Syndromic Monogenic Diabetes Genes Should Be Tested in Patients With a Clinical Suspicion of Maturity-Onset Diabetes of the Young

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At present, outside of infancy, genetic testing for monogenic diabetes is typically for mutations in maturity-onset diabetes of the young (MODY) genes that predominantly result in isolated diabetes. Monogenic diabetes syndromes are usually only tested for when supported by specific syndromic clinical features. How frequently patients with suspected MODY have a mutation in a monogenic syndromic diabetes gene is unknown and thus missed by present testing regimes. We performed genetic testing of 27 monogenic diabetes genes (including 18 associated with syndromic diabetes) for 1,280 patients with a clinical suspicion of MODY who were not suspected of having monogenic syndromic diabetes. We confirmed monogenic diabetes in 297 (23%) patients. Mutations in seven different syndromic diabetes genes accounted for 19% (95% CI 15–24%) of all monogenic diabetes. The mitochondrial m.3243A>G and mutations in *HNF1B* were responsible for the majority of mutations in syndromic diabetes genes. They were also the 4th and 5th most common causes of monogenic diabetes overall. These patients lacked typical features, and their diabetes phenotypes overlapped with patients with nonsyndromic monogenic diabetes. Syndromic monogenic diabetes genes (particularly m.3243A>G and *HNF1B*) should be routinely tested in patients with suspected MODY who do not have typical features of a genetic syndrome.

Maturity-onset diabetes of the young (MODY) is an autosomal dominant form of monogenic diabetes diagnosed outside of infancy. Mutations in *GCK*, *HNF1A*, and *HNF4A* are the most common causes of MODY. The genetic diagnosis is important for determining the most effective treatment. Patients with *HNF1A* and *HNF4A* MODY are better treated with sulphonylurea, whereas *GCK* MODY does not require treatment (1,2). MODY is suspected in nonobese individuals with young-onset diabetes that does not require insulin treatment, lack islet autoantibodies, and have persistent endogenous insulin (3). Syndromic forms of monogenic diabetes are less common and characterized by young-onset diabetes, but unlike MODY, they typically present with additional non-autoimmune extrapancreatic features. These syndromes are caused by mutations that can be autosomal dominant (e.g., *HNF1B*), mitochondrial (e.g., m.3243A>G), and autosomal recessive (e.g., *WFS1*). For example, a patient with an *HNF1B* mutation will commonly have diabetes and renal structural features, such as renal cysts, hypoplasia, and aplasia. Patients with the mitochondrial mutation m.3243A>G commonly have diabetes and bilateral sensorineural deafness (4–6). Patients with syndromic diabetes typically have a similar diabetes phenotype to MODY (young-onset diabetes, nonobese, and negative islet autoantibodies), but unlike those with MODY, these patients are more likely to be treated with insulin (7). Knowledge of the specific subtype has implications for clinical management, disease prognosis, surveillance for extrapancreatic conditions, and genetic counseling for recurrence risk.

At present, outside of infancy, genetic testing for monogenic diabetes focuses on MODY genes. Genetic testing for

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a syndromic diabetes gene is usually undertaken only when the patient presents with characteristic clinical features suggestive of the syndrome (e.g., m.3243A>G testing if the patient has a personal or maternal family history of diabetes, deafness, and other mitochondrial disease features). This testing strategy is reflected by the lack of comprehensive inclusion of syndromic diabetes genes (with the exception of *HNF1B*) in gene panels for MODY testing in the National Center for Biotechnology Information (NCBI) gene testing registry (8).

Monogenic syndromic diabetes has variable expressivity of additional syndromic features and can present with isolated diabetes (9–12). This in conjunction with an overlap of the diabetes phenotype with MODY may result in patients being referred from routine clinical practice for MODY testing rather than for testing for a specific monogenic syndrome (13). However, the proportion of patients with suspected MODY who have a mutation in a syndromic diabetes gene is not known. A high proportion of patients would support changing the current genetic testing strategy to include all syndromic diabetes genes on MODY gene panels, whereas a low proportion would support the current testing strategy of only testing a syndromic diabetes gene if the related clinical features are present. In this study, we analyzed syndromic diabetes genes in a large cohort of patients with suspected MODY in routine clinical care to determine whether syndromic genes should be routinely tested in this population.

RESEARCH DESIGN AND METHODS

Study Cohort

We studied 1,280 unrelated probands who were referred by U.K. clinicians from routine clinical care for MODY genetic testing at the Exeter Genomics Laboratory from 30 November 2011 to 30 November 2018. This cohort represents all probands referred for targeted next-generation sequencing (tNGS) for MODY over this period. Clinical and biological characteristics and family history were provided by clinicians at the time of referral. The suspicion of a MODY diagnosis was made by the referring clinician. In all cases, the referring clinician did not suspect a diagnosis of a monogenic diabetes syndrome, and the clinical features provided by the clinician at the time of testing did not support genetic testing for a specific monogenic diabetes syndrome.

As a comparison cohort, we included 50 patients with an *HNF1B* and 54 with an m.3243A>G mutation who were referred to the Exeter Genomics Laboratory over the same period from routine clinical care with a suspicion of having the respective monogenic diabetes syndrome by the referring clinician.

All probands gave informed consent for genetic studies approved by the North Wales ethics committee (no. 17/WA/0327). The study was performed in accordance with the principles of the Declaration of Helsinki.

Genetic Testing

We performed genetic testing for 27 monogenic diabetes genes, including the m.3243A>G mutation and 17 other syndromic diabetes genes (Supplementary Table 1). The coding regions, 50 nucleotides of flanking intronic sequence of the genes, and the mtDNA nucleotide m.3243 were analyzed for single nucleotide variants, insertions/deletions, and gene deletions by tNGS. Our assay did not target any other mitochondrial mutations or structural rearrangements. We used the Agilent SureSelect custom capture library and an Illumina NextSeq 500 NGS sequencing platform according to the methodology described by Ellard et al. (13). Our assay sequenced 99.7% of bases within the regions of interest at a minimum 30× read depth for all patients. All sequence variants are described using the nomenclature guidelines recommended by the Human Genome Variation Society (14). Interpretation and classification of sequence variants were based on American College of Medical Genetics and Genomics guidelines (15) and recommendations published by Ellard et al. (16). Only variants classified as likely pathogenic (class 4) or pathogenic (class 5) were included in the study. Copy number variant (CNV) analysis was performed using ExomeDepth according to the methodology described by Parrish et al. (17). The estimated sensitivity for CNV detection was >95%. Pathogenic or likely pathogenic CNVs were confirmed by multiplex ligation-dependent probe assay (MLPA) (SALSA MLPA Probemix P241 MODY kit; MRC Holland). *HNF1B* analysis was performed by Sanger sequencing and MLPA dosage analysis as described previously (18). The m.3243A>G mutation was confirmed by TaqMan real-time PCR according to the method described previously (19). Heteroplasmy was measured in peripheral blood, and m.3243A>G levels >3% were considered diagnostic for mitochondrial diabetes (12). The m.3243A>G heteroplasmy level was calculated as the number of sequence reads containing the mutation expressed as a percentage of the total number of reads aligned to the m.3243 locus. Heteroplasmy level was not assessed in any other tissues because of the lower prior likelihood of maternally inherited diabetes and deafness (MIDD) in our study cohort. The blood heteroplasmy level was corrected for age using a published method (20).

Statistical Analyses

Data were analyzed using Stata 16 (StataCorp, College Station, TX). Mann-Whitney *U* and Fisher exact tests were used to compare continuous and categorical variables, respectively.

Data and Resource Availability

All mutations identified in the study are provided in the Supplementary Materials. The clinical data generated and/or analyzed as part of this study are not publicly available because of patient confidentiality but are available from the corresponding authors upon reasonable request. Clinical information on individual patients with specific monogenic diabetes mutations is available upon

request in order to assist other laboratories and clinicians with variant interpretation and genetic diagnosis of their patients.

RESULTS

Characteristics of the Cohort

The clinical characteristics of the 1,280 participants who were referred from routine clinical care with suspected MODY are presented in Supplementary Table 2. The median age of diabetes diagnosis was 20 years (interquartile range [IQR] 14–29), median diabetes duration was 3 years (IQR 1–12), and median BMI was 25.7 kg/m^2 (IQR 22.4–30.0). One-half of the cohort was not treated with insulin (627 of 1,280, 49%), and 68% (873 of 1,280) had a parent with diabetes. None of the patients were clinically suspected of having a mutation in a syndromic diabetes gene.

Mutations in Syndromic Diabetes Genes Accounted for 19% of All Monogenic Cases in Patients With Suspected MODY

We confirmed monogenic diabetes in 23% (297 of 1,280) of patients (Fig. 1 and Supplementary Tables 3 and 4). Mutations in syndromic diabetes genes accounted for 19% (56 of 297 [95% CI 15–24%]) of monogenic cases (Fig. 1). The mitochondrial mutation m.3243A>G was the most common syndromic subtype, accounting for 43% (24 of 56) of all syndromic cases followed by mutations in *HNF1B* (18 of 56, 32%; 14 with a gene deletion and 4 with a single nucleotide variant). These were the 4th and 5th most common monogenic causes overall. Mutations in 6 other genes were responsible for the remaining syndromic cases (14 of 56, 25%) (Fig. 1 and Supplementary Table 3).

Clinical Features of Patients With Mutations in Syndromic Diabetes Genes Overlapped With Patients With Mutations in Nonsyndromic Genes

We next compared the clinical features of patients with a syndromic diabetes gene mutation with patients with a mutation in a nonsyndromic gene (Table 1). Both groups had similar age at diagnosis of diabetes, BMI, and HbA_{1c}. Patients with a mutation in a syndromic gene were more likely to be treated with insulin (71% vs. 39%, $P < 0.001$) and less likely to have a parent with diabetes (53% vs. 76%, $P = 0.001$). They were more likely to have extrapancreatic

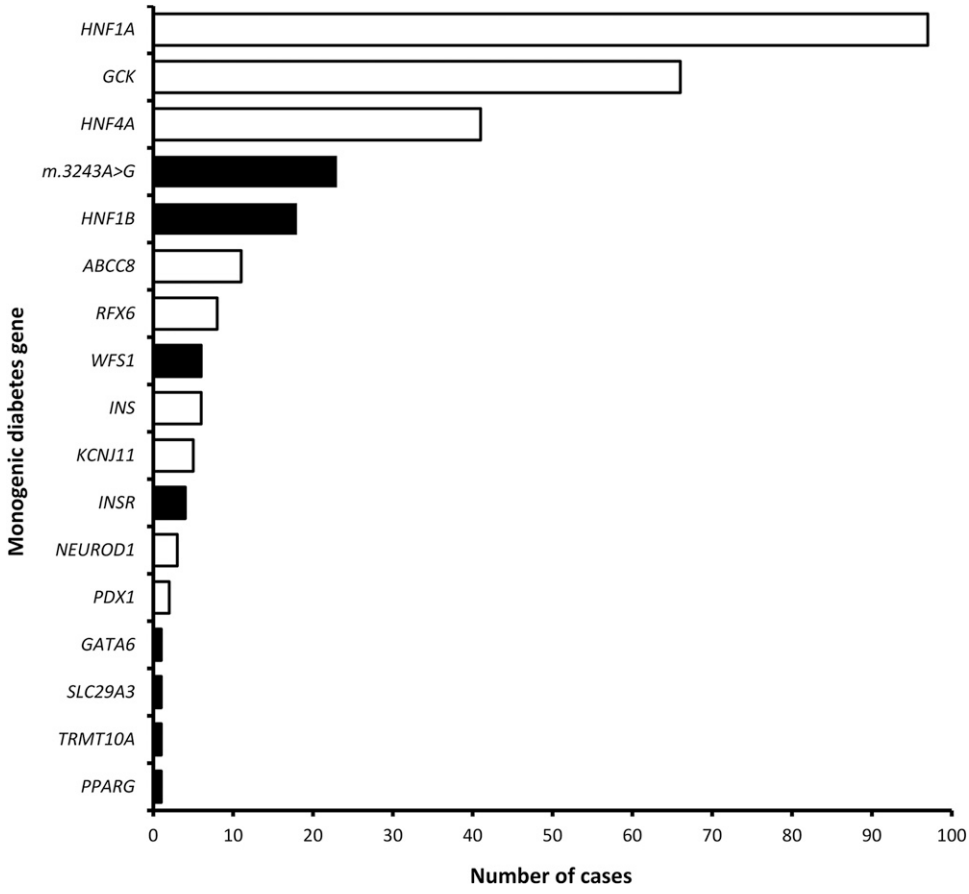

Figure 1—Bar chart showing the number of patients with each monogenic diabetes gene. Filled bars indicate syndromic monogenic diabetes genes, and open bars indicate nonsyndromic monogenic diabetes genes.

Table 1—Characteristics of patients with mutations in syndromic diabetes genes and nonsyndromic diabetes genes

Characteristic	Patients with mutations in syndromic monogenic diabetes genes (*n* = 56)	Patients with mutations in nonsyndromic monogenic diabetes genes (*n* = 241)	*P*
Age at diagnosis of diabetes (years)	20 (13.5–29), 56	17 (13–25), 241	0.09
Diabetes duration (years)	4 (1–8.5), 56	3 (0.5–14), 241	0.89
Female sex	37 (66)	145 (60)	0.44
BMI (kg/m^2)	22.0 (20.0–26.9), 49	23.7 (21.2–27.6), 197	0.05
Extrapancreatic features	13 (23)	15 (6)	<0.001
Parent with diabetes	30 (53)	184 (76)	0.001
Ethnicity (non-White)	13 (23)	38 (16)	0.23
HbA_{1c}, *n*	41	199	
%	7.3 (6.5–9.5)	7 (6.3–8.4)	0.20
mmol/mol	56 (48–80)	53 (45–68)	0.20
Insulin treated	40 (71)	95 (39)	<0.001
Insulin alone, *n*	33	73	
Insulin with oral hypoglycemic drugs, *n*	7	22	

Data are median (IQR), *n*, for continuous variables and *n* (%) for categorical variables unless otherwise indicated.

clinical features (23% vs. 6%, $P < 0.001$), but no patients had a constellation of features that pointed to a specific genetic syndrome.

m.3243A>G Cases Identified in a Suspected MODY Cohort Have Atypical Presentations

We compared the clinical features of m.3243A>G cases identified in our suspected MODY cohort with those of patients who were diagnosed with m.3243A>G when clinically suspected of having MIDD. We found no significant difference in sex, age at diabetes diagnosis, BMI, HbA_{1c}, diabetes treatment, and maternal history of diabetes (Fig. 2 and Supplementary Table 5). Clinician-reported deafness and maternal history of deafness (cardinal features of m.3243A>G) were significantly less common compared with the clinically suspected group (9% vs. 78% [$P < 0.001$] for deafness and 4% vs. 65% [$P < 0.001$] for maternal deafness). No patient in the suspected MODY cohort had any other extrapancreatic features associated with the m.3243A>G mutation. The median blood heteroplasmy level in the 24 patients with m.3243A>G detected by tNGS was 24.4% (IQR 18.1–33.8), and the median age-corrected

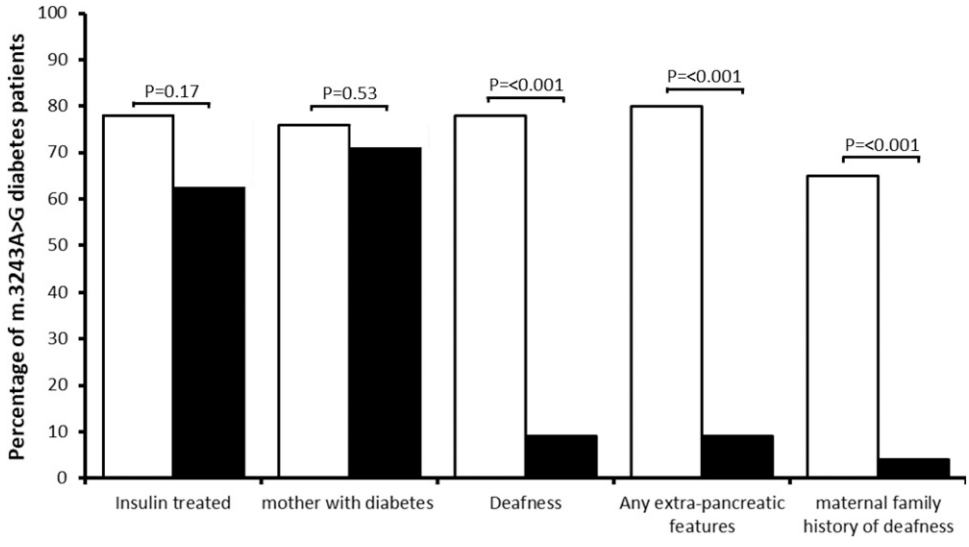

Figure 2—Comparison of clinical features in patients with m.3243A>G diabetes diagnosed by unselected testing using tNGS and by clinically suspected testing using a TaqMan genotyping assay undertaken as requested by the referring clinician. Filled bars indicate patients with diabetes and the m.3243A>G mutation identified by tNGS in a suspected MODY cohort, and unfilled bars indicate patients with m.3243A>G identified when clinically suspected of having MIDD.

blood heteroplasmy was 79.6% (IQR 60.7–92.8). The age-adjusted blood heteroplasmy level was not associated with age at diagnosis of diabetes (β = −0.08 [95% CI −0.24 to 0.75], P = 0.28) (Supplementary Fig. 1) and maternal diabetes status (median 85.5% [IQR 73.7–125] with maternal diabetes vs. 77.6% [IQR 53.8–90] without maternal diabetes, P = 0.13). The two people with deafness had a marginally higher age-adjusted blood heteroplasmy level compared with those without deafness (94.9% and 99.4% vs. median 78.3% [IQR, 58.6–90], P = 0.11).

Patients With an *HNF1B* Mutation in a Suspected MODY Cohort Had Atypical Presentation

We observed no significant difference in age at diabetes diagnosis, BMI, HbA_{1c}, diabetes treatment, or parental diabetes between patients with an *HNF1B* mutation identified in our suspected MODY cohort and patients with an *HNF1B* mutation identified when clinically suspected of having *HNF1B* diabetes (Supplementary Table 6). Extrapancreatic features were less common in patients diagnosed in our cohort than in those who underwent testing based on clinical suspicion (11% vs. 94%, $P <$ 0.001) (Fig. 3 and Supplementary Table 6). Structural kidney disease (renal cysts, dysplasia, and hypoplasia/agenesis, which are the cardinal features of *HNF1B* disease) was not reported in any of the patients diagnosed by unselected genetic testing. Nonkidney features were reported in two patients with a whole-gene deletion; one patient had autism and the other had a rudimentary uterus and hypoplastic ovaries. The lack of extrapancreatic features was still observed when analysis was restricted to patients with a whole-gene deletion (Supplementary Table 7).

Genetic Diagnosis Led to Identification of Extrapancreatic Features in Patients With Mutations in Syndromic Diabetes Genes

Mutations in syndromic diabetes genes other than m.3243A>G and *HNF1B* were identified in 14 patients, but none had clinical features at referral that were suggestive of having mutations in any of these genes (Supplementary Table 8). We contacted again the clinicians of these patients and obtained follow-up information on 12 (5 with *WFS1*, 4 with *INSR*, and 1 each with *GATA6*, *TRMT10A*, and *PPARG*) (Supplementary Table 8). In 7 (58%) of 12 patients, there were unreported clinical features that would have supported the final genetic diagnosis, but there were no known features present in 5 (42%) of 12 patients at the time of genetic testing. With further investigation/follow-up after the genetic diagnosis, all patients were found to have features consistent with their syndrome, except the patient with *GATA6* diabetes. We also contacted again the clinicians of patients with m.3243A>G and *HNF1B* diabetes identified by tNGS and obtained follow-up information on 15 (5 of 18 with *HNF1B* and 10 of 24 with m.3243A>G). We found that only two patients (13%) had characteristic syndromic features that were not reported by the clinician at the time of genetic testing. One clinician failed to report renal cysts for a patient with *HNF1B* diabetes, and deafness in another patient with the m.3243A>G mutation. At median follow-up of 5.4 years (range 3.8–7 years), three of the four remaining patients with *HNF1B* were found to have renal cysts, whereas only one of the remaining nine patients with m.3243A>G developed deafness. In total, after follow-up, 11 (19.6%) of 56 patients had features that would have predicted the presence of the syndromic

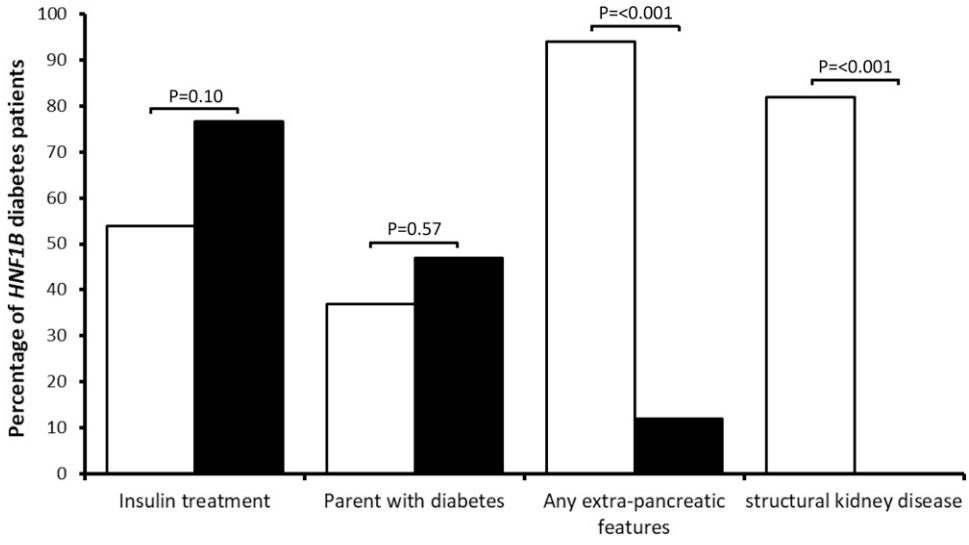

Figure 3—Comparison of clinical features in patients with *HNF1B* diabetes diagnosed by unselected testing using tNGS and by clinically suspected testing using Sanger sequencing and MLPA analysis undertaken as requested by the referring clinician. Filled bars indicate patients with an *HNF1B* mutation identified by tNGS in a suspected MODY cohort, and unfilled bars indicate patients with an *HNF1B* mutation identified when clinically suspected of having *HNF1B*-related disease.

gene mutation. Even if we remove these patients from the study, mutations in syndromic genes still accounted for 16% (46 of 287 [95% CI 12–20%]) of all monogenic cases.

DISCUSSION

Our study in a real-world setting strongly supports routine testing of syndromic diabetes genes in patients with suspected MODY. We showed that one in five patients with suspected MODY had a mutation in a syndromic diabetes gene and lacked typical features. It is the overlapping diabetes features with MODY that results in the referral of these patients for genetic testing. Their diagnosis would be missed using the current strategy that restricts testing of syndromic genes to patients with characteristic clinical features.

The m.3243A>G mutation is the 4th most common cause of monogenic diabetes (8% of all monogenic cases) after mutations in *GCK*, *HNF1A*, and *HNF4A* in patients with suspected MODY. There have been numerous studies of genetic testing in clinically suspected MODY cohorts, but only one small study of 109 patients from Korea included m.3243A>G (21). This lack of m.3243A>G testing is also seen in the NCBI Genetic Testing Registry, where none of the 26 gene panels for MODY included m.3243A>G testing (8). All patients with m.3243A>G in our suspected MODY cohort lacked typical features of MIDD; only two patients had deafness that was reportedly due to drug toxicity and ear infection, and our follow-up of 10 patients identified only 1 additional patient with deafness. Even if we remove these three patients from our calculation, m.3243A>G remains the most common syndromic subtype, accounting for 39% (21 of 53) of syndromic cases and 7% (21 of 294) of all monogenic cases. The low prevalence of deafness in our patients with the m.3243A>G mutation suggests that significant variable expressivity is the most likely reason for the nonsyndromic appearance of patients with MIDD and not the lack of reporting by clinicians. These data are consistent with previous reports of significant variable expressivity in MIDD (22).

Previous studies have suggested that heteroplasmy levels explain up to 27% of the variation in disease burden of m.3243A>G (20). We saw no association of heteroplasmy with age at diabetes diagnosis, maternal diabetes status, and maternal deafness status. However, the small sample size of our study prevents firm conclusions from being made. Most patients had an intermediate level of heteroplasmy, suggesting that the lack of severe hearing loss is not due to a low blood heteroplasmy level. Further studies are needed to compare heteroplasmy levels of patients identified from the MODY cohort with patients diagnosed because of a clinical suspicion of MIDD.

HNF1B mutations were also common in patients with suspected MODY but who lacked renal features suggestive of *HNF1B* disease. This finding was seen in a previous large study but at a lower frequency (10%) (23). *HNF1B* is also included in 24 of 26 MODY gene panels from the NCBI Genetic Testing Registry, highlighting the awareness of testing *HNF1B* in patients with suspected MODY. Seventy-eight percent of patients with *HNF1B* diabetes in our study had a large partial (one or more exons) or whole-gene deletion. Conventional variant calling performed by GATK HaplotypeCaller does not detect these large deletions, and they can only be detected by performing CNV analysis as a part of the NGS bioinformatics pipeline. In our whole study, 16 (5.4%) of 297 of patients had monogenic diabetes due to either a partial or whole-gene deletion (14 *HNF1B*, 1 *HNF1A*, and 1 *HNF4A*) (Supplementary Table 4). CNV analysis is performed on already-available data generated by tNGS, with minimal cost implications, and has the benefit of an additional genetic diagnosis. CNV analysis should therefore be performed as part of NGS testing for MODY. However, this is currently rarely performed in published studies of MODY testing using NGS (9,24).

We also identified 14 patients with mutations in syndromic monogenic diabetes genes other than m.3243A>G and *HNF1B*. In more than half, the characteristic features were present at referral, but the clinician did not associate them with the cause of the diabetes and, thus, did not report these at genetic testing. The lack of any specific features in 40% of patients was due to variable expressivity in these genes as reported previously (7,11,25,26). Our study also shows that the simple clinical features that may suggest a monogenic cause of the diabetes are not reported at least in some patients, highlighting the need for continuing professional education about monogenic diabetes for clinicians who see only a handful of monogenic cases in their careers because of the rarity of the disease.

Including syndromic genes on MODY panels has a number of benefits. It removes the need for clinicians to have detailed knowledge of all monogenic diabetes syndromes and focuses on identifying patients with a clinical suspicion of monogenic diabetes by using tools that are independent of etiology (e.g., C-peptide, islet autoantibodies, and type 1 diabetes genetic risk score) (3,27). A diagnosis of syndromic monogenic diabetes provides prognostic information and may prompt clinicians to screen for the presence of additional features, providing an opportunity to treat early in the disease process (e.g., screening for renal cysts and kidney function in *HNF1B* diabetes or cardiomyopathy in m.3243A>G diabetes). The genetic diagnosis may also explain the presence of additional features and may prevent unnecessary investigations to explain these features (e.g., raised liver enzymes with *HNF1B* diabetes or myopathy with m.3243A>G diabetes). It is recommended that patients with genetic syndromes are reviewed by clinical genetic services. Support from clinical genetics services and specialist clinics are needed, particularly when an unexpected diagnosis of a genetic syndrome is made, to prevent significant anxiety and provide holistic management. This

strategy also requires extra caution in interpreting novel variants in syndromic genes identified in patients who lack typical features of the syndrome (16).

Seventy-seven percent of patients did not receive a genetic diagnosis. MODY has overlapping features with young-onset type 1 and type 2 diabetes, and no single criterion can identify all patients with MODY (28). In the U.K., all children with negative GAD, IA2, and ZnT8 islet autoantibodies and detectable C-peptide and adults diagnosed at age <35 years with >20% prior probability of MODY are recommended to have genetic testing (29) with the aim of identifying the majority of patients with MODY at the expense of a lower positive predictive value due to the testing of patients with polygenic atypical type 1 and type 2 diabetes. The lack of a genetic diagnosis in 77% of patients is therefore more likely due to the inclusion of atypical type 1 and type 2 diabetes, with a minority of patients having yet-unknown novel monogenic diabetes or noncoding mutations in known genes not detected by our assay. We did not include *BLK*, *KLF11*, and *PAX4* in our gene panel because of lack of strong genetic evidence supporting the gene-disease association for MODY (30). *APPL1* is a very rare putative MODY gene that was only tested in 36% of our tNGS cohort and therefore not included in the study. This lower prior likelihood of monogenic diabetes has important implications for the assessment of pathogenicity of a detected novel variant, which is particularly important for the genes that cause syndromic diabetes in our study because the phenotype is used as evidence when classifying variant pathogenicity (15). Novel missense variants in cohorts of patients with a low prior probability of monogenic diabetes are more likely to be benign or have uncertain clinical significance, particularly when patients lack the typical features of the syndrome (31).

A limitation of our study is the lack of long-term clinical follow-up of all patients with m.3243A>G and *HNF1B* to determine whether their cases are truly atypical. However, our limited follow-up on one-third of the patients and the specific request for renal disease and deafness status on our referral form suggest that it is likely that these patients are not severely affected. A further clinical study with longer follow-up duration is needed to assess the stability of the nonsyndromic appearance.

In conclusion, mutations in syndromic monogenic diabetes genes are common in patients with suspected MODY in routine clinical practice. We strongly recommend including syndromic diabetes genes in gene panel tests for MODY to enable early diagnosis of atypical presentations and clinical benefits for diagnosed patients.

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