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Title

Absence of islet autoantibodies and modestly raised glucose values at diabetes diagnosis should lead to testing for MODY: Lessons from a 5-year pediatric Swedish national cohort study.

Short running title: Identifying MODY at diagnosis of diabetes

Authors

Prof A Carlsson MD[1]*, Prof M Shepherd PhD[2]*, Prof S Ellard PhD[2,3], Ass Prof M Weedon PhD[2], Prof Å Lernmark PhD[4], Ass Prof G Forsander MD[5], K Colclough BSc[3], Q Brahimi MSc[4], Dr C Valtonen-Andre MD[6], Prof S.A Ivarsson MD[4], Ass Prof H Elding Larsson MD[4], Ass Prof U Samuelsson MD[7], Dr E Örtqvist MD[8], Prof LGroop MD[9], Prof J Ludvigsson MD[7#], Prof C Marcus MD[10#+], Prof A.T Hattersley MD[2#+]

[1]Department of Clinical Sciences, Lund University, Skåne University Hospital, Lund, Sweden.

[2]Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK

[3]Molecular Genetics, Royal Devon and Exeter NHS Foundation Trust, UK

[4]Department of Clinical Sciences, Malmö, Lund University/CRC, Skåne University Hospital, Sweden

[5]The Queen Silvia Children's Hospital, Sahlgrenska University Hospital, and Instit of Clinical Sciences, University of Gothenburg, Gothenburg, Sweden

[6] University and Regional Laboratories Region Skåne, Department of Clinical Chemistry, Malmö, Sweden

[7]Crown Princess Victoria Children´s Hospital, University Hospital and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden.

[8]Pediatric Endocrinology Unit, Department of Woman and Child Health, Karolinska Institute, Stockholm, Sweden.

[9]Finnish Institute for Molecular Medicine (FIMM), Helsinki University, Helsinki, Finland

[10]Dept of Clinical Science Intervention and Technology, Division of Pediatrics, Karolinska Institutet, Stockholm, Sweden

*= contributed equally to this study

# = contributed equally to this study

+ = Corresponding Authors:

Professor Claude Marcus
Division of Pediatrics, Karolinska Institutet
Blickagången 6A
141 86 Stockholm
Sweden
Email claude.marcus@ki.se
+468058581419
and
Professor Andrew Hattersley
Institute of Biomedical and Clinical Science
University of Exeter Medical School
Exeter, UK
EX2 5DW
Email A.T.Hattersley@exeter.ac.uk
01392 408260

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Structured Abstract

Identifying maturity onset diabetes of the young (MODY) in pediatric populations close to diabetes diagnosis is difficult. Misdiagnosis and unnecessary insulin treatment are common.

Objective

We aimed to identify the discriminatory clinical features at diabetes diagnosis of patients with GCK, HNF1A and HNF4A MODY in the pediatric population.

Research Design and Methods

Swedish patients (n=3933) aged 1-18 years, diagnosed with diabetes May 2005-December 2010 were recruited from the national consecutive prospective cohort 'Better Diabetes Diagnosis' (BDD). Clinical data, islet autoantibodies (GADA, IA-2A, ZnT8A, and IAA), HLA type and C-peptide were collected at diagnosis. MODY was identified by sequencing GCK, HNF1A and HNF4A, either through routine clinical or research testing.

Results

The minimal prevalence of MODY was 1.2%. Discriminatory factors for MODY at diagnosis included 4 islet autoantibody negativity (100%vs11% not known MODY; $p=2x10^{-44}$), HbA1c (7.0vs10.7%, 53vs93 mmol/mol, $p=1x10^{-20}$), plasma glucose (11.7vs26.7mmol/L, $p=3x10^{-19}$), parental diabetes (63%vs12% $p=1x\ 10^{-15}$), and DKA (0/46vs601/3887, p=0.001). Testing 303 autoantibody negative patients identified 46 MODY patients (detection rate 15%). Limiting testing to the 73 antibody negative patients with HbA1c<7.5%(58mmol/mol) at diagnosis identified 36/46(78%) MODY patients (detection rate 49%). On follow-up the 46 MODY patients had excellent glycaemic control, HbA1c 6.4% (47mmol/mol) with42/46(91%) not on insulin treatment.

Conclusions

At diagnosis of pediatric diabetes absence of all islet autoantibodies and modest hyperglycaemia (HbA1c <7.5% (58mmol/mol)) should result in testing for GCK, HNF1A and HNF4A MODY. Testing all 12% patients negative for 4 islet autoantibodies is an effective strategy for not missing MODY but will result in a lower detection rate. Identifying MODY results in excellent long term glycaemic control without insulin.

Maturity onset diabetes of the young (MODY) is a monogenic, dominantly inherited, diabetes that is typically diagnosed young but is not insulin dependent. Recognizing MODY is important as treatment and management is different from type 1 diabetes and type 2 diabetes. Diabetes in children is predominantly type 1 diabetes, but type 2 diabetes and MODY also occur (1, 2). MODY accounts for 1-4% of pediatric diabetes (1, 3-7), but misdiagnosis results in many young people being treated unnecessarily with insulin (1,4) with many years delay from initial diabetes diagnosis to correct genetic diagnosis (8).

The commonest subtypes of MODY are Glucokinase (GCK) MODY which needs no treatment and Hepatocyte Nuclear Factor 1 alpha (HNF1A) MODY and Hepatocyte Nuclear Factor 4 alpha (HNF4A) MODY which are both optimally treated with low dose sulphonylureas when pharmaceutical therapy is needed (2,9).

Identifying MODY in pediatric diabetes populations is diagnostically difficult as no single or combination of commonly used clinical criteria can adequately separate them from type 1 diabetes and type 2 diabetes (1,2,10,11). This is particularly true close to diagnosis, when those with type 1 diabetes continue to produce endogenous insulin. Increasing obesity in all children (12) can also make differential diagnosis from type 2 diabetes challenging (13).

Islet autoantibodies can be useful in identifying 'non type 1 diabetes' and are rarely detected in MODY, being present in only 1% of cases, similar to the healthy population (14). In contrast islet autoantibodies are detected in approximately 90% of children with type 1 diabetes at diagnosis (15,16). Despite this, use of islet autoantibodies is not universally advocated and comprehensive islet autoantibody testing of all 4 sub-types glutamic acid decarboxylase (GADA), insulinoma antigen-2 (IA-2A), ZnT8 transporter (ZnT8A) and insulin (IAA) is not routinely performed in clinical care.

A correct diagnosis of MODY in children and adolescents leads to improved treatment with the avoidance of insulin, no deterioration in HbA1c (17, 18) and cost savings (19). Making the genetic diagnosis as close as possible to the diabetes diagnosis will reduce delays in starting recommended treatment. Approaches to the recognition of MODY are currently predominantly based on clinical features at follow-up rather than at diagnosis (20).

The aim of our study was to identify the discriminatory clinical features of the commonest types of MODY at diagnosis of diabetes in a pediatric national cohort.

Research Design and Methods

Individuals aged between 1-18 years, with a new diagnosis of diabetes were recruited from the national consecutive prospective cohort 'Better Diabetes Diagnosis' (BDD) study, involving 42 hospital pediatric clinics in Sweden, from May 2005 to December 2010 (21). A total of 4574 children and young people between the ages of 1-18 years were diagnosed with diabetes during the study period and 3933 (86%) were recruited into the study cohort (Figure 1). Participants were 45% female (n=1755) and had a mean age of diagnosis of 10.1 years.

Clinical characteristics: Clinical data including symptoms of polyuria, polydipsia and weight loss, family history of diabetes and samples for plasma glucose concentration, HbA1c, islet autoantibodies against GADA, IA-2A, ZnT8A and insulin, HLA type and C-peptide were collected at diagnosis. The routine laboratory tests, plasma-glucose, pH and HbA1c, were analysed locally with results returned within 24 hours and are described as 'early' investigations (Supplementary Table 1). Diabetic ketoacidosis (DKA) was defined as pH <7.3 OR serum bicarbonate <15mEq/L with a plasma glucose >11mmol/L. Blood samples sent to

the reference laboratory for analyses of all islet autoantibodies (GADA, IA-2A, ZnT8A and IAA), HLA genotype and random C-peptide are described as 'delayed' as the results were returned to the clinician within 14-90 days(Supplementary Table 1). Demographic data, symptoms, physical signs and blood analysis at onset were registered in SWEDIABKIDS, a national incidence and longitudinal quality control register for children and adolescents with diabetes (22).

Molecular genetic testing was undertaken to identify the commonest causes of MODY (*GCK, HNF1A* and *HNF4A*), all of which need different treatment from type 1 or type 2 diabetes. The molecular genetic sequencing of the whole coding region and critical noncoding regions of each gene was either performed as a result of a clinical request or as a research test at the diagnostic laboratory at the Royal Devon and Exeter NHS Foundation Trust, Exeter, UK (www.diabetesgenes.org) or at the Department of Clinical Chemistry, Division of Laboratory Medicine, Skåne University Hospital, Malmö, Sweden. Clinical referrals for genetic testing were predominantly requested in autoantibody negative patients 76/81 (94%) (Figure 1).

Of those who were islet autoantibody negative there were a total of 386/462 patients who were not tested clinically. To assess if cases of MODY had been missed that would alter treatment, research sequencing of the *HNF1A*, *HNF4A* and *GCK* genes was undertaken in an additional 404 patients (227 autoantibody negative and 177 autoantibody positive). We 'research tested' all 227 autoantibody negative patients in whom there was sufficient DNA for genetic sequencing. There were 159 islet autoantibody negative patients who were not tested on a clinical or research basis as there was insufficient DNA available (Figure 1). We compared the characteristics of the 159 islet autoantibody negative patients not research tested with the 227 'research tested' patients (supplementary table 2). No variables were

significant after correction for multiple testing. Therefore, the patients 'research tested' were representative of the whole group who were islet autoantibody negative and not tested clinically.

To provide a random collection of autoantibody positive patients we identified 250 consecutive patients. Research sequencing was performed on the 177 of these islet autoantibody positive patients that had not been tested clinically where we had adequate quantity of DNA for genetic testing. We compared the characteristics of the randomly selected 177 autoantibody positive patients who were 'research tested' for MODY to the 3294 antibody positive patients who were not tested (supplementary table 3). No variables except C peptide were significant after correction for multiple testing. Therefore, the patients research tested were representative of the whole group who were islet autoantibody positive and not tested clinically.

Details of testing:

Autoantibodies:

Autoantibodies against GADA, IA-2A, ZnT8A and IAA were analyzed in radio binding assays (23). The cut off values used equated to the level found in only 1% of an age matched population (23). GADA and IA-2A levels were expressed as U/ml derived from the WHO standard 97/550 and were considered positive if GADA were >35U/ml and IA-2A levels >5U/ml. The intra-assay CV for duplicates was 5 % for GADA and 11% for IA-2A. The radio ligand binding assay for all three ZnT8 autoantibodies (ZnT8A) variants (ZnT8RA, ZnT8WA and ZnT8QA) were analysed (24). Cut off values for positive results were ZnT8RA ≥75 U/ml, ZnT8WA ≥75 U/ml and ZnT8QA ≥ 100 U/ml. The intra-assay CV was 5.5% for ZnT8RA, 5.3%

for ZnT8WA, and 4.9% for ZnT8QA, respectively. IAA was considered positive if >0.8 relative units (RU). The intra-assay CV in the IAA assay was 6.0% (23).

The laboratory undertaking the autoantibody analyses participates in the biannual Islet Autoantibody Standardization Program (http://www.immunologyofdiabetssociety.com/).

HLA genotyping

Dried blood spots (DBS) were used for PCR amplification with sequence-specific oligonucleotide probes of HLA-DQB1 and DQA1 alleles using a DELFIA Hybridization assay (Perkin Elmer, Boston, MA) (25). The HLA-DQB1* probes defined the presence of HLA-DQB1*02, 03:02, 03:01, 06:02, 06:03 and 06:04 alleles and the HLA-DQA1 probes the DQA1*02:01, 03 and 05 alleles (25-27).

C-peptide measurement

Serum C-peptide, from the random non-fasting blood sample, was measured at Linkoping University, Sweden, with a time-resolved fluoroimmunoassay (AutoDELFIATM C-peptide kit, Wallac, Turku, Finland), with a detection level of 0.03 nmol/L (28).

Molecular genetic testing for MODY

The coding exons and conserved splice sites of *HNF1A*, *HNF4A* and *GCK* were amplified by PCR and sequenced on an ABI 3730 (Applied Biosystems, Warrington, UK). Sequences were compared to the published reference sequences (NM_000545.6 for *HNF1A*, NM_175914.4 for *HNF4A* and NM_000162.5 for *GCK*) using Mutation Surveyor v3.24 (SoftGenetics, PA, USA) or ABI SeqScape Software v2.5 (Applied Biosystems, Warrington, UK). Variants were classified according to the American College of Medical Genetics and Genomics guidelines

(29). MODY was diagnosed by the identification of heterozygous pathogenic or likely pathogenic variants.

Statistical methods

For statistical testing of binary variables we used Fisher's exact test and for continuous traits we used unpaired t-tests. Where the continuous traits were not normally distributed we log transformed the variable (indicated in tables). All analyses were performed in Stata v14.

Ethical approval

The Regional Ethics Board at the Karolinska Institute, Stockholm, Sweden approved the BDD study (Dnrs 2004-826/1, 2006/1082-32, 2009/1684-32).

The study is written in line with STROBE guidelines (https://strobe-statement.org/) for cohort studies.

Results

88% (3471/3933) of patients were positive for at least one islet autoantibody when all 4 autoantibodies were analyzed (Supplementary Table 1). The characteristics of the autoantibody positive and autoantibody negative patients are provided in Supplementary Table 1. We examined how testing a lower number of autoantibodies would change the number of individuals identified as autoantibody negative (Table 2). This table shows that each additional autoantibody tested result in less autoantibody negative patients being identified but with a reducing impact. So the number of autoantibody negative patients is 49% with GADA testing only, 17% GADA and IA-2A, 13% GADA, IA-2A and ZnT8A and 12%

with all 4 autoantibodies. The detailed breakdown of the distribution of different autoantibodies is in Supplementary Figure 1

MODY was only identified in autoantibody negative patients (Figure 1). There were no cases of MODY identified in the autoantibody positive cases tested either clinically (n=5) or through research testing of consecutively selected individuals, (n=177) who were positive for at least one islet autoantibody (Figure 1).

The clinical features of those with confirmed MODY (Table 1). The strongest discriminatory clinical features of MODY at diagnosis in addition to being negative to all 4 islet autoantibodies (100% vs 11% not known MODY; $p=2x10^{-44}$) were: lower HbA1c 7.0 vs 10.7% (53 vs 93 mmol/mol, $p=1x10^{-20}$), lower random plasma glucose (mean 11.7 vs 26.7mmol/L, $p=3x10^{-19}$), parental diabetes (63% v 12%) $p=1x\ 10^{-15}$ and not having DKA (0/46 vs 601/3887, p=0.001).

There was a high detection rate of MODY, 34/76 (45%), in the autoantibody negative patients tested on clinician's request (Figure 1). Clinical molecular genetic diagnosis of MODY in these 34 patients was made at a median of 9 (IQR 4-21) months post clinical diagnosis of diabetes. The patients clinically tested were more likely to be autoantibody negative, had lower plasma glucose and HbA1c and were more likely to have a parent with diabetes (Supplementary Table 4).

There was a lower rate of MODY detection, 12/227 (5%), in the autoantibody negative patients who had genetic testing as part of this research and were not referred for genetic testing by their clinicians (Figure 1).

The MODY subtypes identified were: GCK MODY in 29 (63%), HNF1A MODY in 10 (22%) and HNF4A MODY in 7 (15%) patients. The distribution of the different MODY types in the

autoantibody negative group, not referred for genetic testing by clinicians and tested on a research basis, was similar. The specific gene variants identified are shown in Supplementary Table 5.

46/303 (15%) of autoantibody negative patients were found by genetic testing to have MODY resulting in a minimal prevalence of 1.2% (46/3933) (Figure 1). If we assume the detection rate of 5% MODY in the 159 autoantibody negative patients who were not referred clinically nor tested on a research basis (n=159) was similar to the 227 who were tested we would expect to find an additional 8 patients with MODY giving an estimated prevalence of 1.4% (54/3933) in this pediatric population.

Autoantibody negativity was by far the strongest discriminatory clinical feature of MODY. Patients with MODY, 46/46 (100%), were negative for all 4 autoantibodies compared to just 416/3887 (11%) subjects not known to have MODY ($p<0.0001$; Table 1).

Among the autoantibody negative patients those with MODY had markedly less severe hyperglycaemia than those without MODY. HbA1c (7.0 vs 10.2% [53 vs 88mmol/mol]) and mean random plasma glucose: (11.7 vs 23.7mmol/L) were less severe in the MODY patients (Figure 2 and Supplementary Figure 2). They were less likely to have osmotic symptoms (polyuria and polydipsia) and weight loss but more likely to have a parent with diabetes (63 vs 27%, $p=4x10^{-6}$) (Supplementary Table 6). In a multiple logistic regression model in this autoantibody negative group of all variables that were significant in univariate analysis ($P<0.05$) only plasma glucose ($p=6x10^{-5}$) and parental history of diabetes ($p=0.02$) remained statistically significant discriminators of MODY. The characteristics of GCK, HNF1A and HNF4A MODY compared to non-MODY patients and to each other are shown in supplementary tables 7, 8 and 9.

Testing using previously suggested clinical criteria for discriminating MODY:

In our study genetic testing in 303 patients who were antibody negative detected 46 patients with MODY which is a detection rate of 15% (46/303). We went on to test how 2 previously defined criteria relating to HbA1c and family history altered the detection rate and the number of patients detected. See supplementry table 10. We used HbA1c rather than plasma glucose even though the latter was slightly more discriminatory as it used in previous clincal criteria and is less variable than glucose.

If testing was limited to the 73 patints who in additon to being antibody negative also had an had an HbA1c below the previously defined upper limit of HbA1c 7.5% (58mmol/mol) for the diagnosis of GCK MODY (30) this improved the detection rate to 49% (36/73) and identified 78% (36/46) patients with MODY. These criteria, as expected, were excellent for detecting GCK MODY (29/29 patients) but also detected 41% (7/17) of HNF1A and HNF4A MODY patients.

A dominant family history has been a defining feature of MODY (9). If testing was limited to patients who were autoantibody negative with a parental family history would result in testing 96 people with a detection rate of 30% (29/96) and would detect 63% (29/46) patients with similar proportions in GCK (18/29) and HNF1A/4A (11/17) MODY.

If those with HbA1c <7.5% (58mmol/mol) OR an affected parent were tested (n=131) then the detection rate was 33% (44/131) with 44/46 (96%) of MODY cases detected (supplementary table 10).

Therefore both glycaemia at diagnosis and family history have a role in selecting which autoantibody negative patients to test but if selecting on a single clinical criteria only then HbA1c <7.5% (58mmol/mol) is both more sensitive and more specific than family history.

Patients identified with a genetic diagnosis of MODY had an excellent outcome.

At a mean of 5.9 years after initial diabetes diagnosis excellent glycaemic control was achieved in all individuals with a genetic diagnosis of MODY with mean (SD) HbA1c 6.4 (1.0)% (47 (8) mmol/mol). A total of 42/46 (91%) patients were not on insulin and were on recommended treatment: no treatment for GCK MODY (29/29, 100%), diet or sulphonylurea for HNF1A MODY (9/10, 90%) and HNF4A MODY (4/7, 57%). 14/18 patients, started on insulin at initial diabetes diagnosis, had discontinued insulin treatment following a positive genetic test (Supplementary Table 11).

Conclusions

This is the first large prospective national study to examine all clinical features at diagnosis of diabetes. Our study provides clear support for identifying pediatric patients for MODY testing by excluding type 1 diabetes through high quality, comprehensive autoantibody testing using 4 autoantibodies. In the autoantibody negative patients the most discriminatory clinical features are low glycaemia (plasma glucose or HbA1c) and family history. Our study suggests that testing autoantibody negative patients with HbA1c <7.5% (<58mmol/mol) will identify over ¾ of GCK, HNF1A and HNF4A MODY with a detection rate of approximately 50%"..

The minimal incidence of MODY in patients aged 1-18 years identified in this Swedish cohort was 1.2% and the estimated prevalence, if all autoantibody negative patients had been tested in this cohort would be 1.4%. The prevalence seen in other studies of pediatric diabetes have been reported as follows: Norway (0.5%) (3), USA (1.2%) (1), Italy (1.6%) or

6.3% including incidental hyperglycaemia (5), Australia 1.9% (6), UK (2.5%) (4) and Poland (3.1-4.2%) (7). However our study included a better coverage of the population (86% of all newly diagnosed cases of pediatric diabetes) and our data was collected prospectively, as opposed to a cross-sectional or selected cohort.

Autoantibody negativity was a key feature of those identified with MODY. This finding was similar to previous studies but within our study autoantibody testing was comprehensive, with four autoantibodies tested and was also performed at diagnosis. This approach efficiently leads to more type 1 diabetes patients being positively identified and reduces the number of cases needing consideration for MODY testing. The fact that our cohort was recruited at diagnosis means that a low (< 200pmol/l) C-peptide result was not found in the majority of patients with antibody positive Type 1 diabetes. C-peptide testing was used in both US and UK studies (1, 4) but both recruited patients who were usually many years after the initial diabetes diagnosis.

Our study indicates that very few cases of MODY will be missed if genetic testing is limited to children who are negative for all 4 autoantibodies. As 1% of the normal population are islet autoantibody positive at the levels used as cut offs, then 1% of MODY patients can also be expected to have autoantibodies. However, as MODY is rare and type 1 diabetes is common in pediatric diabetes populations and approximately 90% type 1 diabetes children are autoantibody positive close to diagnosis the number of patients with MODY predicted in the autoantibody positive patients would be <0.1%. Therefore we consider data from this and other studies means autoantibody positivity is a reasonable exclusion for progressing to genetic testing in a person with diabetes in the pediatric age range (14, 31).

The breakdown of autoantibody positivity indicates that there is a clear benefit for testing 3 islet autoantibodies (GADA, IA-2A and ZnT8A). However only few additional patients will be found to be positive if IAA testing is also performed at diagnosis. The additional technical difficulty of testing IAA only reduces the number that are autoantibody negative from 13% of pediatric diabetes to 12% and may not be considered necessary clinically.

In our study the results of the autoantibody tests were returned to all clinicians and this was a major determinant of which patients the clinicians referred for genetic testing. This led to a very high rate of detection of MODY (45%) compared to 27% in reported routine services (8).

This is the first study performed at the time of diabetes diagnosis. Samples and data were typically collected at diagnosis before insulin was given. The US SEARCH study included patients close to diagnosis but was typically around eight months post initial diabetes presentation (1). Our study enabled accurate recording of initial symptoms and acute investigations at diagnosis of diabetes. The key features of those with MODY that we identified at diabetes diagnosis included, lower HbA1c, lower plasma glucose and less osmotic symptoms which all reflect less severe hyperglycaemia and there were no patients with MODY who presented in ketoacidosis.

A key issue is what is the appropriate level of sensitivity and specificity of the threshold set for systematic testing of patients. The low prevalence of MODY means that even features with a very high odds ratio have a low positive predictive value. This makes it hard to identify likely cases and unduly strict criteria while resulting in a high detection rate but will miss cases. With a reduction in the cost of genetic testing it may be the most effective strategy to sequence all pediatric patients with diabetes who are negative on testing four autoantibodies, leading to a detection rate of ≅15%. Our results support that pre-specified

cut offs of HbA1c <7.5% in antibody negative patients gives a much higher detection rate for the person being tested having MODY (≅50%) of but would miss ≅2% of cases (mainly HNF1A and HNF4A MODY). A compromise might be to test all antibody negative patientswith an HbA1c < 7.5% or a parent with diabetes; this approach had an detection rate ≅ 33% and detected ≅94% MODY.

It is interesting that, after receiving the islet autoantibody results, clinicians chose to test only 76/462 (16%) of the autoantibody negative patients but had a high detection rate 34/76 (45%) in those they did test. The main factors that influenced clinician testing were severity of glycaemia and family history (supplementary table 12). Ultimately a model that integrates all clinical factors may outperform clinician choice but at present either testing all islet autoantibody negative patients or those with a predefined HbA1c cut off of <7.5% at diagnosis performs better than clinician choice with less MODY cases missed.

It is important to detect MODY as our results show improved outcome both in terms of insulin cessation and HbA1c. This study has prospectively has followed-up the impact of a diagnosis of MODY from diabetes diagnosis to effect on clinical outcomes, 42/46 (91%) were not on insulin at follow up, 14/18 of these had ceased insulin treatment (which had been started at initial diabetes diagnosis) and excellent glycaemic control was achieved, HbA1c mean (SD) 6.4 (1.0)% (47 (8) mmol/mol) (Supplementary table 11). 2/4 of the individuals still on insulin chose to remain on this treatment. Stopping insulin can be a major challenge for some patients and this highlights the importance of identifying the correct genetic diagnosis as soon as possible (32).

The major strengths of this study is that it is a large, consecutive series recruiting 86% of cases of newly diagnosed diabetes in the pediatric population at diagnosis allowing assessment of both clincal features and antibodies at diagnosis.

It is also a strength that all autoantibody tests were carried out for the entire country at a central BDD laboratory at the Lund University CRC at Skåne University Hospital in Malmö. The laboratory is participating in the Islet Autoantibody Standardization program and has very good results. In addition, all samples are subjected to end point titration to better define cut off levels compared to a large number of serum and plasma samples from healthy individuals. All three isoforms of ZnT8A (R, W or Q at position 325) were analyzed to ensure that children single positive for any of the three variants were accounted for (15, 23). However most commonly clincally used ELISA assays for ZnT8A will detect as positive > 99% of samples positve for the 3 separate isoforms as they detect the common R and W variants. Only pathogenic or likely pathogenic variants have been included in this study (Supplementary table 3). The only likely pathogenic variants are in GCK, and so further investigations could be performed by testing other family members to check for segregation of the variant with fasting hyperglycaemia and raised HbA1c. Variants of uncertain significance were not included since they cannot be used to diagnose MODY.

A weakness of this study is that only the three commonest subtypes of MODY, that can alter treatment, were tested. If a next generation sequencing approach is used in children allowing testing of all potential monogenic subtypes cases then there is a slight increase (approximately 15%) more monogenic diabetes cases than the common GCK, HNF1A and HNF4A MODY cases alone) (4). The data presented applies to the Swedish population and

will vary in other populations especially when there is a higher representation of ethnic groups with a lower prevalence of Type 1 diabetes in the population.

In conclusion at diagnosis of pediatric diabetes comprehensive autoantibody testing and degree of glycaemia are key clinical features of MODY allowing differentiation from type 1 diabetes. Establishing negativity to four islet autoantibodies at diabetes diagnosis in the pediatric population efficiently identifies which individuals should be considered for genetic testing. Within the autoantibody negative patients modest hyperglycaemia, indicated by an HbA1 <58mmol/mol (<7.5%), and family history are further features that can be used to guide testing. Identifying patients for genetic testing at diabetes diagnosis will prevent delays in the correct molecular genetic diagnosis of patients. This will lead to improvements in treatment, quality of life and reductions in treatment and monitoring costs and should be universally advocated in pediatric diabetes patients at diagnosis.

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Author Contributions

AC, MHS, SE, GF, AL, LG, JL, CM and ATH were all involved in the conception and design of this study. AC, AL, GF, KC, QB, CV-A, SAI, HEL, US, EO, LG, JL and CM provided data, AL, AC, SE, MW, EO, KC analyzed data, AC, MHS, SE, MW, AL, KC, JL,CM and ATH interpreted data. MS, ATH, AC and CM wrote the early drafts of the paper and all authors critically reviewed and modified the manuscript and have agreed the final submitted version and agree to be accountable for the work.

There are no conflicts of interest to declare

References

1. Pihoker C, Gilliam LK, Ellard S et al. Prevalence, characteristics and clinical diagnosis of maturity onset diabetes of the young due to mutations in HNF1A, HNF4A, and glucokinase: results from the SEARCH for Diabetes in Youth. *J Clin Endocrinol Metab.* 2013; 98(10):4055-62.
2. Hattersley AT, Greeley SAW, Polak M et al. ISPAD 2018 Consensus Guidelines: The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes.* 2018; 19 Suppl 27:47-63
3. Johansson BB, Irgens HU, Molnes J et al. Targeted next-generation sequencing reveals MODY in up to 6.5% of antibody-negative diabetes cases listed in the Norwegian Childhood Diabetes Registry. *Diabetologia.* 2017; 60(4):625-35.
4. Shepherd M, Shields B, Hammersley S et al. Systematic Population Screening, Using Biomarkers and Genetic Testing, Identifies 2.5% of the U.K. Pediatric Diabetes Population with Monogenic Diabetes*. Diabetes Care.* 2016; 39(11):1879-88.
5. Delvecchio M, Mozzillo E, Salzano G et al. Monogenic diabetes accounts for 6.3% of cases referred to 15 Italian pediatric diabetes centres during 2007-2012. *J Clin Endocrinol Metab.* 2017; 102(6):1826-1834
6. Johnson SR, Ellis JJ, Leo PJ et al. Comprehensive genetic screening: The prevalence of maturity-onset diabetes of the young gene variants in a population-based childhood diabetes cohort. *Pediatric Diabetes.* 2018; 1-8
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(10):2631-5.
8. 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(12):2504-8.
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(4):200-13
10. Pearson ER, Pruhova S, Tack CJ net al. Molecular genetics and phenotypic characteristics of MODY caused by hepatocyte nuclear factor 4alpha mutations in a large European collection. *Diabetologia.* 2005; 48(5):878-85.
11. Bellanne-Chantelot C, Levy DJ, Carette C et al. Clinical characteristics and diagnostic criteria of maturity-onset diabetes of the young (MODY) due to molecular anomalies of the HNF1A gene. *J Clin Endocrinol Metab.* 2011; 96(8):E1346-51.
12. Collaboration NCDRF. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet.* 2017; 16;390(10113):2627-2642
13. Kleinberger JW, Copeland KC, Gandica RG et al. Monogenic diabetes in overweight and obese youth diagnosed with type 2 diabetes: the TODAY clinical trial. *Genet Med.* 2018; 20(6):583-90.
14. 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(9):1028-33.

15. Andersson C, Vaziri-Sani F, Delli A et al. Triple specificity of ZnT8 autoantibodies in relation to HLA and other islet autoantibodies in childhood and adolescent type 1 diabetes. *Pediatr Diabetes.* 2013; 14(2):97-105.
16. Bravis V, Kaur A, Walkey HC et al.Relationship between islet autoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort. *BMJ Open.* 2018. 4;8(4):e020904
17. Bacon S, Kyithar MP, Rizvi SR et al. Successful maintenance on sulphonylurea therapy and low diabetes complication rates in a HNF1A-MODY cohort. *Diabet Med.* 2016. 33(7):976-84
18. Shepherd MH, Shields BM, Hudson M et al. A UK nationwide prospective study of treatment change in MODY: genetic subtype and clinical characteristics predict optimal glycaemic control after discontinuing insulin and metformin. *Diabetologia.* 2018. 61(12):2520-2527
19. Naylor RN, John PM, Winn AN et al. Cost-effectiveness of MODY genetic testing: translating genomic advances into practical health applications. Diabetes Care. 2014; 37:202-209
20. Shields BM, McDonald TJ, Ellard S, Campbell MJ, Hyde C, Hattersley AT. The development and validation of a clinical prediction model to determine the probability of MODY in patients with young-onset diabetes. *Diabetologia.* 2012 May;55(5):1265-72
21. Persson M, Becker C, Elding Larsson H et al. The Better Diabetes Diagnosis (BDD) study - A review of a nationwide prospective cohort study in Sweden. *Diabetes Res Clin Pract.* 2018; 140:236-44.
22. Hanberger L, Samuelsson U, Lindblad B, Ludvigsson J. Swedish Childhood Diabetes Registry SWEDIABKIDS. A1c in children and adolescents with diabetes in relation to certain clinical parameters: the Swedish Childhood Diabetes Registry SWEDIABKIDS. *Diabetes Care.* 2008 31(5):927-9.
23. Delli AJ, Vaziri-Sani F, Lindblad B et al. Zinc transporter 8 autoantibodies and their association with SLC30A8 and HLA-DQ genes differ between immigrant and Swedish patients with newly diagnosed type 1 diabetes in the Better Diabetes Diagnosis study. *Diabetes.* 2012; 61(10):2556-64.
24. 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
25. Kiviniemi M, Hermann R, Nurmi J et al. A high-throughput population screening system for the estimation of genetic risk for type 1 diabetes: an application for the TEDDY (the Environmental Determinants of Diabetes in the Young) study. Diabetes Technol Ther 2007;9:460-472
26. Larsson HE, Lynch K, Lernmark B et al. Relationship between increased relative birthweight and infections during pregnancy in children with a high-risk diabetes HLA genotype. Diabetologia 2007;50:1161-1169
27. Hagopian WA, Erlich H, Lernmark A et al. The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. Pediatric diabetes 2011;12:733-743
28. Ludvigsson J, Carlsson A, Forsander G et al. C-peptide in the classification of diabetes in children and adolescents. *Pediatr Diabetes.* 2012; 13(1):45-50.

29. Richards S, Aziz N, Bale et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5.
30. Steele AM, Wensley KJ, Ellard S et al. Use of HbA1c in the identification of patients with hyperglycaemia caused by a glucokinase mutation: observational case control studies. *PLoS One.* 2013; 14;8(6):e65326
31. Patel KA, Weedon MN, Shields BM et al. Zinc Transporter 8 Autoantibodies (ZnT8A) and a Type 1 Diabetes Genetic Risk Score Can Exclude Individuals With Type 1 Diabetes From Inappropriate Genetic Testing for Monogenic Diabetes. Diabetes Care. 2019 Feb;42(2):e16-e17. doi: 10.2337/dc18-0373. Epub 2018 Nov 8.
32. Shepherd M. Stopping insulin injections following genetic testing in diabetes: impact on identity. *Diabet Med.* 2010; 27(7):838-43

	HNF1A/HNF4A/ GCK MODY (n=46)		Not known MODY (n=3887)^		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P
Clinical features					
N	46		3887		
Sex (% female)	46	54 (25)	3887	45 (1730)	0.23
Age at diagnosis (yrs)	46	12.1 (4.4)	3887	10.1 (4.4)	$3x10^{-3}$
Parental Diabetes (%)	46	63 (29)	3887	12 (471)	$1x10^{-15}$
Polyuria (%)	38	34 (13)	3570	95 (3378)	$2x10^{-22}$
Polydipsia (%)	38	34 (13)	3559	94 (3350)	$2x10^{-21}$
Weight loss (%)	38	16 (6)	3449	76 (2619)	$1x10^{-14}$
BMI (SDS)	37	0.54 (1.2)	3341	-0.36 (1.6)	$7x10^{-4}$
Acanthosis Nigricans (%)	37	0 (0)	3453	100 (44)	1
Investigations- early*					
Plasma glucose (mmol/L)	41	11.7 (4.1)	3528	26.7 (9.0)	$3x10^{-19}$
HbA1c (%)	46	7.0 (3.7)	3495	10.7 (4.5)	$1x10^{-20}$
DKA (%)	46	0 (0)	3887	15 (601)	0.001
Investigations – delayed†					
4 Autoantibody negative (%)	46	100 (46)	3887	11 (416)	$2x10^{-44}$
High risk HLA (%)	46	20 (9)	3830	70 (2684)	$3x10^{-12}$
C peptide (nmol/mol)	41	0.99 (0.63)	3555	0.34 (0.43)	$1x10^{-14}$
C Peptide <0.2 (nmol/mol)	41	2 (1)	3555	40 (1433)	$4x10^{-8}$

Table 1: Clinical features and investigation results of MODY (*HNF1A, HNF4A and GCK*) and Not known MODY patients.

^ Not known MODY consist of n=3471 autoantibody positive (182 MODY tested) and 416 antibody negative (257 MODY tested).

Plasma glucose and C peptide results based on log10 transformation.

*early local testing with results 0-24 hours

†testing at reference laboratories where results were delayed (14-90 days)

Number of autoantibodies tested	Autoantibody positive to:	N positive (%) (/3933)	N (%) of people who tested negative with this testing of antibody combination
1 antibody	GAD	2081 (53)	1852 (47)
	IA2	2718 (69)	1215 (31)
2 antibodies	GAD and/or IA2	3263 (83)	670 (17)
3 antibodies	GAD and/or IA2 and /or ZnT8	3428 (87)	505 (13)
4 antibodies	GAD and/or IA2 and/or ZnT8 and/or IAA	3471 (88)	462 (12)

Table 2: Combinations of commonly tested autoantibodies, illustrating number and percentage of individuals positive to at least one autoantibody and percentage of patients negative depending on combination tested.

Figure 1. Study flow diagram: MODY was only identified in islet autoantibody negative patients

Figure 2. MODY patients had lower HbA1c at diagnosis than those without MODY.

Data shown as a Box and Whisker plot: the ends of the box are the upper and lower quartiles, the median is marked as the vertical line inside the box. The vertical lines indicate the maximum and minimum values excluding extreme outliers shown as dots. HbA1c of 7.5% (58mmol/mol) cut off for GCK MODY (ref 30) shown as red dotted line.

Online only supplemental material

	All Patients		Autoantibody +ve		Autoantibody -ve		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P
Clinical features							
N			3471		462		
Sex (% female)	3933	45 (1755)	3471	45 (1566)	462	41 (189)	0.09
Age at diagnosis (yrs)	3933	10.1 (4.4)	3471	9.9 (4.4)	462	11.6 (4.5)	$5x10^{-14}$
Parental Diabetes (%)	3933	13 (500)	3471	11 (379)	462	26 (121)	$4x10^{-17}$
Polyuria (%)	3608	94 (3392)	3233	96 (3104)	375	77 (288)	$2x10^{-33}$
Polydipsia (%)	3597	93 (3363)	3225	95 (3077)	372	77 (286)	$1x10^{-29}$
Weight loss (%)	3487	75 (2625)	3129	77 (2423)	358	56 (202)	$2x10^{-16}$
BMI (SDS)	3378	-0.35 (1.55)	3020	-0.44 (1.49)	358	0.43 (1.83)	$1x10^{-16}$
Acanthosis Nigricans (%)	3490	1 (44)	3136	1 (17)	354	8 (27)	$8x10^{-17}$
Investigations- early*							
Plasma glucose (mmol/L)	3569	26.5 (9.1)	3198	26.9 (8.9)	371	23.1 (10.8)	$1x10^{-15}$
HbA1c (%)	3541	10.6 (4.5)	3161	10.7 (4.5)	380	9.9 (5.1)	$9x10^{-8}$
DKA (%)	3933	15 (601)	3471	17 (574)	462	6 (27)	$6x10^{-11}$
Investigations – delayed†							
High risk HLA (%)	3876	69 (2693)	3419	73 (2486)	457	45 (207)	$2x10^{-30}$
C peptide (nmol/mol)	3596	0.35 (0.44)	3184	0.28 (0.23)	412	0.85 (0.98)	$3x10^{-36}$
C Peptide <0.2 (nmol/mol) (%)	3596	40 (1434)	3184	42 (1337)	412	24 (97)	$1x10^{-13}$

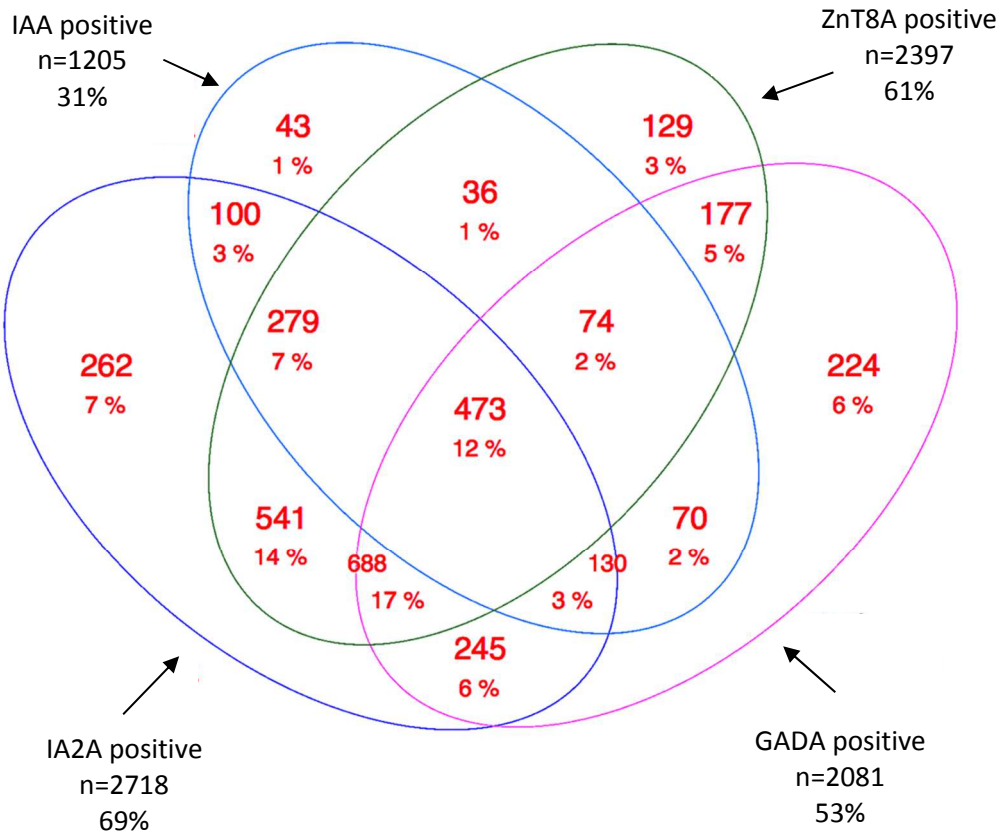
Supplementary Table 1. Clinical characteristics of subjects. Plasma glucose and C peptide results based on log10 transformation.
* local testing with results 0-24 hours
† testing at reference laboratory, results within 14-90 days

	Research tested Autoantibody negative		Not tested Autoantibody negative		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P value *
Clinical features					
N	227		159		
Sex (% female)	227	37 (85)	159	42 (66)	0.46
Age at diagnosis (yrs)	227	12.06 (4.47)	159	10.88 (4.57)	0.01
Parental Diabetes (%)	227	25 (56)	159	15 (24)	0.03
Polyuria (%)	189	79 (150)	122	87 (106)	0.10
Polydipsia (%)	186	80 (149)	122	85 (104)	0.29
Weight loss (%)	178	58 (104)	118	69 (81)	0.09
BMI (SDS)	178	0.42 (1.96)	118	0.28 (1.85)	0.55
Acanthosis Nigricans (%)	178	9 (16)	115	8 (9)	0.83
Investigations- early					
Plasma glucose (mmol/L)	186	23.6 (10.2)	121	25.9 (11.2)	0.05
HbA1c (%)	186	10.3 (5.0)	123	10.3 (5.1)	0.88
DKA (%)	227	6 (13)	159	8 (13)	0.41
Investigations - delayed					
High risk HLA (%)	226	46 (103)	155	53 (82)	0.18
C peptide (nmol/mol)	213	0.86 (1.01)	131	0.83 (1.07)	0.08

Supplementary table 2. Comparing the research tested autoantibody negative group with those not tested to assess if representative
Plasma glucose and C-peptide results based on log10 transformation.
* to correct for the 13 variables analysed a p value of < 0.004 should be considered significantly different.

	Research tested Autoantibody positive		Not tested Autoantibody positive		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P value *
Clinical features					
N	177		3294		
Sex (% female)	177	49 (87)	3294	45 (1479)	0.28
Age at diagnosis (yrs)	177	9.54 (4.43)	3294	9.91 (4.39)	0.27
Parental Diabetes (%)	177	8 (15)	3294	11 (364)	0.32
Polyuria (%)	156	95 (148)	3077	96 (2956)	0.40
Polydipsia (%)	156	94 (147)	3069	95 (2930)	0.43
Weight loss (%)	146	73 (107)	2983	78 (2316)	0.22
BMI (SDS)	143	-0.17 (1.56)	2877	-0.45 (1.49)	0.03
Acanthosis Nigricans (%)	154	1 (2)	2982	1 (15)	0.20
Investigations- early					
Plasma glucose (mmol/L)	155	25.3 (7.4)	3043	27.0 (8.9)	0.02
HbA1c (%)	154	10.5 (4.5)	3007	10.7 (4.5)	0.22
DKA (%)	177	12 (21)	3294	17 (553)	0.10
Investigations - delayed					
High risk HLA (%)	177	72 (128)	3242	73 (2358)	0.93
C peptide (nmol/mol)	166	0.34 (0.263)	3018	0.28 (0.231)	$4x10^{-4}$
Antibodies					
1 antibody +ve	177	23 (40)	3294	19 (618)	0.20
2 antibody +ve	177	35 (61)	3294	34 (1108)	0.81
3 antibody +ve	177	31 (54)	3294	34 (1117)	0.37
4 antibody +ve	177	12 (22)	3294	14 (451)	0.74

Supplementary table 3. Comparing the research tested autoantibody positive group with those not tested to assess if representative. Plasma glucose and C peptide results based on log10 transformation. * to correct for the 17 variables analysed a p value of < 0.003 should be considered significantly different.



Supplementary Figure 1: Breakdown of autoantibody positivity in 3933 individuals tested

Venn diagram produced using venndiag stata package

	Patients Tested Clinically		Patients Not Tested Clinically		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P
Clinical features					
N	81		3852		
Sex (% female)	81	49 (40)	3852	45 (1715)	0.43
Age at diagnosis (yrs)	81	11.8 (4.02)	3852	10.1 (4.44)	$3x10^{-4}$
Parental Diabetes (%)	81	54 (44)	3852	12 (456)	$1x10^{-19}$
Polyuria (%)	69	54 (37)	3539	95 (3355)	$5x10^{-22}$
Polydipsia (%)	69	55 (38)	3528	94 (3325)	$1x10^{-19}$
Weight loss (%)	67	31 (21)	3420	76 (2604)	$2x10^{-14}$
BMI (SDS)	67	0.78 (1.46)	3311	-0.37 (1.55)	$2x10^{-8}$
Acanthosis Nigricans (%)	66	3 (2)	3424	1 (42)	0.20
Investigations- early					
Plasma glucose (mmol/L)	69	17.0 (8.9)	3500	26.7 (9.0)	$3x10^{-13}$
HbA1c (%)	76	8.2 (4.8)	3465	10.7 (4.5)	$9x10^{-12}$
DKA (%)	81	1 (1)	3852	16 (600)	$3x10^{-5}$
Investigations – delayed					
4 Autoantibody negative (%)	81	94 (76)	3852	10 (386)	$9x10^{-67}$
High risk HLA (%)	81	28 (23)	3795	70 (2670)	$2x10^{-14}$
C peptide (nmol/mol)	72	0.85 (0.69)	3524	0.34 (0.43)	$2x10^{-12}$
C Peptide < 0.2 (nmol/mol) (%)	72	13 (9)	3524	40 (1425)	$5x10^{-7}$

Supplementary Table 4. Characteristics of the patients referred for genetic testing (and not referred for genetic testing) by clinicians. Plasma glucose and C peptide results based on log10 transformation.

Study Number	Gene	DNA description*	Protein Description*	Predicted Effect	No. of heterozygotes in GnomAD†	Variant Classification‡	References	Clinician requested test?
BDD0024	*GCK*	c.772G>T	p.(Gly258Cys)	Missense	0/123027	Pathogenic	Mantovani (2003) Hum Mutat 22, 338	No
BDD0068	*GCK*	c.571C>T	p.(Arg191Trp)	Missense	2/123005	Pathogenic	Ellard (2000) Diabetologia 43, 250	Yes
BDD0377	*HNF4A*	c.47dup	p.(Tyr16Ter)	Nonsense	0/15464	Pathogenic	Novel	Yes
BDD0387	*GCK*	c.1016A>G	p.(Glu339Gly)	Missense	0/118808	Pathogenic	Sagen (2006) Diabetes 55, 1713	Yes
BDD0422	*GCK*	c.766G>A	p.(Glu256Lys)	Missense	1/123047	Pathogenic	Gidh-Jain (1993) Proc Natl Acad Sci U S A 90, 1932	Yes
BDD0647	*GCK*	c.704T>C	p.(Met235Thr)	Missense	0/122988	Pathogenic	Gloyn (2003) Hum Mutat 22, 353	Yes
BDD0664	*HNF1A*	c.872dup	p.(Gly292fs)	Frameshift	0/132382	Pathogenic	Yamagata (1996) Nature 384, 455	Yes
BDD0665	*GCK*	c.675C>G	p.(Ile225Met)	Missense	0/123114	Likely Pathogenic	Massa (2001) Diabetologia 44, 898	Yes
BDD0717	*HNF1A*	c.872dup	p.(Gly292fs)	Frameshift	0/132382	Pathogenic	Yamagata (1996) Nature 384, 455	Yes
BDD0809	*HNF4A*	c.46_49+6delinsG	p.(?)	Aberrant splicing	0/15464	Pathogenic	Novel	Yes
BDD0840	*GCK*	c.766G>A	p.(Glu256Lys)	Missense	1/123047	Pathogenic	Gidh-Jain (1993) Proc Natl Acad Sci U S A 90, 1932	Yes
BDD0842	*GCK*	c.854G>A	p.(Gly285Asp)	Missense	0/120800	Likely Pathogenic	Novel (1 family in MODY DB)	Yes
BDD0911	*GCK*	c.766G>A	p.(Glu256Lys)	Missense	1/123047	Pathogenic	Gidh-Jain (1993) Proc Natl Acad Sci U S A 90, 1932	Yes
BDD1002	*HNF4A*	c.956_958dup	p.(Leu319dup)	In-frame amino acid deletion	0/122264	Pathogenic	Pearson (2005) Diabetologia 48, 878	Yes
BDD1107	*HNF1A*	c.814C>T	p.(Arg272Cys)	Missense	0/121947	Pathogenic	Yoshiuchi (1999) Diabetologia 42, 621	Yes
BDD1337	*GCK*	c.680-2A>G	p.(?)	Aberrant splicing	0/122599	Pathogenic	Osbak (2009) Hum Mutat 30, 1512	Yes
BDD1433	*GCK*	c.442T>A	p.(Phe148Ile)	Missense	0/123131	Likely Pathogenic	Osbak (2009) Hum Mutat 30, 1512	Yes
BDD1470	*GCK*	c.878T>G	p.(Ile293Arg)	Missense	0/122348	Pathogenic	Novel (3 families in MODY DB)	Yes
BDD1515	*HNF4A*	c.956_958dup	p.(Leu319dup)	In-frame amino acid deletion	0/122264	Pathogenic	Pearson (2005) Diabetologia 48, 878	Yes
BDD1526	*GCK*	c. 704T>C	p.(Met235Thr)	Missense	0/122988	Pathogenic	Gloyn (2003) Hum Mutat 22, 353	Yes
BDD1557	*HNF4A*	c.1A>G	p.(?)	Start-loss	0/122240	Pathogenic	Novel	Yes
BDD1586	*GCK*	c.623C>T	p.(Ala208Val)	Missense	1/123121	Pathogenic	Osbak (2009) Hum Mutat 30, 1512	Yes
BDD1930	*GCK*	c.490C>T	p.(Leu164Phe)	Missense	0/15478	Likely Pathogenic	Nam (2000) Diabetes Res Clin Pract 50, 169	Yes
BDD2002	*GCK*	c.766G>A	p.(Glu256Lys)	Missense	1/123047	Pathogenic	Gidh-Jain (1993) Proc Natl Acad Sci U S A 90, 1932	Yes
BDD2035	*GCK*	c.1144T>C	p.(Cys382Arg)	Missense	0/115895	Pathogenic	Osbak (2009) Hum Mutat 30, 1512	Yes
BDD2161	*GCK*	c.680-2A>G	p.(?)	Aberrant splicing	0/122599	Pathogenic	Osbak (2009) Hum Mutat 30, 1512	No
BDD2282	*HNF1A*	c.493T>C	p.(Trp165Arg)	Missense	0/138536	Pathogenic	Tatsi (2013) Pediatr Diabetes epub, epub	Yes
BDD2362	*GCK*	c.929T>G	p.(Val310Gly)	Missense	1/121775	Likely Pathogenic	Novel (2 families in MODY DB)	Yes

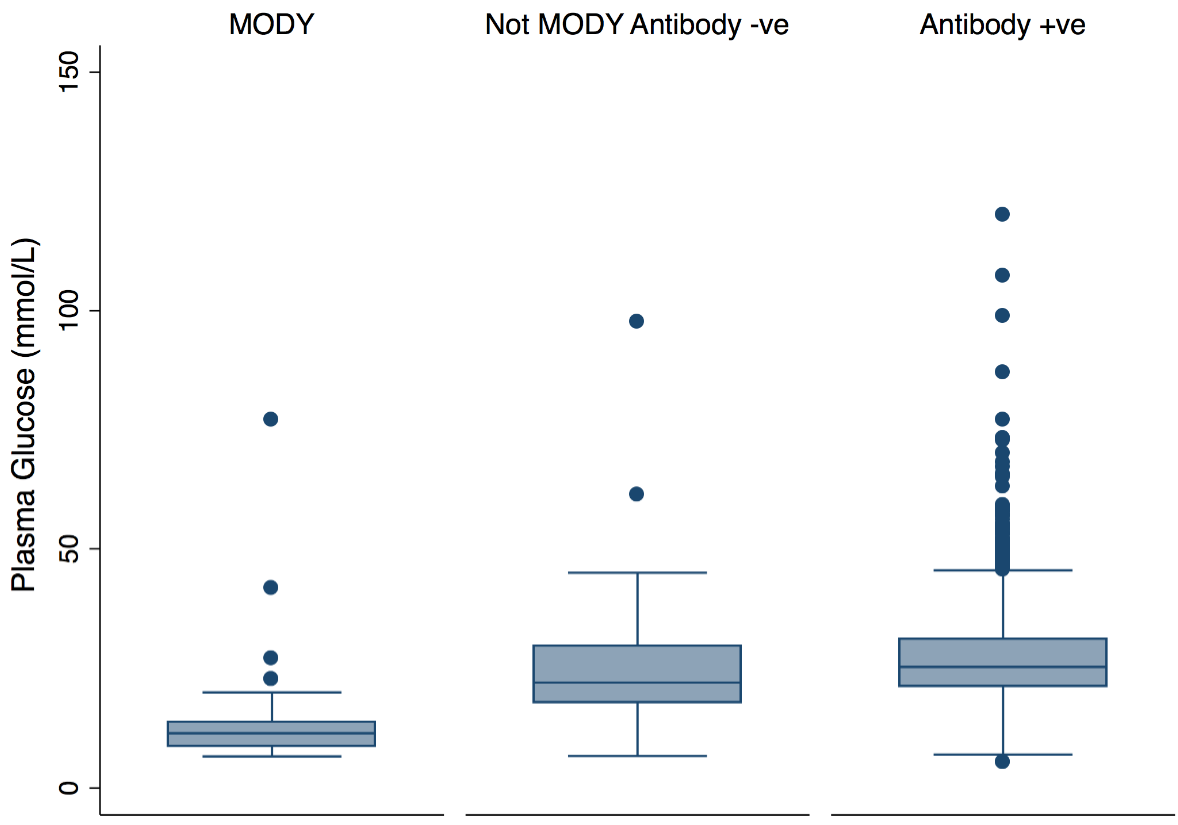
BDD2549	*HNF4A*	c.931C>T	p.(Arg311Cys)	Missense	0/121920	Pathogenic	Yorifuji (2012) Pediatr Diabetes 13, 26	No
BDD2655	*GCK*	c.869A>G	p.(Glu290Gly)	Missense	0/121651	Likely Pathogenic	Novel	No
BDD2788	*HNF1A*	c.1340C>T	p.(Pro447Leu)	Missense	0/15467	Pathogenic	Yamagata (1996) Nature 384, 455	No
BDD2801	*GCK*	c.1305dup	p.(Ile436fs)	Frameshift	0/120231	Pathogenic	Novel	No
BDD2844	*GCK*	c.1142T>C	p.(Met381Thr)	Missense	0/115895	Pathogenic	Osbak (2009) Hum Mutat 30, 1512	Yes
BDD2952	*GCK*	c.162T>G	p.(Ser54Arg)	Missense	3/123132	Likely Pathogenic	Novel (2 families in MODY DB)	Yes
BDD3040	*GCK*	c. 704T>C	p.(Met235Thr)	Missense	0/122988	Pathogenic	Gloyn (2003) Hum Mutat 22, 353	No
BDD3079	*GCK*	c.766G>A	p.(Glu256Lys)	Missense	1/123047	Pathogenic	Gidh-Jain (1993) Proc Natl Acad Sci U S A 90, 1932	Yes
BDD3233	*GCK*	c.571C>T	p.(Arg191Trp)	Missense	2/123005	Pathogenic	Ellard (2000) Diabetologia 43, 250	Yes
BDD3254	*GCK*	c. 704T>C	p.(Met235Thr)	Missense	0/122988	Pathogenic	Gloyn (2003) Hum Mutat 22, 353	Yes
BDD3490	*HNF4A*	c.47dup	p.(Tyr16Ter)	Nonsense	0/15464	Pathogenic	Novel	No
BDD3591	*HNF1A*	c.160C>T	p.(Arg54Ter)	Nonsense	0/116545	Pathogenic	Lambert (2003) Diabetes Care 26, 333	No
BDD3640	*GCK*	c.854G>A	p.(Gly285Asp)	Missense	0/120800	Likely Pathogenic	Novel (1 family in MODY DB)	No
BDD3791	*HNF1A*	c.25C>T	p.(Gln9Ter)	Nonsense	0/121466	Pathogenic	Novel	Yes
BDD3798	*HNF1A*	c.431T>C	p.(Leu144Pro)	Missense	0/123112	Pathogenic	Colclough (2013) Hum Mutat 34, 669	No
BDD3961	*HNF1A*	c.366C>G	p.(Tyr122Ter)	Nonsense	0/123098	Pathogenic	Novel	Yes
BDD3973	*GCK*	c.1167_1168dupCA	p.(Ile390fs)	Frameshift	0/112573	Pathogenic	Novel	Yes
BDD3980	*HNF1A*	c.872dup	p.(Gly292fs)	Frameshift	0/132382	Pathogenic	Yamagata (1996) Nature 384, 455	No

Supplementary table 5: Pathogenic MODY gene variants identified in the cohort

*Variants described according to Human Genome Variation Society (HGVS) nomenclature guidelines v15.11 and using the reference sequences NM_000162.3 for *GCK*, NM_000545.6 for *HNF1A*, NM_175914.4 for *HNF4A* and NM_000458.3 for *HNF1B*.

†GnomAD data is number of heterozygous individuals identified out of the total number of individuals with genotype quality (GQ) >= 20 and depth (DP) >= 10 over a 10bp window containing the variant. GnomAD data accessed on 03/11/2017.

‡Variants classified according to the ACGS and ACMG best practice guidelines for variant interpretation (http://www.acgs.uk.com/media/1092626/uk_practice_guidelines_for_variant_classification_2017.pdf and https://www.acmg.net/docs/Standards_Guidelines_for_the_Interpretation_of_Sequence_Variants.pdf).



Supplementary Figure 2 (REPLACE WITH NEW VERSION). Fasting plasma glucose in both known MODY patients (both GCK and HNF1A/HNF4A) and those without known MODY (antibody negative and antibody positive)..

	MODY		Not MODY		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P
Clinical features					
N	46		257		
Sex (% female)	46	54 (25)	257	38 (98)	0.05
Age at diagnosis (yrs)	46	12.1 (4.39)	257	12.0 (4.39)	0.84
Parental Diabetes (%)	46	63 (29)	257	27 (68)	$4x10^{-6}$
Polyuria (%)	38	34 (13)	215	79 (169)	$1x10^{-7}$
Polydipsia (%)	38	34 (13)	212	80 (169)	$6x10^{-8}$
Weight loss (%)	38	16 (6)	202	57 (115)	$2x10^{-6}$
BMI (SDS)	37	0.54 (1.21)	203	0.50 (1.92)	0.85
Acanthosis Nigricans (%)	37	0 (0.00)	202	9 (18)	0.08
Investigations- early~					
Plasma glucose (mmol/L)	41	11.7 (4.13)	209	23.7 (10.0)	$7x10^{-18}$
HbA1c (%)	46	7.0 (3.7)	211	10.2 (5.0)	$4x10^{-19}$
DKA (%)	46	0 (0)	257	5 (14)	0.14
Investigations – delayed#					
High risk HLA (%)	46	20 (9)	256	45 (116)	$1x10^{-3}$
C peptide (nmol/mol)	41	0.81 (0.63)	240	0.48 (0.99)	$1x10^{-4}$
C Peptide < 0.2 nmol/mol (%)	41	2 (1)	240	23 (54)	$1x10^{-3}$

Supplementary Table 6: Differences in autoantibody negative patients with or without MODY. This table consist of only the 303 antibody negative patients who were sequenced for MODY. Plasma glucose and C peptide results based on log10 transformation.
~ local testing with results 0-24 hours
# testing at reference laboratory, results within 14-90 days

	GCK MODY		Not known MODY		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P
Clinical features					
N	29		3887		
Sex (% female)	29	41 (12)	3887	45 (1730)	0.85
Age at diagnosis (yrs)	29	11.0 (4.8)	3887	10.1 (4.4)	0.25
Parental Diabetes (%)	29	62 (18)	3887	12 (471)	$4x10^{-10}$
Polyuria (%)	23	26 (6)	3570	95 (3379)	$4x10^{-17}$
Polydipsia (%)	23	26 (6)	3559	94 (3350)	$2x10^{-16}$
Weight loss (%)	23	13 (3)	3449	76 (2619)	$4x10^{-10}$
BMI (SDS)	22	0.50 (1.23)	3341	-0.36 (1.55)	$4x10^{-3}$
Acanthosis Nigricans (%)	22	0 (0)	3453	1 (44)	1
Investigations- early					
Plasma glucose (mmol/L)	24	9.9 (2.5)	3528	26.7 (9.0)	$7x10^{-16}$
HbA1c (%)	29	6.3 (2.5)	3495	10.7 (4.5)	$2x10^{-47}$
DKA (%)	29	0 (0)	3887	15 (601)	0.02
Investigations – delayed					
4 Antibody negative (%)	29	100 (29)	3887	11 (416)	$2x10^{-28}$
High risk HLA (%)	29	14 (4)	3830	70 (2684)	$6x10^{-10}$
C peptide (nmol/mol)	26	0.92 (0.62)	3555	0.34 (0.43)	$3x10^{-8}$
C Peptide < 0.2 nmol/mol (%)	26	4 (1)	3555	40 (1433)	$1x10^{-4}$

Supplementary table 7: GCK-MODY only vs. non-MODY. Plasma glucose and C peptide results based on log10 transformation.

	HNF1A/HNF4A MODY		Not known MODY		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P
Clinical features					
N	17		3887		
Sex (% female)	17	76 (13)	3887	45 (1730)	0.01
Age at diagnosis (yrs)	17	13.8 (3.2)	3887	10.1 (4.4)	$2x10^{-4}$
Parental Diabetes (%)	17	65 (11)	3887	12 (471)	$6x10^{-7}$
Polyuria (%)	15	47 (7)	3570	95 (3379)	$4x10^{-7}$
Polydipsia (%)	15	47 (7)	3559	94 (3350)	$7x10^{-7}$
Weight loss (%)	15	0.2 (3)	3449	76 (2619)	$9x10^{-6}$
BMI (SDS)	15	0.61 (1.2)	3341	-0.36 (1.6)	0.009
Acanthosis Nigricans (%)	15	0 (0)	3453	1 (44)	1
Investigations- early					
Plasma glucose (mmol/L)	17	14.2 (4.8)	3528	26.7 (9.0)	$7x10^{-7}$
HbA1c (%)	17	8.3 (2.4)	3495	10.7 (4.5)	$1x10^{-4}$
DKA (%)	17	0 (0)	3887	15 (601)	0.09
Investigations – delayed					
4 Antibody negative (%)	17	100 (17)	3887	11 (416)	$4x10^{-17}$
High risk HLA (%)	17	29 (5)	3830	70 (2684)	$7x10^{-4}$
C peptide (nmol/mol)	15	1.10 (0.63)	3555	0.34 (0.43)	$6x10^{-8}$
C Peptide < 0.2 nmol/mo) (%)	15	0 (0)	3555	40 (1433)	$7x10^{-4}$

Supplementary table 8: HNF1A/HNF4A MODY vs. Not known MODY.

Plasma glucose and C peptide results based on log10 transformation.

	GCK MODY		HNF1A/4A MODY		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P
Clinical features					
N	29		17		
Sex (% female)	29	41 (12)	17	76 (13)	0.03
Age at diagnosis (yrs)	29	11.0 (4.8)	17	13.8 (3.2)	0.04
Parental Diabetes (%)	29	62 (18)	17	65 (11)	1.00
Polyuria (%)	23	26 (6)	15	47 (7)	0.30
Polydipsia (%)	23	26 (6)	15	47 (7)	0.30
Weight loss (%)	23	13 (3)	15	20 (3)	0.66
BMI (SDS)	22	0.50 (1.23)	15	0.61 (1.2)	0.79
Acanthosis Nigricans (%)	22	0 (0)	15	0 (0)	1.00
Investigations- early					
Plasma glucose (mmol/L)	24	9.9 (2.5)	17	14.2 (4.8)	$6x10^{-4}$
HbA1c (%)	29	6.3 (2.5)	17	8.3 (2.4)	0.01
DKA (%)	29	0 (0)	17	0 (0)	1.00
Investigations – delayed					
4 Antibody negative (%)	29	100 (29)	17	100 (17)	1.00
High risk HLA (%)	29	14 (4)	17	29 (5)	0.26
C peptide (nmol/mol)	26	0.92 (0.62)	15	1.10 (0.63)	0.38
C Peptide < 0.2 (nmol/mol) (%)	26	4 (1)	15	0 (0)	1.00

Supplementary table 9: HNF1A/HNF4A MODY vs. GCK MODY. Plasma glucose and C peptide results based on log10 transformation.

Criteria	Total number autoantibody negative	Total number of individuals tested for MODY	Number (%) of the 46 MODY patients detected
All	380	257	46 (100%)
HbA1c<7.5% (58mmol/mol)	100 (26%)	73 (28%)	36 (78%)
Parent affected	120 (32%)	96 (37%)	29 (63%)
HbA1c < 7.5% (58mmol/mol) or parent affected	174 (46%)	131 (51%)	44 (96%)
HbA1c < 7.5% (58mmol/mol) and parent affected	46 (12%)	38 (15%)	21 (46%)

Supplementary Table 10: Effectiveness of HbA1c and family history on identifying MODY in autoantibody negative individuals.

*Note this table only includes the patients who had HbA1c at diagnosis data. This was available on 380 of 462 patients that were negative for all 4 antibodies and 257 of 303 patients that were negative for all 4 antibodies and were sequenced. Parental history was available on all subjects. The percentages shown in are of the patients in whom HbA1c was available

No	Study Number	Sex	Age	Initial diagnosis	Initial treatment	Clinical / Research Diagnostic Test	Current Treatment	HbA1c mmol/mol	Follow up years
1	BDD0024	M	5.4	Unclassified	None	Clinical *GCK*	None	42	8
2	BDD0068	M	11.1	Unclassified	None	Clinical *GCK*	None	47	7.5
3	BDD0377	M	15	susp MODY	Insulin	Clinical *GCK*	None	40	3
4	BDD0387	F	15.6	Type 2	Diet	Clinical *GCK*	None	47	9.5
5	BDD0422	M	16.1	susp MODY	Diet	Clinical *GCK*	None	51	3
6	BDD0647	M	16.4	susp MODY	Diet	Clinical *GCK*	None	38	4.5
7	BDD0664	F	16	susp MODY	Diet	Clinical *GCK*	None	42	2
8	BDD0665	F	13.7	susp MODY	Diet	Clinical *GCK*	None	34	2
9	BDD0717	M	6.5	susp MODY	Diet	Clinical *GCK*	None	51	4
10	BDD0809	F	16.2	susp MODY	Diet	Clinical *GCK*	None	42	2
11	BDD0840	F	5.8	susp MODY	Diet	Clinical *GCK*	None	47	10
12	BDD0842	F	6.6	Type 1	Diet	Clinical *GCK*	None	46	8
13	BDD0911	M	9.9	susp MODY	Insulin	Clinical *GCK*	None	46	10
14	BDD1002	F	17	Type 2	Metformin	Clinical *GCK*	None	51	1.5
15	BDD1107	M	7.8	susp MODY	Diet	Clinical *GCK*	None	47	8.5
16	BDD1337	M	5.5	susp MODY	Diet	Clinical *GCK*	None	41	9
17	BDD1433	F	14.4	susp MODY	Insulin	Clinical *GCK*	None	40	8

18	BDD1470	F	11.8	susp MODY	Diet	Clinical	*GCK*	None	42	6
19	BDD1515	M	16.9	susp MODY	None	Clinical	*GCK*	None	47	0
20	BDD1526	M	15	susp MODY	Diet	Clinical	*GCK*	None	48	0
21	BDD1557	M	13.9	Type 1	Insulin	Clinical	*GCK*	None	44	2
22	BDD1586	F	3.7	susp MODY	None	Clinical	*GCK*	None	44	6.5
23	BDD1930	M	4.9	Type 1	Insulin	Clinical	*GCK*	None	48	1
24	BDD2002	F	11.5	Type 1	Insulin	Clinical	*HNF1A*	Sulphonylurea+Insulin*	47	6.5
25	BDD2035	F	15.5	Type 2	Metformin	Clinical	*HNF1A*	Sulphonylurea	36	5
26	BDD2161	F	15.5	susp MODY	Insulin	Clinical	*HNF1A*	None	45	8
27	BDD2282	F	11	Type 2	Diet	Clinical	*HNF1A*	Sulphonylurea	59	9.5
28	BDD2362	M	13.9	susp MODY	Insulin	Clinical	*HNF1A*	None	40	11
29	BDD2549	M	7.9	susp MODY	None	Clinical	*HNF1A*	Sulphonylurea	68	10
30	BDD2655	F	15.1	Type 1	Insulin	Clinical	*HNF4A*	None	52	10
31	BDD2788	F	7.2	susp.MODY	Diet	Clinical	*HNF4A*	Sulphonylurea	55	10
32	BDD2801	F	11.8	susp MODY	Insulin	Clinical	*HNF4A*	Insulin**	72	4.5
33	BDD2844	F	15.4	Type 1	Insulin	Clinical	*HNF4A*	Insulin***	60	9
34	BDD2952	M	16.5	Type 1	Insulin	Clinical	*HNF4A*	Insulin****	67	7.5
35	BDD3040	M	11	unclass.	Diet	Research	*GCK*	None	46	7
36	BDD3079	M	15.5	Type 1	Diet	Research	*GCK*	None	36	2.5

37	BDD3233	F	2.8	Type 1	Insulin	Research *GCK*	None	46	7
38	BDD3254	F	4	Type 1	Insulin	Research *GCK*	None	53	7
39	BDD3490	M	13.2	Type 2	Metformin	Research *GCK*	None	43	5
40	BDD3591	M	9.7	susp MODY	None	Research *GCK*	None	42	11
41	BDD3640	F	10.8	susp MODY	None	Research *HNF1A*	None	49	6.5
42	BDD3791	F	15.8	Type 1	Insulin.	Research *HNF1A*	Sulphonylurea+Januvia	40	9
43	BDD3798	F	17.1	Type 2	Insulin+Metformin	Research *HNF1A*	Sulphonylurea	56	1
44	BDD3961	M	15.7	Type 1	Insulin	Research *HNF1A*	Sulphonylurea	69	2.5
45	BDD3973	F	16.9	Type 2	Diet	Research *HNF4A*	None	33	1
46	BDD3980	F	17	Type 1	Insulin	Research *HNF4A*	Sulphonylurea	50	6.5

Supplementary table 10: Initial and present treatment
Age = Initial diagnosis of diabetes, susp = suspected, HbA1c after molecular genetic diagnosis
*Patient chose to stay on insulin in addition to sulphonylurea, **reported worse control on sulphonylurea so recommended insulin, *** has not tried Sulphonylurea, ****tried glibenclamide, but chose to continue Insulin

	Antibody Negative Patients Clinically Tested		Antibody Negative Patients Not Clinically Tested		
Phenotype	N	mean (SD) or % (N)	N	mean (SD) or % (N)	P
Clinical features					
N	76		386		
Sex (% female)	76	50 (38)	386	39 (151)	0.10
Age at diagnosis (yrs)	76	11.7 (4.1)	386	11.6 (4.6)	0.75
Parental Diabetes (%)	76	54 (41)	386	21 (80)	$2x10^{-8}$
Polyuria (%)	64	50 (32)	311	82 (256)	$2x10^{-7}$
Polydipsia (%)	64	52 (33)	308	82 (253)	$9x10^{-7}$
Weight loss (%)	62	27 (17)	296	63 (185)	$5x10^{-7}$
BMI SDS	62	0.75 (1.38)	296	0.37 (1.91)	0.07
Acanthosis Nigricans (%)	61	3 (2)	293	9 (25)	0.19
Investigations - early					
Plasma glucose (mmol/L)	64	16.3 (8.5)	307	24.6 (10.7)	$1x10^{-9}$
HbA1c (%)	71	8.1 (4.8)	309	10.3 (5.0)	$1x10^{-8}$
DKA (%)	76	1 (1)	386	7 (26)	0.10
Investigations – delayed					
High risk HLA (%)	76	29 (22)	381	45 (185)	0.002
C peptide (nmol/mol)	68	0.89 (0.69)	344	0.85 (1.03)	0.02
C Peptide < 0.2 (nmol/mol)	68	12 (8)	344	26 (89)	0.012

Supplementary Table 11. Characteristics of the patients referred for genetic testing and antibody negative patients not referred for genetic testing by clinicians. Plasma glucose and C peptide results based on log10 transformation.