematic assessment, and/or not making a robust molecular genetic diagnosis (confirmed mutations not polymorphisms).

Our study indicated a higher proportion of known cases of MODY versus new cases identified through systematic screening. The 28 patients with previously confirmed MODY (15 who took part and 13 who did not take part in the study) reflect the high levels of awareness of monogenic diabetes in these geographical regions. The 13 patients previously identified who did not take part in the study included 9 with GCK MODY (who had been discharged from clinic follow-up), 3 with HNF1A MODY, and 1 with Wolfram syndrome. This study shows that clinical recognition of key phenotypes in patients and their family members can identify the majority of pediatric patients (15 of 20 in this study). However, the five new patients identified through this pathway of screening indicate the need for a systematic approach. If this approach were used in other areas where the recognition of monogenic diabetes is not so apparent, then a greater proportion of new cases would be identified. We have based our prevalence figures only on the population recruited in this study; however, if the 13 patients who did not take part were taken into account, this could give a prevalence as high as 3.3% (33 of 1,016 patients). There are estimated to be ~35,000 children and young people with diabetes who are under 19 years of age in the U.K. (31,32). If the prevalence of 2.5% found in those patients who took part in this six-clinic survey reflects the whole of the U.K., then this suggests at least 875 expected patients with MODY in this age group (95% CI 560–1,365), of whom 468 have received a diagnosis to date with ~50% still likely to be misdiagnosed as having type 1 diabetes.

This approach of systematic testing combined with clinical criteria can result in a diagnosis in >99% of patients, and this is an advantage of this approach beyond the recognition of monogenic diabetes. We were able to use C-peptide, autoantibody, and genetic testing to give a clear diagnosis in 92.3% of patients. Clinical criteria suggest that 3.3% had type 2 diabetes, a figure that is very similar to the number of individuals with monogenic diabetes, as seen in other European populations (7,24,33). A total of 0.2% had secondary diabetes due to cystic fibrosis, which probably reflects an underestimate as many of these patients will not attend a pediatric diabetes clinic. A further 3.2% were within 3 years of having received a diagnosis and probably had antibody-negative type 1 diabetes in the honeymoon period. There remained five patients (0.6%) who were atypical and hard to classify—they may represent atypical type 1 diabetes (antibody negative and significant C-peptide levels >3 years after diagnosis) or a presently unrecognized subtype of monogenic diabetes.

There were limitations to this study. The geographical areas where the study was undertaken already had a high awareness of MODY, so the number of new cases was low (25%) relative to those already known (75%), while elsewhere in the U.K. we estimate that this figure is ~50% detected and 50% undetected. We subjected only those patients who had significant endogenous insulin (C-peptide) levels and did not have autoantibodies to systematic genetic testing, although previous research (14,17,18) and our failure to find any mutations in 65 patients who did have significant C-peptide levels but were antibody positive support the idea that this approach would miss very few cases. UCPCR calculation was performed irrespective of the duration of diabetes, and it is acknowledged that some of the patients tested close to receiving a diagnosis could be producing endogenous insulin during the honeymoon period, and, if retested over time, that those patients with type 1 diabetes would be expected to have declining C-peptide levels. The calculation of UCPCR is best for excluding patients >3 years after diabetes is diagnosed, while autoantibody testing is best for excluding patients close to diagnosis. In this study, we wanted to test everyone, regardless of disease duration, so a testing method that used both biomarkers worked well. If a study was performed of purely incident cases, which would have an advantage of making the correct diagnosis early, then measuring C-peptide levels would have little value, and further testing could be performed on those individuals who had negative results from testing for multiple autoantibodies. Although patients were asked to send a "postmeal" urine sample, the prandial state of the patient was assumed (and not observed); therefore, we cannot be certain that all UCPCR tests were stimulated. Our population consisted predominantly (96%) of whites, and systematic studies in other, especially high-prevalence, populations are also needed.

There are many strengths of this study. The result is likely to be representative of the clinics studied because 79.5% of the eligible population took part, a result that shows the high acceptability of this approach in pediatric clinics. The systematic biomarker–based approach that is independent of clinical features allows atypical patients to be detected (e.g., those with no family history of diabetes). This is the first study that has used next-generation sequencing to assess all known causes of monogenic diabetes, although the majority of patients (85%) had the most common types of MODY (GCK, HNF1A, and HNF4A), so studies that have not used this approach will have missed only a few patients.

This systematic, high-uptake study gives a prevalence of 2.5% (95% CI 1.6–3.9%) for monogenic diabetes in the U.K. pediatric population. Patients with monogenic diabetes were identified in every pediatric clinic. The successful identification of patients with monogenic diabetes is crucial because they require different treatment than those with type 1 or type 2 diabetes. The vast majority (>99%) of pediatric patients can be

successfully classified by UCPCR, antibody testing, genetic testing, and clinical criteria. UCPCR is a noninvasive and inexpensive test, which could be more widely used in the pediatric age group where it has a high acceptability. This screening algorithm is a practical approach to determining the prevalence of cases in a clinic to ensure the correct diagnosis of subtypes of diabetes. Confirming a prevalence of MODY of 2.5% in the pediatric population indicates that all those involved in pediatric diabetes care should be aware of the possibility of an alternative diagnosis and know how to refer patients for genetic testing.

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References

1. Pihoker C, Gilliam LK, Ellard S, et al.; SEARCH for Diabetes in Youth Study Group. 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
2. Irgens HU, Molnes J, Johansson BB, et al. Prevalence of monogenic diabetes in the population-based Norwegian Childhood Diabetes Registry. Diabetologia 2013;56:1512–1519
3. Carmody D, Lindauer KL, Naylor RN. Adolescent non-adherence reveals a genetic cause for diabetes. Diabet Med 2015;32:e20–e23
4. Gandica RG, Chung WK, Deng L, Goland R, Gallagher MP. Identifying monogenic diabetes in a pediatric cohort with presumed type 1 diabetes. Pediatr Diabetes 2015;16:227–233
5. Lambert AP, Ellard S, Allen LI, et al. Identifying hepatic nuclear factor 1alpha mutations in children and young adults with a clinical diagnosis of type 1 diabetes. Diabetes Care 2003;26:333–337
6. Thirumalai A, Holing E, Brown Z, Gilliam LK. A case of hepatocyte nuclear factor-1β (TCF2) maturity onset diabetes of the young misdiagnosed as type 1 diabetes and treated unnecessarily with insulin. J Diabetes 2013;5:462–464
7. Fendler W, Borowiec M, Baranowska-Jazwiecka A, et al. Prevalence of monogenic diabetes amongst Polish children after a nationwide genetic screening campaign. Diabetologia 2012;55:2631–2635
8. Rubio-Cabezas O, Edghill EL, Argente J, Hattersley AT. Testing for monogenic diabetes among children and adolescents with antibody-negative clinically defined type 1 diabetes. Diabet Med 2009;26:1070–1074
9. Murphy R, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. Nat Clin Pract Endocrinol Metab 2008;4:200–213
10. Rubio-Cabezas O, Hattersley AT, Njølstad PR, et al.; International Society for Pediatric and Adolescent Diabetes. 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
11. Craig ME, Jefferies C, Dabelea D, Balde N, Seth A, Donaghue KC; International Society for Pediatric and Adolescent Diabetes. ISPAD Clinical Practice Consensus Guidelines 2014. Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes 2014;15(Suppl. 20):4–17
12. Colclough K, Saint-Martin C, Timsit J, Ellard S, Bellanné-Chantelot C. Clinical utility gene card for: maturity-onset diabetes of the young. Eur J Hum Genet. 12 February 2014 [Epub ahead of print]. DOI: 10.1038/ejhg.2014.14
13. Shields BM, Hicks S, Shepherd MH, Colclough K, Hattersley AT, Ellard S. Maturity-onset diabetes of the young (MODY): how many cases are we missing? Diabetologia 2010;53:2504–2508
14. Besser RE, Shepherd MH, McDonald TJ, et al. Urinary C-peptide creatinine ratio is a practical outpatient tool for identifying hepatocyte nuclear factor 1-alpha/hepatocyte nuclear factor 4-alpha maturity-onset diabetes of the young from long-duration type 1 diabetes. Diabetes Care 2011;34:286–291
15. McDonald TJ, Knight BA, Shields BM, Bowman P, Salzmann MB, Hattersley AT. Stability and reproducibility of a single-sample urinary C-peptide/creatinine ratio and its correlation with 24-h urinary C-peptide. Clin Chem 2009;55:2035–2039
16. Besser RE, Ludvigsson J, Jones AG, et al. Urine C-peptide creatinine ratio is a noninvasive alternative to the mixed-meal tolerance test in children and adults with type 1 diabetes. Diabetes Care 2011;34:607–609
17. Besser RE, Shields BM, Hammersley SE, et al. Home urine C-peptide creatinine ratio (UCPCR) testing can identify type 2 and MODY in pediatric diabetes. Pediatr Diabetes 2013;14:181–188
18. McDonald TJ, Colclough K, Brown R, et al. Islet autoantibodies can discriminate maturity-onset diabetes of the young (MODY) from type 1 diabetes. Diabet Med 2011;28:1028–1033
19. Kanakatti Shankar R, Pihoker C, Dolan LM, et al.; SEARCH for Diabetes in Youth Study Group. Permanent neonatal diabetes mellitus: prevalence and genetic diagnosis in the SEARCH for Diabetes in Youth Study. Pediatr Diabetes 2013;14:174–180
20. Hameed S, Ellard S, Woodhead HJ, et al. Persistently autoantibody negative (PAN) type 1 diabetes mellitus in children. Pediatr Diabetes 2011;12:142–149
21. Wheeler BJ, Patterson N, Love DR, et al. Frequency and genetic spectrum of maturity-onset diabetes of the young (MODY) in southern New Zealand. J Diabetes Metab Disord 2013;12:46
22. Redondo MJ, Rodriguez LM, Escalante M, Smith EO, Balasubramanyam A, Haymond MW. Types of pediatric diabetes mellitus defined by anti-islet autoimmunity and random C-peptide at diagnosis. Pediatr Diabetes 2013;14:333–340
23. Ehtisham S, Hattersley AT, Dunger DB, Barrett TG; British Society for Paediatric Endocrinology and Diabetes Clinical Trials Group. First UK survey of paediatric type 2 diabetes and MODY. Arch Dis Child 2004;89:526–529
24. Neu A, Feldhahn L, Ehehalt S, Hub R, Ranke MB; DIARY group Baden-Württemberg. Type 2 diabetes mellitus in children and adolescents is still a rare disease in Germany: a population-based assessment of the prevalence of type 2 diabetes and MODY in patients aged 0-20 years. Pediatr Diabetes 2009;10:468–473
25. Amed S, Dean HJ, Panagiotopoulos C, et al. Type 2 diabetes, medication-induced diabetes, and monogenic diabetes in Canadian children: a prospective national surveillance study. Diabetes Care 2010;33:786–791
26. Galler A, Stange T, Müller G, et al.; Childhood Diabetes Registry in Saxony, Germany. Incidence of childhood diabetes in children aged less than 15 years and its clinical and metabolic characteristics at the time of diagnosis: data from the Childhood Diabetes Registry of Saxony, Germany. Horm Res Paediatr 2010;74:285–291
27. Schober E, Rami B, Grabert M, et al.; DPV-Wiss Initiative of the German Working Group for Paediatric Diabetology and. 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 multicentre database. Diabet Med 2009;26:466–473
28. Freeman JV, Cole TJ, Chinn S et al. Cross sectional stature and weight reference curves for the UK, 1990. Arch Dis Child 1995;73:17–24
29. Ellard S, Lango Allen H, De Franco E, et al. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. Diabetologia 2013;56:1958–1963
30. Awa WL, Thon A, Raile K, et al.; DPV-Wiss. Study Group. Genetic and clinical characteristics of patients with HNF1A gene variations from the German-Austrian DPV database. Eur J Endocrinol 2011;164:513–520
31. Health & Social Care Information Centre. National diabetes audit 2011-12 report 1: care processes and treatment targets: CCG and LHG data tables [Internet], 2013. Available from http://www.hscic.gov.uk/searchcatalogue?productid=13129&q=%22National+diabetes+audit%22&sort=Relevance&size=10&page=1#top. Accessed 3 January 2016
32. Diabetes in Scotland. Scottish Diabetes Survey 2014 [Internet], 2014. Available from http://www.diabetesinscotland.org.uk/Publications/SDS2014.pdf. Accessed 3 January 2016
33. Vaziri-Sani F, Delli AJ, Elding-Larsson H, et al. A novel triple mix radiobinding assay for the three ZnT8 (ZnT8-RWQ) autoantibody variants in children with newly diagnosed diabetes. J Immunol Methods 2011;371:25–37
34. Yorifuji T, Fujimaru R, Hosokawa Y, et al. Comprehensive molecular analysis of Japanese patients with pediatric-onset MODY-type diabetes mellitus. Pediatr Diabetes 2012;13:26–32
35. 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