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Systematic population screening, using biomarkers and genetic testing, identifies 2.5% of the UK pediatric diabetes population with monogenic diabetes

Maggie Shepherd PhD^{1,2}, Beverley Shields PhD¹, Suzanne Hammersley², Michelle Hudson BSc², Timothy J McDonald PhD^{1,3}, Kevin Colclough BSc(Hons)⁴, Richard A Oram PhD¹, Bridget Knight PhD², Christopher Hyde MRCP⁵, Julian Cox FRCPCH⁶, Katherine Mallam MRCPCH⁷, Christopher Moudiotis MRCPCH⁸, Rebecca Smith MBChB⁹, Barbara Fraser FRCPCH¹⁰, Simon Robertson MRCPCH¹¹, Stephen Greene FRCPCH¹², Sian Ellard PhD¹, Ewan R Pearson PhD¹³, Andrew T Hattersley DM¹ on behalf of the UNITED team

Affiliations

1. Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter UK.
2. Exeter NIHR Clinical Research Facility, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
3. Blood Sciences, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK.
4. Molecular Genetics Laboratory, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
5. Exeter Test Group, Institute of Health Research, University of Exeter Medical School, Exeter, UK
6. J Cox, Department of Paediatrics, Northern Devon Healthcare NHS Trust, Barnstaple, UK
7. K Mallam, Department of Paediatrics, Royal Cornwall Hospitals NHS Trust, Truro, UK
8. C Moudiotis, Department of Paediatrics, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
9. R Smith, Children & Young People's Outpatient Department, Plymouth Hospitals NHS Trust, Plymouth, UK
10. B Fraser, Department of Paediatrics, South Devon Healthcare NHS Foundation Trust, Torquay, UK
11. S Robertson, Department of Paediatrics, Royal Cornwall Hospitals NHS Trust, Truro, UK
12. S Greene, Child Health, School of Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee, Scotland, UK
13. Division of Cardiovascular & Diabetes Medicine, School of Medicine, University of Dundee, Dundee, UK.

Corresponding author:

Maggie Shepherd

L3.11, RILD

Barrack Road

Exeter, EX6 7QX

01392 408261

m.h.shepherd@exeter.ac.uk

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Abstract

Objective

Monogenic diabetes is rare but an important diagnosis in pediatric diabetes clinics. These patients are often not identified as this relies on recognition of key clinical features by an alert clinician. Biomarkers (islet autoantibodies and C-peptide) can assist in 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 UK 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 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 IA-2) testing, with negative cases undergoing genetic testing for all 29 identified causes of monogenic diabetes.

Results

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

Conclusion

This large systematic study confirms a prevalence of 2.5% with monogenic diabetes aged <20yrs in 6 UK clinics. This figure suggests that around 50% of the estimated 875 UK pediatric patients with monogenic diabetes are still not diagnosed. This biomarker screening pathway is a practical approach that can be used to identify pediatric patients most appropriate for genetic testing.

Background

Monogenic diabetes is often not recognised 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 as the management of the commonest subtypes (GCK, HNF1A and HNF4A maturity onset diabetes of the young (MODY)) is markedly different from 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). Due to the predominance of Type 1 diabetes in children, the potential significance of a parent with diabetes or possible non-insulin 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 (unpublished data KC, SE), (based on 1240 patients initially diagnosed with diabetes <20 years and having 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. As 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 under diagnosis of MODY in some regions in the UK reflects reduced testing rather than inappropriate testing (13).

Biomarker tests can help 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 consideration of genetic testing. The lack of significant endogenous insulin production (stimulated serum C peptide <200pmol/l) is seen in Type 1 diabetes outside the honeymoon period. Urinary C-peptide creatinine ratio (UCPCR) provides a simple, stable, reliable, non-invasive 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). UCPCR ≥ 0.2 nmol/mol indicates endogenous insulin and has been used to differentiate patients with MODY from Type 1 diabetes >5 years post diagnosis (17). Islet autoantibodies are found in the majority of Type 1 patients 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 do not use a systematic approach or are 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: i) a US multicentre systematic study (SEARCH) identified a minimum prevalence of 1.2% with MODY (1) and a further 0.2% with neonatal diabetes (19), ii) a Norwegian nationwide study identified a minimum prevalence of monogenic diabetes in children of 1.1% (2), iii) 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 clinical features of monogenic diabetes in pediatric populations state widely varying prevalence 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 UK pediatric population using a systematic screening algorithm and genetic testing for all subtypes of monogenic diabetes.

Aims

To identify the prevalence of monogenic diabetes in the UK pediatric diabetes population by systematic screening.

Methods

Study eligibility: All patients with diabetes who were less than 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 NRES Committee South West - Central Bristol. Participants under 16 were asked to provide assent and their parents provided consent.

The total number of potential recruits (n=1016) were ascertained by the local pediatric clinical teams from their clinic records i.e. all their patients with diabetes <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, participants aged 16 or over 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: gender, ethnic group, current age / age at diagnosis, initial / current treatment, time to insulin, family history of diabetes, most recent / highest HbA1c, height / weight at diagnosis and time of recruitment and presence of learning difficulties or deafness. BMI was reported as age adjusted centiles to enable comparison across age groups (28).

Screening method: The study comprised of three potential stages which systematically identified those patients eligible for genetic testing (Figure 1).

Stage 1 consisted of a urine sample for measurement of urinary c-peptide creatinine ratio (UCPCR) (14-16). Participants on insulin treatment were asked to mail a urine sample collected two hours 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 ≥ 0.2 nmol/mmol and those not on 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 of a blood sample which tested for the presence of islet auto antibodies (GAD and IA-2) to identify those with autoimmune diabetes. This was performed on all those with significant endogenous insulin (either a UCPCR ≥ 0.2 nmol/mmol on insulin treatment or not on 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 IA-2 levels $> 99^{\text{th}}$ centile 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 usually taken 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 (MLPA) 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 HbA1c of $\leq 7.6\%$ (≤ 60 mmol/mol). This testing strategy was performed initially as these are the commonest genes implicated in MODY accounting for $>95\%$ of all UK MODY cases (Shields 2010), and are amenable to treatment change. Patients with no pathogenic mutation identified by Sanger sequencing and MLPA 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 was double entered onto a database and subsequently cleaned. Data are presented as proportions, and median (IQR) where appropriate, due to non-normality of data. Prevalence was calculated as the proportion of positive cases out of the total number studied. Data was analysed using Stata 13.1.

Results

79.5% (n=808/1016) of the eligible population completed the study (Figure 2). 15 of these had previously had genetic testing and were already known to have monogenic diabetes (Table 2).

Patient characteristics: 54% participants were male (441 male: 376 female). Median age at recruitment was 13 years, [10,16 IQR], median age at diagnosis was 8 years [4,11 IQR] and all individuals were diagnosed with diabetes >6 months of age. Median duration of diabetes was 4.3 years [1.6,7.9 IQR]. The majority, 788 (96%) of the cohort were white Caucasian, reflecting the population demographics in these areas. 792 (97%) were insulin treated at time of recruitment, including 4 patients taking insulin in addition to metformin. 25 (3%) patients were non insulin treated with 11 taking oral agents only and 14 on diet alone. Median HbA1c was 8.6% (70 mmol/mol) [7.7,9.7 IQR] (61,83 IQR) and median BMI centile 79 [56,94 IQR].

Stage 1 - UCPCR: 547/817 (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 have further testing. 261 (32%) had significant endogenous insulin production (≥ 0.2 nmol/mol); this included 236 patients who were insulin treated and 25 non-insulin treated patients.

Stage 2 - Antibodies: 253 patients with significant endogenous insulin underwent islet autoantibody testing, this included 236 patients who were insulin treated and confirmed UCPCR positive through stage 1 of the study and 17 of the non-insulin treated patients. 8/15 patients previously diagnosed with monogenic diabetes did not have antibody testing (but were all non insulin treated) and 9 patients did not return their blood sample for antibody testing.

179/253 participants were islet autoantibody positive confirming a diagnosis of Type 1 diabetes. 45 of these were positive to both GAD and IA-2, 28 positive to GAD only, 21 positive to GAD but IA-2 not tested and 85 positive to IA-2 only, indicating the importance of testing 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 UK pediatric diabetes population <20 years was 2.5% (95% CI of 1.5-3.9%). 82/808 (10.1%) patients had genetic testing and 20 (24%) of these (1 in 4) had monogenic diabetes (Table 2). 15/20 patients were previously known to have monogenic diabetes (7 GCK MODY, 5 HNF1A 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 <99th centile and therefore proceeded to genetic testing, but GAD titre 25.9 >97.5th centile, UCPCR 0.21nmol/mol two years post diagnosis, on continuous insulin treatment from diagnosis). Cases of monogenic diabetes were found in all 6 clinics with a prevalence varying between 1.2-3.7%.

To assess if we had missed cases of monogenic diabetes in those with islet autoantibodies, 65/179 patients with positive autoantibodies underwent Sanger sequencing analysis of the commonest 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 2 individuals with a previously recorded diagnosis of cystic fibrosis related diabetes. 27/62 of these patients (3.3% of the cohort) met diagnostic criteria for Type 2 diabetes: no monogenic or secondary cause, BMI ≥85th centile and antibody negative (<http://public.ukcrn.org.uk/search/StudyDetail.aspx?StudyID=7574>) 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 as they were close to diagnosis (median duration 0.8 years [0.4,2.8 IQR]) and were not overweight (median BMI centile 51st [43,67 IQR]). 26/33 of these had diabetes duration of <3 years, so could be considered within the honeymoon phase and repeating UCPCR in these individuals over time could prove useful. However 5/33 had diabetes duration of median 6.1 years (range 5-10), median BMI 53rd centile (range 46-81) with a UCPCR median 0.36nmol/mmol (range 0.21-1.27) and therefore the diabetes in these individuals should be considered atypical and not fitting a clear diagnostic category.

Only 19.4% (n=198) of the eligible patients within these pediatric diabetes populations did not take part in this study. This included 13 known cases of monogenic diabetes (10 of whom 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 with known monogenic diabetes. The prevalence of monogenic diabetes in those recruited was 2.5% compared to 6.6% (p=0.0038) in those not taking part.

Discussion

We found a prevalence of monogenic diabetes in patients diagnosed under 20 years 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 IA-2 autoantibodies would identify a sub-population of around 10% where 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 identification of those most likely to have MODY, as in our study 18/20 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 three other large systematic population studies: two from predominantly European white populations (Poland (3.1-4.2%), Norway (1.1%)) and one from a multi-ethnic population from the USA (1.4%) (7,2,1, 19) (Table 1). The Polish study used targeted case finding predominantly using clinical criteria supported by the lack of autoantibodies and measurable C peptide. The Norwegian population based study predominantly used antibody negativity combined with a parental history of diabetes or lack of insulin therapy and HbA1c<7.5% (58mmol/mol) (2). The lower prevalence (1.1%) probably reflected that they studied children aged 0-14 years rather than 0-20 years (mean age of diagnosis 10.6 years in our study) and only 10 patients were tested for glucokinase. The US study, like our study, used systematic biomarker screening with genetic testing performed in all patients who had measurable C peptide and did not have GAD and IA-2 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, as the combined prevalence of MODY in minorities was very similar to the prevalence of MODY in non-Hispanic whites (1). There are many other less comprehensive studies of the prevalence of monogenic diabetes (Table 1): these are limited by studying a single clinic, using a non-systematic assessment and/or not making a robust molecular genetic diagnosis (confirmed mutations not polymorphisms) (25,27,30).

Our study indicated a higher proportion of known cases of MODY versus new cases identified through systematic screening. The 28 cases of previously confirmed MODY (15 who took part and 13 who did not take part in the study) reflects the high levels of awareness of monogenic diabetes in these geographical regions. The 13 cases previously identified who did not take part 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 the cases and their family members can identify the majority of paediatric patients: (15/20) in this study. However the 5 new cases identified through this pathway of screening indicates the need for a systematic approach. If this approach was used in other areas where 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% (33/1016). There are estimated to be around 35,000 children and young people with diabetes, under the age of 19, in the UK (31,32). If the prevalence of 2.5% found in those who took part in this 6 clinic survey reflects the whole of the UK then this suggests at least 875 (95% CI 560-1365) expected cases of MODY in this age group, of whom 468 have been diagnosed to date with approximately 50% still likely to be misdiagnosed with Type 1 diabetes.

This approach of systematic testing combined with clinical criteria can result in a diagnosis in over 99% of cases and this is an advantage of this approach above 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 cases. Clinical criteria suggests that 3.3% had Type 2, a figure very similar to the amount of monogenic diabetes, as seen in other European populations (7, 24, 33). 0.2% had secondary diabetes due to cystic fibrosis which probably reflects an underestimate as many of these patients will not

attend a paediatric diabetes clinic. A further 3.2% were within three years of diagnosis and were probably antibody negative Type 1 diabetes in the honeymoon period. There remained 5 patients (0.6%) who are atypical and hard to classify – they may represent atypical Type 1 diabetes (antibody negative and significant C peptide more than 3 years after diagnosis) or a presently unrecognised 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 UK we estimate this figure is approximately 50% detected and 50% undetected. We only systematically genetically tested patients who had significant endogenous insulin (C peptide) and did not have autoantibodies although previous research (14,17,18) and our failure to find any mutations in 65 patients who did have significant C peptide but were antibody positive supports that this approach would miss very few cases. UCPCR was conducted irrespective of duration of diabetes and it is acknowledged that some of the patients tested close to diagnosis could be producing endogenous insulin during the honeymoon period and if retested over time those with Type 1 diabetes would be expected to have declining c-peptide levels. UCPCR is best for excluding patients 3 years after diabetes is diagnosed while autoantibodies are best close to diagnosis. In this study we wanted to test everyone irrelevant of disease duration so a 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 would have little value and further testing could be performed on those that were negative to multiple autoantibodies. Although patients were asked to send a 'post meal' urine sample the prandial state of the patient was assumed (and not observed) and therefore we cannot be certain that all UCPCR tests were stimulated. Our population were predominantly (96%) white Caucasians 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 as 79.5% of the eligible population took part; a result that shows the high acceptability of this approach in paediatric clinics. The systematic biomarker based approach that is independent of clinical features allows atypical cases 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 (85%) had the most common MODY genes *GCK*, *HNF1A* and *HNF4A* so studies that have not used this approach will have only missed a few patients.

Conclusion

This systematic, high uptake study gives a prevalence of 2.5% (95% CI 1.6%, 3.9%) of monogenic diabetes in the UK pediatric population. Patients with monogenic diabetes were identified in every pediatric clinic. The successful identification of patients with monogenic diabetes is crucial as they require different treatment than 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 non-invasive and inexpensive test which could be more widely used in the paediatric age group where it has a high acceptability. This screening algorithm is a practical approach to determining the prevalent cases in a clinic to ensure 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.

Author contributions

MS wrote the manuscript, collected and analysed the data. BS analysed the data and reviewed / edited the manuscript. SH collected the data and reviewed the manuscript. MH co-ordinated the project, assisted with the data and reviewed the manuscript, TJM co-ordinated the UCPCR and antibody testing and reviewed the manuscript. KC assisted with genetic data and reviewed / edited the manuscript, RO contributed to the discussion and reviewed the manuscript, BK developed the protocol, submitted the ethics application and reviewed the manuscript, CH reviewed the manuscript, JC, KM, CM, RS, BF, SR, and SG facilitated patient recruitment within their pediatric clinics and reviewed the manuscript, SE co-ordinated the genetic testing and reviewed the manuscript, ERP co-ordinated the Tayside arm of the project and reviewed / edited the manuscript, ATH designed the study, contributed to the discussion, reviewed and edited the manuscript, the UNITED team collected the data. MS 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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Figure Legends:

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

Figure 1. Pathway of testing

Figure 2. Patient progression through pathway

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