This is a pre-copyedited, author-produced version of an article accepted for publication in 'The Journal of Clinical Endocrinology & Metabolism' following peer review. The version of record [Katharina Warncke et al. Frequency and Characteristics of MODY 1 (HNF4A Mutation) and MODY 5 (HNF1B Mutation): Analysis From the DPV Database, The Journal of Clinical Endocrinology & Metabolism, Volume 104, Issue 3, March 2019, Pages 845–855] is available online at:

[https://academic.oup.com/jcem/article/104/3/845/5235618;](https://academic.oup.com/jcem/article/104/3/845/5235618)

<https://doi.org/10.1210/jc.2018-01696>

Frequency and characteristics of MODY 1 (*HNFB4A* mutation) and MODY 5 (*HNFB1B* mutation)

- Analysis from the DPV database

Short title: Features of MODY 1 and 5 in young people

Katharina Warncke, MD¹, Sebastian Kummer, MD², Klemens Raile, MD³, Jürgen Grulich-Henn, MD⁴, Joachim Woelfle, MD⁵, Elisabeth Steichen, MD⁶, Nicole Prinz^{7,8}, Reinhard W. Holl, MD^{7,8}

(KW and SK contributed equally to the article as shared first authors)

¹Department of Pediatrics, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, ²Department of General Pediatrics, Neonatology and Pediatric Cardiology, University Children's Hospital, Duesseldorf, Germany, ³Department of Paediatric Endocrinology and Diabetology, Charité, Berlin, Germany, ⁴Department of Pediatrics, University of Heidelberg, Heidelberg, Germany, ⁵Pediatric Endocrinology Division, Children's Hospital, University of Bonn, Bonn, Germany, ⁶Department of Pediatrics, Medical University of Innsbruck, Innsbruck, Austria, ⁷Institute of Epidemiology and Medical Biometry, Central Institute for Biomedical Technology, University of Ulm, Ulm, Germany, ⁸German Center for Diabetes Research (DZD), München-Neuherberg, Germany

Key words: diabetes, MODY 1, HNF4A, HNF1B, MODY 5, registry

Corresponding author and contact for reprint requests:

Katharina Warncke, MD, Department of Pediatrics, Klinikum rechts der Isar, Technische Universität München, Kölner Platz 1, 80804 Munich, Germany

Phone number: +49-89-3068-2350; Fax number: +49-89-3068-3849

E-Mail: katharina.warncke@mri.tum.de

Word count: 4231

Disclosure summary: The authors have nothing to disclose in connection with the work on this publication.

Abstract

Objective. To characterize initial presentation and clinical course of patients with rare HNF4A- and HNF1B-MODY in a large multinational registry.

Design, setting and participants. Within the DPV (Diabetes Patienten Verlaufsdocumentation) registry, 44 patients with HNF4A- and 35 patients with HNF1B-MODY were characterized and compared to type 1 diabetes (T1D) / type 2 diabetes (T2D) in patients < 20 years.

Main outcome measure. Clinical and laboratory parameters, therapy, and quality of metabolic control, extrapancreatic symptoms in HNF1B-MODY patients.

Results. Patients with both MODY types were significantly older than T1D patients at time of diagnosis (HNF4A 13.8 years and HNF1B 13.5 years, vs. T1D 8.8 years, $p < 0.0001$). Mean C-peptide at diagnosis was higher for HNF4A-MODY than T1D (1.8 ng/ml vs. 0.9 ng/ml, $p < 0.01$). 36.4% of patients with HNF4A-MODY and 65.7% of patients with HNF1B-MODY are treated with insulin, while 20.5% and 8.6% receive oral antidiabetics (OADs) only ($p < 0.05$ and $p < 0.01$ vs. T2D). At the most recent visit, HbA1c levels were lower in HNF4A and HNF1B-MODY compared to T1D (mean 6.5% and 6.1% vs 7.9% in T1D, $p < 0.0001$). In 40% of patients with HNF1B-MODY extrapancreatic symptoms were reported. Several clinical predictors previously described to differentiate between MODY and T1D or T2D could be revalidated by logistic regression analyses in this cohort.

Conclusion

The population-based DPV registry enabled us to precisely characterize phenotype and treatment in these two rare MODY-types. Although phenotype of HNF4A-and HNF1B-MODY shows distinct differences to T1D and T2D, 38% of patients are initially misclassified as T1D or T2D.

Précis

We investigated patients with the rare diabetes forms HNF4A- (n=44) and HNF1B-MODY (n=35) in the DPV registry and characterize phenotype and treatment of these two rare forms of MODY diabetes.

Introduction

Approximately 1-4% of people with diabetes suffer from monogenic diabetes [1-3]. Monogenic diabetes may be differentiated into severe/early onset forms diagnosed within the first 6 months of life (neonatal diabetes), Maturity Onset Diabetes of the Young (MODY), and rare syndromic diabetes.

With the recent identification of ABCC8-, KCNJ11-, and APPL1-MODY (MODY 12-14), 14 different genetic subtypes of MODY have been described [4-6].

The most common genetic causes are mutations in the glucokinase (*GCK*) gene (GCK-MODY, MODY 2) and mutations in the Hepatocyte nuclear factor 1A (*HNF1A*) gene (HNF1A-MODY, MODY 3) [7]. Other genetic etiologies such as HNF4A-MODY (MODY 1) and HNF1B-MODY (MODY 5) are less frequent, with *HNF4A* accounting for 10% of MODY cases in the UK [8] and *HNF1B* accounting for 1%. Both, HNF4A-MODY and HNF1B-MODY, have a first peak of diagnosis in adolescence or early adulthood [7]. In contrast to GCK-MODY, hyperglycemia of MODY forms caused by mutations in HNF-transcription factors aggravate over time, and most patients require treatment with oral antidiabetics or insulin over time [9]. Initially, patients are usually responsive to oral sulfonylurea analogues, but later insulin treatment may become necessary, when beta cell function progressively declines. Patients with HNF1B-MODY often have additional extrapancreatic features, such as renal and/or hepatic cysts, elevated liver enzymes, genitourinary malformations, mental retardation or eye defects [10]. *HNF1B* mutations may also present without diabetes, but only nonpancreatic/nonmetabolic features. Many patients with HNF1B-MODY have additional hepatic insulin resistance [10, 11]. Congenital hyperinsulinism can also be associated with mutations in the *HNF4A*-gene, and patients with HNF4A-MODY may have episodes of hypoglycemia in early childhood. Recent data show that predisposition to hypoglycemia may persist into early adulthood [12,13].

However, the phenotype of HNF4A- and HNF1B-MODY varies considerably, and large cohort studies defining the clinical spectrum are still scarce. Because of the rarity of patients with these MODY subtypes, data aggregation from multiple centers or large registries is necessary for representative surveys of clinical and metabolic characteristics of these entities. Previously, in the DPV cohort we evaluated the so far largest cohort of patients with HNF1A-MODY [14, 15], and pediatric or

adolescent onset of HNF4A-, GCK-, and HNF1A-MODY in comparison to type 2 diabetes [16]. In the US, the SEARCH study also reported on patients with HNF4A-, GCK-, and HNF1A-MODY in detail [7].

This manuscript will add an extensive overview of a large number of patients with HNF4A-MODY and HNF1B-MODY of the DPV registry, describing clinical characteristics at the time of diagnosis, epidemiologic factors, treatment, metabolic control and extrapancreatic clinical features of these rare MODY entities compared to type 1 diabetes (T1D) and type 2 diabetes (T2D).

Research Design and Methods

Study Design

This observational study was based on data from the DPV Diabetes Registry (“Diabetes Patienten Verlaufsdokumentation”). Anonymous, standardized, prospective data are transmitted by diabetes centers in Germany, Austria, Switzerland, and Luxemburg for central validation and analysis twice a year. For optimal data validity, inconsistent data are reported back to participating centers, corrected if necessary, and reentered into the database as previously described [17]. 439 centers (listed as supporting information [18]) contributed data to this analysis. Analysis of anonymized routine data within the DPV initiative was approved by the Ethics Committee of the Medical Faculty of the University of Ulm, Germany, and the institutional review boards at the participating centers. Data from 76,836 children and adolescents with diabetes <20 years of age were recorded in the database between 1995 and 2017 and included into this analysis. The primary focus was to characterize patients with HNF4A-MODY and HNF1B-MODY and compare these to patients with T1D and T2D. Clinical characteristics were compared at the time of diagnosis (age, family history of diabetes, typical symptoms, HbA1c, presence of beta-cell antibodies, C-peptide, initial treatment after diagnosis, insulin dose/kg). Furthermore, we analyzed clinical characteristics and metabolic control at the most recent visit (age, BMI-SDS, duration of disease, HbA1c, presence of beta-cell antibodies, C-peptide, treatment, insulin dose/kg, frequency of hypoglycemia, concomitant medication, presence of diabetes complications).

Diabetes classification / case definition

Diabetes subtype was classified and reported to the registry by local specialized diabetes experts according to ISPAD/DDG guidelines [19, 20]. For case definition of MODY patients we decided to restrict to cases that were defined by the local clinicians as genetically confirmed. If available, we additionally revalidated sequence information in the database. If there was no detailed genetic analysis result readily available in a patient's dataset, data were reinquired from the local treatment centers. However, as we were limited to reanalysis of genetic results entered into the DPV database by local clinicians, detailed segregation analysis, careful clinical profiling of family members etc. were not available in all cases. We considered truncating mutations or large deletions to be disease causing. Regarding missense or splice-site mutations, complete reassessment according to the most recent ACMG standards [21] or using the 2008 guidelines for the molecular genetic diagnosis of MODY [22] was impossible because segregation data are not reliably available in this retrospective database. Instead, reported variants were reviewed using the Ensembl Variant Effect Predictor VEP [23], accessed on Dec 21 2017, using database version GRCh38 (<https://www.ensembl.org/info/docs/tools/vep/index.html>). This tool combines analysis of different criteria for variant assessment (in silico prediction models [SIFT, PolyPhen2], species conservation, prediction of effects on splicing, population allele frequency [ESP, Exome Aggregation Consortium, and 1000 genomes], and previous reporting of mutation in the literature or in databases). When these different criteria for variant assessment consistently suggested pathogenicity for a specific variant, we judged the mutation to be "likely pathogenic". When different criteria for variant assessment were inconsistent, we concluded the variant to be of unclear significance. When the sequence information given in the DPV database diverged with the most recent reference sequence in the GRCh38 database, we concluded this variant to be "not assignable". Results of this reassessment are presented in supplementary Table 1 [24].

Change of diabetes subtype classification after diabetes diagnosis was identified by comparing the classification at the time of diagnosis with the classification at the most recent visit. If there was a change of classification during follow-up, we further analysed at what time after diabetes diagnosis the reclassification was documented.

Laboratory parameters

Total cholesterol, HDL- and LDL cholesterol and triglycerides were measured in local laboratories following the RILIBÄK requirements, i.e. the German Medical Association's current guideline (Richtlinie der Bundesärztekammer [RiliBÄK]), including internal and external quality control [25, 26]. Dyslipidemia was defined as elevated total cholesterol (> 200 mg/dl), LDL cholesterol (> 130 mg/dl), triglycerides (> 150 mg/dl), and/ or low levels of HDL cholesterol (< 35 mg/dl). HbA1c was used as an indicator of glycemic control. Levels were mathematically standardized to the Diabetes Control and Complications Trial (DCCT) reference range of 4.05–6.05% with the MOM (multiple of mean) transformation to correct for different laboratory methods among the study centers [27]. Quality of glycemic control was assessed according to ISPAD criteria (optimal: HbA1c < 58 mmol/mol [$< 7.5\%$]; suboptimal: HbA1c 58–75 mmol/mol [$7.5\text{--}9.0\%$]; poor: HbA1c > 75 mmol/mol [$> 9.0\%$]) [28]. Pancreatic β -cell antibodies (Abs) were classified as “positive” if at least two antibodies were present among islet cell Abs, insulinoma antigen 2 Abs, insulin autoantibody (IAA), zinc transporter antibody (ZnT8), and glutamic acid decarboxylase Abs; IAA measurements were only included at onset of diabetes. Pancreatic β -cell Abs and C-peptide were measured in local laboratories.

Demographics and non-laboratory variables

BMI, Height, weight, blood pressure

All clinical and anthropometric measurements were performed in the participating study centers according to local protocols. Results were reported to the registry and analyzed using contemporary national reference data for height, weight and BMI [29, 30]. We used recent reference values and calculated BMI-SDS values using the LMS method [31]. Blood pressure levels were measured in a relaxed, sitting position at the upper arm with proper cuff size using sphygmomanometer or semi-automated Dinamap (Critikon, Tampa, FL, USA) [32]. Systolic/ diastolic BP SDS-values were calculated according to KIGGS and current guidelines [33, 34], BP values were defined as increased if they were above the 95th percentile. Severe hypoglycemia was defined according to the American Diabetes Association Workgroup on Hypoglycemia [35] as an event requiring assistance of another person to actively administer carbohydrates, glucagon or intravenous glucose. Antihyperglycemic

therapy was categorized as follows: (i) non-pharmacological treatment/lifestyle only (physical activity, dietary advice), (ii) OADs only, (iii) insulin therapy with/without OADs. Insulin therapy was categorized as conventional insulin therapy (CT) (1–3 daily injection time points), intensified insulin therapy (ICT; >3 daily injection time points) or insulin pump therapy.

Extrapancreatic symptoms

Extrapancreatic symptoms in patients with HNF1B-MODY were analyzed by searching for the following key words in the datasets of all patients: exocrine pancreatic failure, renal cysts, renal hypoplasia, single kidney, renal malformation, nephropathy, horseshoe kidney, polycystic kidney disease, renal insufficiency, pancreatic hypoplasia, pancreatic fibrosis, liver cysts, cholestasis, hypotension, hypospadias, hyperuricaemia, elevation of transaminases, hepatopathy, coloboma, cataract, autism, developmental delay.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). A case was only included in the analysis if at least the date of birth, date of diabetes diagnosis, gender and type of diabetes were known. If data for another variable were not available in individual cases, the case was not considered for the analysis of that variable. Data are presented as percentage or median and interquartile range (IQR). For group comparisons of continuous variables, non-parametrical statistical tests (Kruskal-Wallis) were used. Categorical variables were compared by the chi-squared test. False Discovery Rate was used to correct for multiple comparisons. Logistic regression models were performed to adjust for age, gender, and BMI-SDS. Parameters were calculated using the mmpl estimation method (based on maximum likelihood, mean of the random effects, pseudo-likelihood). The between-within method was used to calculate degrees of freedom. Adjusted mean values were based on observed marginal frequencies. To identify clinically useful discriminatory potential of different variables to distinguish between MODY subtypes and T1D or T2D odds ratios were estimated using logistic regression models. For all statistical analyses, two-sided p-values < 0.05 were considered statistically significant.

Results

In the DPV-Wiss database, anthropometric and metabolic data of 76,836 patients with diabetes and a current age < 20 years were available for analysis. A total of 1,047 patients (1.4%) are documented with the diagnosis “MODY” in the database, although in 118 patients the diagnosis could not be clearly confirmed by molecular genetic testing. Excluding these 118 we further analyzed the remaining 929 patients, representing 1.2% of all patients under the age of 20 years. Further subclassification based on the information provided by the local clinicians revealed the following subgroups for further analysis in this work: (1) patients with HNF4A-MODY (MODY 1; n=44), (2) patients with Glukokinase (*GCK*)-MODY (MODY 2; n = 609), (3) patients with HNF1A-MODY (MODY 3; n=230), and (4) patients with HNF1B-MODY (MODY 5; n=35). These were compared to patients with T1D (n=74,087), and (6) patients with T2D (n=1,667). Patients with other forms of diabetes (diabetes in cystic fibrosis, neonatal diabetes or other rare forms of diabetes) were excluded. For this analysis, we focused on patients with HNF4A-diabetes or HNF1B-diabetes compared to patients with T1D or T2D.

Revalidation of genetic classification

Recalls and validation of disease-causing mutations

If a diagnosis of monogenic HNF1B and HNF4A-MODY was given without entering the specific mutation, we performed a second recall to the local clinicians, asking for entering the disease-causing mutation that led to the respective MODY-classification. Finally, any genetic variant information was available for 26 of 44 patients with HNF4A-MODY, and 16 of 35 patients with HNF1B-MODY (supplementary Table 1). Limitations were caused by the long period over that clinical and genetic information had been entered into DPV locally. Sometimes, local clinicians were unable to obtain consent for transfer of sequence information from some patients. In some patients, genetic diagnosis was made several years ago and no contact could be made to patients or to clinicians that originally entered the genetic diagnosis. Some entries could not be clearly assigned to one, specific base exchange, e.g. when older nomenclatures or reference sequences had been used, potential typing errors occurred or unspecific and incomplete/inconsistent amino acid changes had been entered. Therefore, in

13/44 cases sequencing results could not be clearly verified because of any data incongruences (e.g. nomenclature, reference sequence). In summary, for HNF1B-MODY, most patients had large 17q21 gene deletions that were clearly assumed to be disease causing. For HNF4A-MODY, 14 of 44 patients had rare variants that were clearly classified as disease-causing, whereas 12 variants were not assignable and one was of unclear significance (supplementary Table 1).

Characteristics of patients with HNF4A-MODY (Table 1)

At the time of diabetes diagnosis, patients with HNF4A-MODY were older than patients with T1D (median 13.8 years vs. 8.8 years for T1D, $p < 0.00001$; n.s. vs. T2D). The majority of HNF4A-patients was female (72.7% vs. 47% in T1D; $p < 0.01$). At the most recent visit, the BMI-SDS was higher in HNF4A-MODY patients compared to T1D patients ($p < 0.01$), but lower than in T2D-patients ($p < 0.00001$). Height-SDS was lower patients with HNF4A-MODY compared to patients with T1D ($p < 0.05$); this difference was most pronounced in males with HNF4A-MODY ($p < 0.005$ vs. males with T1D) or T2D ($p < 0.01$). Positive pancreatic β -cell Abs were reported in only one patient with HNF4A-MODY. Dyslipidemia was present in 10% of patients with HNF4A-MODY, whereas 42.2% of patients with T1D and 68.8% of patients with T2D were affected by dyslipidemia ($p < 0.01$ vs. T1D and $p < 0.001$ vs. T2D after adjusting for age, sex and BMI-SDS). The percentage of patients with elevated blood pressure values was not significantly different after adjusting for age, sex and BMI-SDS.

Treatment of patients with HNF4A-MODY

At the most recent visit, 36.4% ($n=16$) of all patients with HNF4A-MODY were treated with insulin (vs. 99.8% in T1D, $p < 0.00001$; n.s. vs. T2D). Of these, five are treated with CT and 11 are treated with ICT. Insulin pump therapy is currently not used in any patient with HNF4A-MODY. 43.2% of patients ($n=19$) are currently treated by lifestyle therapy only, while nine patients take OADs only and four patients combine insulin therapy with OADs (Table 1 and Figure 1). Of all 13 patients taking OADs, five take metformin, six sulfonylurea and two take glinides.

At the most recent visit, the median HbA1c level was significantly lower in patients with HNF4A-MODY compared to patients with T1D ($p < 0.0001$; n.s. to T2D). There was no significant difference

regarding the rate of severe hypoglycemia between patients with HNF4A-MODY patients vs. patients with T1D or T2D.

Characteristics of patients with HNF1B-MODY (Table 1)

Age at diabetes diagnosis was higher in patients with HNF1B-MODY compared to T1D (median 13.5 years vs. 8.8 years for T1D, $p < 0.00001$; n.s. versus T2D). The BMI-SDS was lower in HNF1B-MODY patients compared to T2D patients ($p < 0.00001$). There were no significant differences regarding height-SDS between patients with HNF1B-MODY, T1D and T2D. The percentage of patients with dyslipidemia was similar in patients with HNF1B-MODY and T2D. Elevated blood pressure values were found in 23.3% of patients with HNF1B-MODY vs. 10.7% with T1D ($p < 0.05$ after adjusting for age, sex and BMI-SDS; n.s. vs. patients with T2D). Positive pancreatic β -cell Abs were found in only one patient with HNF1B-MODY. Extrapaneatic symptoms were reported in 40% of patients ($n=14$) with HNF1B-MODY. In detail, kidney disease was reported in nine patients, structural pancreas anomaly or any other concomitant disease in three patients, respectively. One patient suffered from combined kidney and pancreas disease.

Treatment of patients with HNF1B-MODY

65.7% ($n=23$) of all patients with HNF1B-MODY are currently treated with insulin (vs. 99.8% of T1D patients, $p < 0.00001$ and 30% of T2D patients, $p < 0.0001$), the majority of these (78.3%; $n=18$) with ICT. Insulin pump therapy is used in one patient. 22.9% of patients ($n=8$) are currently treated by lifestyle therapy only, while four patients take OADs only and three patients combine insulin therapy with OADs. Of all seven patients taking OADs, five take metformin and two take glinides. At the most recent visit, the median HbA1c level was significantly lower in patients with HNF1B-MODY compared to patients with T1D (6.1%, i.e. 43.2 mmol/mol vs. 7.9%, i.e. 62.8 mmol/mol in T1D; $p < 0.00001$; n.s. vs. T2D).

Clinical and laboratory characteristics of patients with HNF4A- and HNF1B-diabetes at time of diabetes diagnosis (Table 2)

Compared to patients with T1D, patients with HNF4A-MODY had lower mean HbA1c-levels (6.9%, i.e. 51.9 mmol/mol vs. 9.5%, i.e. 80.33 mmol/mol in T1D; $p<0.001$), were less frequently treated with insulin (48.1% vs. 93.9% in T1D; $p<0.00001$), and, if insulin-treated, with a lower insulin dosage (0.3 vs. 0.6 IE/kg/d; $p<0.01$) at diabetes onset. Mean C-peptide at diagnosis was significantly higher compared to patients with T1D (1.8 ± 0.4 ng/ml vs. 0.9 ng/ml in T1D; $p<0.01$). Slight differences between HNF4A-MODY- and T2D-patients were not statistically significant.

At diabetes onset, median HbA1c levels were lower in patients with HNF1B-MODY vs. patients with T1D (7.0%, i.e. 53.0 mmol/mol; $p<0.01$) and they were less frequently treated with insulin (71.4% vs. 93.9%; $p<0.0001$). In contrast, compared to T2D patients, insulin therapy was more frequently used at onset in HNF1B-MODY (29.8% of T2D patients; $p<0.001$). There was a trend towards higher mean c-peptide at diagnosis for HNF1B-MODY (1.5 ± 0.5 ng/dl vs. 0.9 ± 0.5 ng/dl in T1D), but this trend did not reach statistical significance.

Discriminatory potential of different variables between MODY subtypes and T1D or T2D

Many of the previously established markers [36] could be revalidated in our cohort to contribute significantly to the odds of having a specific MODY type instead of T1D or T2D (Table 3).

Interestingly, there were more variables showing significant differences comparing either MODY subtype to T1D, as opposed to the comparison between MODY and T2D, when there were less variables showing distinct predictive potential. Furthermore, at the time of diagnosis (Table 3) there were more variables significantly different than at the most recent visit (data on supplementary Table 2, [37]), pointing towards a better discriminating potential at the time of diagnosis than later in the follow-up).

Reclassification during follow-up

33 of 44 patients (75%) with HNF4A-MODY and 16 of 35 patients (45.7%) with HNF1B-MODY were classified as “MODY diabetes” already at the time of diabetes diagnosis. However, 11 patients with HNF4A-MODY were reclassified from T1D ($n=8$) or T2D ($n=3$) to MODY. This reclassification was documented after an average of 1.3 years in patients with HNF4A-MODY. 19 patients with

HNF1B-MODY were initially classified as T1D (n=14) or T2D (n=5). Reclassification as MODY was documented after an average time of 1.6 years in patients with HNF1B-MODY.

Discussion

Within the DPV registry, we identified 44 patients with HNF4A- and 35 patients with HNF1B-MODY. With respect to the rarity of the diseases, this represents a large number of cases allowing a comprehensive characterization with a focus on clinical data, treatment and metabolic control.

Mutations in the transcription factor *HNF4A* results in reduced insulin secretory response and overall, HNF4A-MODY is a very rare disease accounting for a minority of cases with MODY diabetes.

Glucose intolerance and diabetes usually starts during adolescence or early adulthood [38]. Regarding diabetes therapy, identical recommendations apply as for the 10 times more frequent HNF1A-MODY (MODY3): Patients with HNF4A-MODY can initially be treated by diet, but the majority will need pharmacological treatment such as sulfonylureas, as they show progressive hyperglycemia over time [38].

To our knowledge, this is one of the largest series of children and adolescents with HNF4A- and HNF1B-MODY in literature. As expected, HNF4A-MODY was diagnosed in adolescence at a median age of 13.8 years. C-peptide levels were significantly higher than in T1D, which reveals a less impaired insulin secretion at the time of diagnosis. The percentage of patients with dyslipidemia was lowest among HNF4A-MODY patients. However, it is unclear if this is related to the molecular pathomechanism, or just reflects the fact that insulin deficiency of HNF4A-MODY patients was less advanced compared to HNF1B-MODY or T1D at the most recent visit (insulin treatment required in only 36.4% vs. 65.4% in HNF1B-MODY or 99.8% in T1D). Furthermore, 75% of HNF4A-MODY patients were classified as “MODY diabetes” already at the time of diabetes diagnosis. The percentage of patients with a short duration of symptoms was higher and the HbA1c levels were lower compared to patients with T1D onset. These findings might be due to the fact that the mechanism of disease is different than in T1D and insulin secretion deteriorates more slowly than in T1D. However, our results may also indicate that the molecular diagnosis of “MODY” may have been previously known – e.g. if

other family members were affected - and diabetes was therefore recognized at a very early stage of disease.

Comparable to patients with neonatal diabetes due to *ABCC8* or *KCNJ11* mutations [39, 40], patients with *HNF4A*-MODY often respond to sulfonylureas [38]. Still, a relatively high proportion of our cohort is treated with insulin. However, the percentage of patients with insulin therapy decreased from diabetes onset (48.1%) to the most recent visit (36.4%) which indicates that at least some patients were switched from insulin to OAD, presumably after correct classification as MODY diabetes was made. The high proportion of patients on insulin therapy might reflect the fact that some diabetologists may not be aware of the possibility to treat patients with *HNF4A*-MODY with OADs/lifestyle therapy, maybe due to the rarity of this disease.

Interestingly, the height SDS was lowest in patients with *HNF4A*-mutations; this finding was most pronounced in males. This finding supports the hypothesis that *HNF4A* mediates the sex-dependent effect of growth hormone on liver gene expression [41], which may have an effect on height-SDS. For *HNF1B*-MODY, extrapancreatic symptoms were reported in 40% of patients, primarily renal symptoms. This percentage is lower compared to recent adult data showing renal disease in the majority of adult *HNF1B*-MODY patients [42]. This finding underlines the hypothesis that the diagnosis of diabetes may precede the occurrence of kidney disease and periodic screening of kidney abnormalities is indicated in patients with *HNF1B*-MODY. However, some aspects are prone to underreporting, as extrapancreatic findings might be present and potentially even known to the local clinician, but still not being reported into the registry. Compared to *HNF1A*-MODY, the percentage of patients with *HNF1B*-MODY being treated with insulin is significantly higher, suggesting that pathogenic *HNF1B* mutations lead to earlier/more severe impairment of insulin secretion compared to *HNF4A* mutations. This is in line with adult data, also showing that the majority of *HNF1B*-MODY patients require insulin treatment [42].

62% of patients were classified as MODY already at the time of diagnosis. The remaining patients were reclassified to MODY from initially being diagnosed with T1D or T2D after a median of 1.3 (*HNF4A*-MODY) and 1.6 years (*HNF1B*-MODY). Interestingly, 27% of these patients were initially classified as T2D, and 73% were reclassified from T1D to MODY, not reflecting the prevalence

proportions of T1D/T2D. This remarkably underlines that especially the clinical diagnosis of early onset T2D has to be carefully revalidated over time, as prevalence of MODY is higher than T2D in diabetics before age 20 in Europe. As the DPV group showed earlier, about 10% of patients with early onset of T2D were reclassified [43]. These findings strongly underline the need for clinical indicators raising the suspicion of a monogenic diabetes form instead of T1D or T2D. As we analysed only two rare subtypes of MODY, we cannot derive a specific set of indicators aiming to differentiate between MODY in general and T1D or T2D from our data. However, such has been previously published [34], and we could in general confirm their discriminatory potential in our cohort. Interestingly, the discriminatory potential seems to be more robust for differentiation between MODY and T1D than between MODY and T2D, and it was generally better at the time of diabetes diagnosis compared to analysis at the most recent visit documented in the database. Thus, it is useful to consider MODY right at the time of diabetes diagnosis, or – if addressing this issue later - at least strongly reconsider the clinical phenotype at the time of diagnosis to look for indicators pointing towards MODY.

Taking together all MODY subtypes, currently 1.4% of patients in the DPV registry aged < 20 years have MODY-diabetes. The proportion of patients with MODY is similar to that found in a Norwegian registry [1]. However, studies offering systematic and extensive molecular testing for all suspicious/potential MODY cases, higher percentages of MODY are reported [2, 7, 44].

Our study is limited by being based on a retrospective diabetes registry, with the contribution of data from many diabetes centers. Therefore, it is not possible to guarantee that all patients with the phenotype “MODY-diabetes” (such as young patients without T1D-associated autoantibodies and detectable C-peptide) are tested for MODY-diabetes and “MODY-diabetes” might be underreported in our registry. Furthermore, not all centers agreed to offer genetic data to the registry due to aspects of data protection, and available registry data are frequently insufficient to clearly revalidate genetic diagnoses according to the most recent and accurate standards. This aspect is reflected by the proportion of patients in our cohort in which we were not able to revalidate the genetic diagnosis based on requested information. However, all clinicians who contribute data are qualified according to national/international standards. With a number of 44 patients with HNF4A-MODY and 35 patients with HNF1B-MODY that were reported to be *genetically* diagnosed, this is one of the largest series of

data regarding these rare two forms of MODY-diabetes in young patients. In contrast to other studies, our registry collects clinical and laboratory data, as well as aspects on diabetes therapy, which enables us to provide a comprehensive characterization of these rare forms of diabetes.

Acknowledgements

The authors acknowledge all 439 participating centers in Germany and Austria (see supporting information). Special thanks to A. Hungele and R. Ranz for DPV documentation software support, and K. Fink and E. Bollow for DPV data management support (all clinical data manager, University of Ulm).

Funding. Sources of funding: Supported by the Federal Ministry of Education and Research within the German Center for Diabetes Research (DZD; grant number 82DZD0017G); the Innovative Medicines Initiative 2 Joint Undertaking (INNODIA; grant number 115797) supported by the European Union's Horizon 2020 research and innovation program and "EFPIA", "JDRF" and "The Leona M. and Harry B. Helmsley Charitable Trust"; the German Diabetes Association (DDG), and the German Robert Koch Institute.

Author Contributions. R.W.H. conceived the study and designed it. K.W., S.K., K.R., J.G.-H., J.W., and E.S. participated in data collection. N.P. contributed to genetic classification and data analysis. R.W.H. performed data analysis. K.W. and S.K. wrote the draft manuscript, which was reviewed and revised by all authors. R.W.H. coordinates the DPV initiative. All authors read and approved the final manuscript.

Duality of Interest. The authors have no conflicts of interest relevant to this article to disclose.

References

- 1) Irgens HU, Molnes J, Johansson BB, Ringdal M, Skrivarhaug T, Undlien DE, Søvik O, Joner G, Molven A, Njølstad PR. Prevalence of monogenic diabetes in the population-based Norwegian Childhood Diabetes Registry. *Diabetologia*. 2013;56(7):1512-1519.
- 2) Fendler W, Borowiec M, Baranowska-Jazwiecka A, Szadkowska A, Skala-Zamorowska E, Deja G, Jarosz-Chobot P, Techmanska I, Bautembach-Minkowska J, Mysliwiec M, Zmyslowska A, Pietrzak I, Malecki MT, Mlynarski W. Prevalence of monogenic diabetes amongst Polish children after a nationwide genetic screening campaign. *Diabetologia*. 2012;55(10):2631-2635.
- 3) Shepherd M, Shields B, Hammersley S, Hudson M, McDonald TJ, Colclough K, Oram RA, Knight B, Hyde C, Cox J, Mallam K, Moudiotis C, Smith R, Fraser B, Robertson S, Greene S, Ellard S, Pearson ER, Hattersley AT; UNITED Team. 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):1879-1888.
- 4) Ovsyannikova AK, Rymar OD, Shakhtshneider EV, Klimontov VV, Koroleva EA, Myakina NE, Voevoda MI. ABCC8-Related Maturity-Onset Diabetes of the Young (MODY12): Clinical Features and Treatment Perspective. *Diabetes Ther*. 2016;7(3):591-600.
- 5) Yorifuji T, Nagashima K, Kurokawa K, Kawai M, Oishi M, Akazawa Y, Hosokawa M, Yamada Y, Inagaki N, Nakahata T. The C42R mutation in the Kir6.2 (KCNJ11) gene as a cause of transient neonatal diabetes, childhood diabetes, or later-onset, apparently type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2005;90(6):3174-3178.
- 6) Vaxillaire M, Froguel P. Monogenic diabetes: Implementation of translational genomic research towards precision medicine. *J Diabetes*. 2016;8(6):782-795.
- 7) Pihoker C, Gilliam LD, Ellard S, Dabelea D, Davis C, Dolan LM, Greenbaum CJ, Imperatore G, Lawrence JM, Marcovina SM, Mayer-Davis E, Rodriguez BL, Steck

- AK, Williams DE, Hattersley AT. 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):4055–4062.
- 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):2504-2508.
 - 9) Pearson ER, Starkey BJ, Powell RJ, Gribble FM, Clark PM, Hattersley AT. Genetic cause of hyperglycaemia and response to treatment in diabetes. *Lancet.* 2003;362(9392):1275-1281.
 - 10) Raile K, Klopocki E, Holder M, Wessel T, Galler A, Deiss D, Müller D, Riebel T, Horn D, Maringa M, Weber J, Ullmann R, Grüters A. Expanded clinical spectrum in hepatocyte nuclear factor 1b-maturity-onset diabetes of the young. *J Clin Endocrinol Metab.* 2009;94(7):2658-2664.
 - 11) 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):200-213.
 - 12) Glaser B. Familial hyperinsulinism. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Ledbetter N, Mefford HC, Smith RJH, Stephens K, editors. GeneReviews®. Seattle (WA): University of Washington, Seattle; 1993-2018; 2003 Aug 19 [updated 2013 Jan 24].
 - 13) Bacon S, Kyithar MP, Condron EM, Vizzard N, Burke M, Byrne MM. Prolonged episodes of hypoglycaemia in HNF4A-MODY mutation carriers with IGT. Evidence of persistent hyperinsulinism into early adulthood. *Acta Diabetol.* 2016;53(6):965-972.
 - 14) Awa WL, Thon A, Raile K, Grulich-Henn J, Meissner T, Schober E, Holl RW; DPV-Wiss. Study Group. Genetic and clinical characteristics of patients with HNF1A gene variations from the German-Austrian DPV database. *Eur J Endocrinol.* 2011;164(4):513-520.

- 15) Raile K, Schober E, Konrad K, Thon A, Grulich-Henn J, Meissner T, Wölflé J, Scheuing N, Holl RW; DPV Initiative the German BMBF Competence Network Diabetes Mellitus. Treatment of young patients with HNF1A mutations (HNF1A-MODY). *Diabet Med.* 2015;32(4):526-530.
- 16) Schober E, Rami B, Grabert M, Thon A, Kapellen T, Reinehr T, Holl RW; DPV-Wiss Initiative of the German Working Group for Paediatric Diabetology. Phenotypical aspects of maturity-onset diabetes of the young (MODY diabetes) in comparison with Type 2 diabetes mellitus (T2DM) in children and adolescents: experience from a large multicenter database. *Diabet Med.* 2009;26(5):466-473.
- 17) Rosenbauer J, Dost A, Karges B, Hungele A, Stahl A, Bächle C, Gerstl EM, Kastendieck C, Hofer SE, Holl RW; DPV Initiative and the German BMBF Competence Network Diabetes Mellitus. Improved metabolic control in children and adolescents with type 1 diabetes: a trend analysis using prospective multicenter data from Germany and Austria. *Diabetes Care.* 2012;35:80-86.
- 18) Materials uploaded to Dryad.
- 19) Craig ME, Jefferies C, Dabelea D, Balde N, Seth A, Donaghue KC; International Society for Pediatric and Adolescent Diabetes. ISPAD Clinical Practice Consensus Guidelines 2014. Definition, epidemiology, and classification of diabetes in children and adolescents. *Pediatr Diabetes.* 2014;15 Suppl 20:4-17.
- 20) Neu A, Beyer P, Bürger-Büsing J, Danne T, Etspüler J, Heidtmann B, Holl RW, Karges B, Kiess W, Knerr I, Kordonouri O, Lange K, Lepler R, Marg W, Näke A, Petersen M, Podeswik A, Stachow R, von Sengbusch S, Wagner V, Ziegler R, Holterhus PM; German Diabetes Association. Diagnosis, therapy and control of diabetes mellitus in children and adolescents. *Exp Clin Endocrinol Diabetes.* 2014;122(7):425-434.
- 21) Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. 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;17(5):405-424.
- 22) Ellard S, Bellanné-Chantelot C, Hattersley AT; European Molecular Genetics Quality Network (EMQN) MODY group. Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. *Diabetologia.* 2008;51(4):546-553.
 - 23) Yates A, Akanni W, Amode MR, Barrell D, Billis K, Carvalho-Silva D, Cummins C, Clapham P, Fitzgerald S, Gil L, Girón CG, Gordon L, Hourlier T, Hunt SE, Janacek SH, Johnson N, Juettemann T, Keenan S, Lavidas I, Martin FJ, Maurel T, McLaren W, Murphy DN, Nag R, Nuhn M, Parker A, Patricio M, Pignatelli M, Rahtz M, Riat HS, Sheppard D, Taylor K, Thormann A, Vullo A, Wilder SP, Zadissa A, Birney E, Harrow J, Muffato M, Perry E, Ruffier M, Spudich G, Trevanion SJ, Cunningham F, Aken BL, Zerbino DR, Flicek P. Ensembl 2016. *Nucleic Acids Res.* 2016;44(D1):D710-D716.
 - 24) Materials uploaded to Dryad.
 - 25) Schlebusch H. Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen (RiliBÄK). In: Luppä B, Schlebusch H (eds.). POCT 2008. Patientennahe Labordiagnostik. 1. Aufl. Springer, Berlin und Heidelberg 2008.
 - 26) Schwab KO, Doerfer J, Scheidt-Nave C, Kurth BM, Hungele A, Scheuing N, Krebs A, Dost A, Rohrer TR, Schober E, Holl RW; German/Austrian Diabetes Documentation and Quality Management System (DPV) and the German Health Interview and Examination Survey for Children and Adolescents (KiGGS). Algorithm-based cholesterol monitoring in children with type 1 diabetes. *J Pediatr.* 2014;164:1079-1084.
 - 27) Schütt M, Kern W, Krause U, Busch P, Dapp A, Grziwotz R, Mayer I, Rosenbauer J, Wagner C, Zimmermann A, Kerner W, Holl RW; DPV Initiative. Is the frequency of self-monitoring of blood glucose related to long-term metabolic control? Multicenter

- analysis including 24,500 patients from 191 centers in Germany and Austria. *Exp Clin Endocrinol Diabetes*. 2006;114:384-388.
- 28) Rewers MJ, Pillay K, de Beaufort C, Craig ME, Hanas R, Acerini CL, Maahs DM; International Society for Pediatric and Adolescent Diabetes. ISPAD Clinical Practice Consensus Guidelines 2014. Assessment and monitoring of glycemic control in children and adolescents with diabetes. *Pediatr Diabetes*. 2014;15 Suppl 20:102-114.
 - 29) Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiss HC, Hesse V, von Hippel A, Jaeger U, Johnsen D, Korte W, Menner K, Mueller G, Mueller JM, Niemann-Pilatus A, Remer T, Schaefer F, Wittchen HU, Zabransky S, Zellner K, Ziegler A, Hebebrand J. Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschrift Kinderheilkunde*. 2001;149:807.
 - 30) Kromeyer-Hauschild K, Moss A, Wabitsch M. Referenzwerte für den Body-Mass-Index für Kinder, Jugendliche und Erwachsene in Deutschland. Anpassung der AGA-BMI-Referenz im Altersbereich von 15 bis 18 Jahren. *Adipositas*. 2015;9:123-127.
 - 31) Cole TJ, Green PJ: Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med*. 1992;11(10):1305-1319.
 - 32) Knerr I, Dost A, Lepler R, Raile K, Schober E, Rascher W, Holl RW; Diabetes Data Acquisition System for Prospective Surveillance (DPV) Scientific Initiative Germany and Austria. Tracking and prediction of arterial blood pressure from childhood to young adulthood in 868 patients with type 1 diabetes: a multicenter longitudinal survey in Germany and Austria. *Diabetes Care*. 2008;31:726-727.
 - 33) Neuhauser HK, Thamm M, Ellert U, Hense HW, Rosario AS. Blood pressure percentiles by age and height from nonoverweight children and adolescents in Germany. *Pediatrics*. 2011;127(4):e978-e988.
 - 34) de Man SA, André JL, Bachmann H, Grobbee DE, Ibsen KK, Laaser U, Lippert P, Hofman A. Blood pressure in childhood: pooled findings of six European studies. *J Hypertens*. 1991;9(2):109-114.

- 35) American Diabetes Association, Workgroup on Hypoglycemia. Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia. *Diabetes Care*. 2005;28:1245-1249.
- 36) 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;55(5):1265-1272.
- 37) Materials uploaded to Dryad.
- 38) Rubio-Cabezas O, Hattersley AT, Njølstad PR, Mlynarski W, Ellard S, White N, Chi DV, Craig ME; International Society for Pediatric and Adolescent Diabetes. ISPAD Clinical Practice Consensus Guidelines 2014. The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*. 2014;15 Suppl 20:47-64.
- 39) Bowman P, Sulen Å, Barbetti F, Beltrand J, Svalastoga P, Codner E, Tessmann EH, Juliusson PB, Skrivarhaug T, Pearson ER, Flanagan SE, Babiker T, Thomas NJ, Shepherd MH, Ellard S, Klimes I, Szopa M, Polak M, Iafusco D, Hattersley AT, Njølstad PR; Neonatal Diabetes International Collaborative Group. Effectiveness and safety of long-term treatment with sulfonylureas in patients with neonatal diabetes due to KCNJ11 mutations: an international cohort study. *Lancet Diabetes Endocrinol*. 2018;6(8):637-646.
- 40) Rafiq M, Flanagan SE, Patch AM, Shields BM, Ellard S, Hattersley AT, Neonatal Diabetes International Collaborative Group. Effective treatment with oral sulfonylureas in patients with diabetes due to sulfonylurea receptor 1 (SUR1) mutations. *Diabetes Care*. 2008;31(2):204–209.
- 41) Holloway MG, Laz EV, Waxman DJ. Codependence of growth hormone-responsive, sexually dimorphic hepatic gene expression on signal transducer and activator of transcription 5b and hepatic nuclear factor 4alpha. *Mol Endocrinol*. 2006;20(3):647-660.

- 42) Dubois-Laforgue D, Cornu E, Saint-Martin C, Coste J, Bellanné-Chantelot C, Timsit J; Monogenic Diabetes Study Group of the Société Francophone du Diabète. Diabetes, Associated Clinical Spectrum, Long-term Prognosis, and Genotype/Phenotype Correlations in 201 Adult Patients With Hepatocyte Nuclear Factor 1B (HNF1B) Molecular Defects. *Diabetes Care*. 2017;40(11):1436-1443.
- 43) Awa WL, Schober E, Wiegand S, Herwig J, Meissner T, Schmidt F, Molz E, Holl RW. Reclassification of diabetes type in pediatric patients initially classified as type 2 diabetes mellitus: 15 years follow-up using routine data from the German/Austrian DPV database. *Diabetes Res Clin Pract*. 2011;94(3):463-467.
- 44) Mozzillo E, Salzano G, Barbetti F, Maffei C, Lombardo F, Franzese A, Delvecchio M, Marigliano M. Survey on etiological diagnosis of diabetes in 1244 Italian diabetic children and adolescents: impact of access to genetic testing. *Diabetes Res Clin Pract*. 2015;107(3):e15-e18.

Table 1. Clinical and laboratory data and treatment modalities at most recent visit.

	HNF4A n=44	HNF1B n=35	GCK n=609	HNF1A N=230	T1D n=74,087	T2D n=1,667	p ¹	p ²	p ³	p ⁴
Current age	14.7 (12.1-15.8)	13.8 (12.4-16.3)	11.1 (7.7-14.0)	13.9 (11.3-15.8)	10.8 (7.1-13.8)	14.9 (13.2-16.7)	<0.00001	n.s.	<0.00001	n.s.
Age at diabetes onset	13.8 (11.5-15.3)	13.5 (11.2-15.7)	10.1 (6.3-13.3)	12.5 (10.1-14.6)	8.8 (5.2-12.1)	14.2 (12.3-16.0)	<0.00001	n.s.	<0.00001	n.s.
Male sex (%)	27.3	45.7	53.9	33.0	53.0	38.6	<0.01	n.s.	n.s.	n.s.
BMI-SDS	1.1 (-0.04-1.8)	0.6 (-0.3-1.4)	0.1 (-0.6-0.8)	0.8 (-0.01-1.5)	0.3 (-0.4-0.9)	2.6 (2.0-3.0)	<0.01	<0.00001	n.s.	<0.00001
Height SDS	-0.4 (-0.9-0.3)	-0.3 (-1.2-0.5)	-0.2 (-0.8-0.5)	-0.1 (-0.8-0.7)	0.1 (-0.6-0.8)	0.1 (-0.7-0.9)	<0.05	n.s.	n.s.	n.s.
Height SDS in male patients	-0.9 (-1.2 - 0.1)	-0.3 (-1.6 - 0.2)	-0.1 (-0.7-0.6)	0.1 (-0.6-0.9)	0.1 (-0.5-0.8)	0.2 (-0.7-1.1)	<0.005	<0.01	n.s.	n.s.
% of patients with dyslipidemia	10.0	65.0	23.3	45.9	42.2	68.8	<0.01	<0.001	n.s.	n.s.
% of patients with elevated blood pressure	7.7	23.3	8.3	13.3	10.7	33.2	n.s.	<0.01	n.s.	n.s.

Treatment										
OAD only (%)	20.5	8.6	5.6	18.3	0	38.8	<0.00001	<0.05	<0.00001	<0.01
% with insulin therapy	36.4	65.7	8.5	48.3	99.8	30.0	<0.00001	n.s.	<0.00001	<0.0001
Insulin + OAD (%)	9.1	8.6	0	9.6	0.3	14.3	<0.00001	n.s.	<0.00001	n.s.
Lifestyle only (%)	43.2	22.9	85.9	33.5	0.2	31.2	<0.00001	n.s.	<0.00001	n.s.
Metabolic control										
HbA1c (%)	6.5 (6.0-8.0)	6.1 (5.8-7.1)	6.2 (5.9-6.5)	6.4 (5.7-7.6)	7.9 (6.9-9.3)	6.6 (5.8-8.5)	<0.0001	n.s.	<0.00001	n.s.
HbA1c (mmol/mol)	47.5 (42.1-63.9)	43.2 (39.9-54.1)	44.3 (41.0-47.5)	46.5 (38.8-59.6)	62.8 (51.9-78.1)	48.6 (39.9-69.4)	<0.0001	n.s.	<0.00001	n.s.
Severe hypoglycemia/ 100 patient years	3.4	0	0.2	2.1	24.9	10.1	n.s.	n.s.	n.s.	n.s.

Values are expressed as medians with interquartile ranges [25th–75th percentile] or as percentage of cases. T1D, type 1 diabetes; T2D, type 2 diabetes; HbA1c, hemoglobin A1c;

GCK, glucokinase. p¹ compares HNF4A to T1D, p² HNF4A to T2D, p³ HNF1B to T1D and p⁴ HNF1B to T2D; the stated p-values are unadjusted.

Table 2. Clinical and laboratory characteristics at diagnosis; data at diabetes onset were not available for all patients (available n in brackets).

	HNF4A (n=27)	HNF1B (n=21)	GCK MODY (n=329)	HNF1A (n=139)	T1D (n=49,299)	T2D (n=1,053)	p ¹	p ²	p ³	p ⁴
HbA1c (%) at diagnosis	6.9 (6.4-9.0)	7.0 (6.0-12.6)	6.3 (6.0-6.5)	6.8 (6.0-8.0)	9.5 (7.7-11.6)	7.1 (6.0-9.5)	<0.001	n.s.	<0.01	n.s.
HbA1c (mmol/mol)	51.9 (46.4-74.9)	53.0 (42.1-114.2)	45.4 (42.1-47.5)	50.8 (42.1-63.9)	80.3 (60.7-103.3)	54.1 (42.1-80.3)	<0.001	n.s.	<0.01	n.s.
C-peptide at diagnosis (ng/ml)	1.8 ± 0.4	1.5 ± 0.5	1.8 ± 0.2	2.7 ± 0.7	0.9 ± 0.02	4.4 ± 0.5	<0.01	n.s.	n.s.	n.s.
% of patients with insulin therapy	48.1	71.4	11.6	48.2	93.9	29.8	<0.00001	n.s.	<0.0001	<0.001
Insulin dosage (IE/kg/d)	0.3 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.5 ± 0	0.6 ± 0	0.5 ± 0.0	<0.001	n.s.	n.s.	n.s.

Values are expressed as medians with interquartile ranges (25th–75th percentile), as means ± standard error, or as percentage of cases. T1D, type 1 diabetes; T2D, type 2

diabetes; HbA1c, hemoglobin A1c; GCK, glucokinase. p¹ compares HNF4A to T1D, p² HNF4A to T2D, p³ HNF1B to T1D and p⁴ HNF1B to T2D; the stated p-values are unadjusted.

Table 3. Logistic regression analysis, given as odds ratio (OR) in favor of the respective MODY subtype vs. T1D/T2D for each variable at diabetes diagnosis.

	HNF4A vs. T1D		HNF4A vs. T2D		HNF1B vs. T1D		HNF1B vs. T2D	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age (OR for each year increase)	1.3 (1.2-1.5)	<0.0001	0.9 (0.8-1.1)	n.s.	1.4 (1.2-1.6)	<0.0001	1.0 (0.8-1.2)	n.s.
Male sex (vs. female)	0.4 (0.1-0.9)	<0.05	0.6 (0.2-1.7)	n.s.	0.7 (0.3-1.8)	n.s.	1.1 (0.4-3.0)	n.s.
HbA1c (OR for each percent increase)	0.7 (0.6-0.9)	<0.01	0.8 (0.6-1.0)	n.s.	0.8 (0.7-0.97)	<0.05	0.9 (0.7-1.1)	n.s.
BMI-SDS (OR for each SDS increase)	1.6 (1.2-2.2)	<0.01	0.4 (0.3-0.6)	<0.0001	1.6 (1.2-2.2)	<0.01	0.4 (0.3-0.6)	<0.0001
Insulin treatment (yes vs. no)	0.04 (0.02-0.1)	<0.0001	5.1 (1.6-16.1)	<0.01	0.1 (0.0-0.3)	<0.0001	8.6 (2.4-30.8)	<0.001

Figure 1. Percentage of patients with HNF4A-MODY/ HNF1B-MODY, T1D or T2D and insulin therapy, or exclusive lifestyle/ OAD therapy. Insulin was most frequently used in patients with T1D ($p<0.00001$ vs. HNF4A- and HNF1B-MODY), and exclusive lifestyle therapy or OADs only were more frequently used in HNF4A-MODY and HNF1B-MODY ($p<0.00001$ vs. T1D).