Precision medicine for monogenic diabetes: from a survey to the development of a next-generation diagnostic panel

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Summary

Monogenic diabetes (MD) accounts for 1–2% of all diabetes cases. Because of its wide phenotypic spectrum, MD is often misdiagnosed as type 1 or type 2 diabetes. While clinical and biochemical parameters can suggest MD, a definitive diagnosis requires genetic analysis. We conducted a survey among clinicians specialising in diabetes to document the cases with MD. Of 74 clinically suspected MD patients, 46% had undergone genetic analysis, which was mostly conducted using Sanger’s classical sequencing method. The most common recorded mutations were located in the \textit{GCK} gene, followed by the mitochondrial genome (m.3243A>G mutation) and the \textit{HNF1B} and \textit{HNF1A} genes. The remaining 54% of patients only had a clinical diagnosis, mostly because genetic analysis was not easily accessible. Here, we designed a new diagnostic panel of 42 genes that was developed based on the survey. The panel was validated with an independent sample of nine known MD patients. Our survey confirms the need for a comprehensive analytical instrument for the diagnosis of MD, which will be met by the proposed panel. The diagnosis of MD is crucial because it dictates treatment and may improve metabolic control and reduce long-term complications as proposed by precision medicine.

Key words: next-generation sequencing, pancreas, personalised medicine, diabetes, neonatal diabetes, precision medicine, genetic diabetes, autoimmune, type 1 diabetes, type 2 diabetes, monogenic diabetes

Introduction

Monogenic diabetes (MD), in contrast to polygenic type 1 and type 2 diabetes, is due to a single gene defect and has traditionally been referred to as maturity onset diabetes of the young (MODY). MODY has been defined as an autosomal dominant, non-insulin dependent form of diabetes that occurs before the age of 25 due to an underlying defect in beta cells [1]. At least 13 genes have now been discovered to cause MODY [2]. In neonatal diabetes, an additional form of MD, the genetic cause is now identified in over 85% of cases and involves over 20 genes [3]. Given that the clinical features of MD are often non-specific, more than 80% of MD cases remain undiagnosed or are misdiagnosed as type 1 or type 2 diabetes [4, 5]. Precision medicine through genetic analyses leads to the correct diabetes classification, which permits tailoring of treatment regimens and optimisation of health outcomes. To estimate the need for such a tool, we conducted a survey among centres and private practices specialising in diabetes treatment to document how many patients had been diagnosed with MD, either clinically or genetically. Total diabetes prevalence in Switzerland is assessed at 6.5% [6]. Overall, MD is estimated to account for 1–2% of all diabetes cases. In countries with a widespread screening policy, such as the UK, the minimal prevalence of the most frequent MODY subtypes was 108 cases per 1 million inhabitants [4]. In the paediatric diabetes population, the prevalence of MD was 2.5% in the UK [7] and 1.1% in the Norwegian childhood diabetes registry [8]. We developed and validated a diagnostic tool using next-generation sequencing (NGS) technology to identify the genetic defect underlying suspected cases efficiently. This technology was not yet widely available.

Our study intends to improve the tools for clinicians to make a precise diagnosis of MD since treatment options may depend on the specific gene defect, ranging from diet-only treatment to oral anti-diabetic agents and the need for insulin replacement [9].

Methods

Questionnaire

We conducted a survey by sending a questionnaire to the members of the Swiss Society of Endocrinology and Diabetes (SGED/SSED) (n = 219) and to the members of the Swiss Society of Paediatric Endocrinology and Diabetes (SPGED/SSED) (n = 39) to collect anonymous data on diabetic subjects with either a clinical suspicion of MD or genetically confirmed MD. Subjects with mito-
Mitochondrial diabetes were also included in our study. We requested the following data in the questionnaire: age at diagnosis; method of diagnosis of MD (clinical or genetic analysis); family history of diabetes; ethnic origin; birth weight; weight loss and body mass index (BMI) at diagnosis; glycosylated haemoglobin (HbA1c), glycaemia, C-peptide, ketosis and treatment at diagnosis; autoimmune anti-diabetes antibodies; lipid profile; liver enzymes; other health problems or congenital malformations; age, BMI, HbA1c, microvascular or macrovascular complications; and treatment at the last medical visit.

The data of the participants were then entered into the MODY probability calculator to establish a risk score for MD and to compare the results with the available genetic analyses [10].

No approval from the ethics committee was needed for this study. Informed consent was obtained from each patient for the use of anonymised DNA for the development of the NGS panel.

Diagnostic tool: Haloplex technology

This custom assay, designed based on liquid-phase capture (Haloplex HS, Agilent, Santa Clara, CA, USA), allows for the trapping of all coding regions of the 42 genes (CCDS exon reference sequence) and splicing regions (±10 nucleotides apart from each intron-exon junction). The sequence of the selected DNA fragments from each patient is then resolved with a next-generation sequencer (PGM, Ion Torrent, ThermoFisher, USA; or NextSEQ500, Illumina, La Jolla, USA). The raw sequencing data obtained are analysed through a series of processes that include the alignment of the readings (BWA), variant calling (SAM- TOOL), and variant annotation (ANNOVAR). The last two steps of the process are automated using a locally developed bioinformatic pipeline (Pyltine). The pipeline tracks all existing information to classify the variant (e.g., the frequency in the general population, report in a mutation database, and bioinformatic pathogenicity prediction (including PolyPhen2, SIFT, MutationTester). Each variant that is ultimately considered pathogenic is then validated by another sequencing method (Sanger) and reported to a physician. We did not incorporate mitochondrial genes in this panel; the request for mitochondrial DNA analysis will have to be done separately.

Results

Survey

We received a total of 74 answers corresponding to 74 subjects from hospitals, medical centres and private practices in different regions. The geographical locations are depicted in figure S1 (appendix 1). Among the subjects, there were 44 females and 30 males with a median age at diabetes onset of 24.5 years (range 0.03–49). Two subjects had neonatal diabetes (<6 months of age), 8 had childhood diabetes (≥6 months to <11 years), and 14 had adolescent diabetes (≥11 to <18 years). The remaining 50 patients were adults, and 19 (25.7%) were 35 years or older at diabetes onset. The clinical characteristics of the patient cohort are depicted in table 1.

Mode of diabetes diagnosis

Diabetes was an incidental finding in 50% of the participants and was diagnosed during a familial screening in 26.2% of the subjects and after weight loss in 23.8% of the subjects. In 32 subjects (43.2%), the discovery mode was not reported (table 1). Forty-seven subjects (63.5%) had been tested for at least diabetes autoantibodies, and three subjects were positive (tables 2a and 2b). As an indicator of the persistence of endogenous insulin secretion, we used C-peptide levels (>200 pmol/l), which were analysed in 21 subjects (28.4%). Most values were measured fasting (in 86%), but no patient had a glucose level <4.7 mmol/l (range 4.7–16.2 mmol/l). All but one subject presented with levels >200 pmol/l. Five subjects presented with ketosis at the time of diagnosis.

Genetic results

Overall, 34 participants (46%) of the survey already had a genetically confirmed diagnosis of MD, all obtained by

| Table 1: Clinical characteristics of the subjects at diabetes onset. |
|---------------------------------------------------------------|
| **Number of patients** | 74 |
| **Age at onset years (range)** | 24.5 (0.03–49) |
| **HbA1c mmol/mol (range) / % (range)** | 63.9 ± 33.7 / 8.0 ± 3.08 |
| **Subjects (n)** | Fraction (%) |
| Female gender | 44/74 | 59.5 |
| Age at onset years (range) | | |
| Neonatal onset <6 months | 2 | 2.7 |
| Childhood onset ≥6 months to <11 years | 8 | 10.8 |
| Adolescent onset ≥11 to <18 years | 14 | 18.9 |
| Young adult onset ≥18 to <35 years | 31 | 41.9 |
| Adult onset ≥35 years | 19 | 25.7 |
| Subjects with ketones | 5/42 | 11.9 |
| Family screening | 11/42 | 26.2 |
| Weight loss | 10/42 | 23.8 |
| Incidental diagnosis | 21/42 | 50 |
| Clinical diagnosis of MD | 40/74 | 54 |
| Genetic analysis | 34/74 | 46 |

HbA1c = glycosylated haemoglobin; MD = monogenic diabetes Data are presented as the mean (range) or ± SD. Number of counts: n. Data on ketones, family screening, weight loss and incidental diagnosis were available for only 42 patients.
classical Sanger sequencing. GCK gene mutations were the most frequent, followed by mutations in the HNF1A and HNF1B, HNF4A, KCNJ11 and PDX1 genes. 8 of the 34 subjects (23.5%) suffered from mitochondrial diabetes (fig. 1). The clinical characteristics of the different diabetes subtypes are listed in tables 2a and 2b. In the other 40 subjects (54%), the diagnosis was based only on clinical features and biochemical criteria, without genetic analysis (table 2b).

### Diabetes treatment
Among the patients with identified genetic mutations, 29.4% were treated with oral hypoglycaemic agents only (OHA), 38.2% received insulin only, 8.8% were prescribed a combination therapy with insulin plus OHA, 2.9% had insulin plus exocrine enzymes, and 20.6% were on a diet only (tables 2a and 2b). In the GCK diabetes group, two subjects were treated with insulin injections, and five were treated with OHA. Insulin therapy was prescribed in two of the subjects with HNF1A diabetes and in all but one subject with HNF1B diabetes. One patient with a KCNJ11 mutation was put on insulin at diagnosis and was switched to oral treatment with sulfonylurea after obtaining the genetic results. The patient with pancreatic agenesis caused by a homozygous PDX1 mutation required exocrine pancreatic enzymes in addition to insulin [11].

All but one subject with mitochondrial diabetes were treated with insulin, and 2 patients received additional treatment with OHA (table 2b).

Overall, 35.9% of the clinically diagnosed subjects were managed with insulin only, 35.9% were treated with OHA, and 2.6% received a combination of both. 25.6% were on the OHA. The mean BMI was 25.1 (10–48) kg/m². 25.6% of the subjects were with diabetes for >10 years, and 20.6% of the subjects were with diabetes for ≥15 years.

#### Table 2a: Subject characteristics according to genotype.

| Genotype | Age at diabetes diagnosis (years) | HbA1c at diagnosis (mmol/mol) (%) | BMI at last visit (kg/m²) | Family Hx (%) (n) | Auto-antibodies positive/ tested | Treatment at last visit | Complications |
|----------|----------------------------------|----------------------------------|--------------------------|------------------|-------------------------------|-------------------------|---------------|
| GCK      | 25.6 (3–45)                      | 46.9 ± 4.0                       | 22.7 ± 3.46 (15–24.7)    | 85.7 (12/14)     | 1/10                          | 5 OHA (3 Glucides,1 Met, 1 SU+Met) | 1 N (*)        |
| HNF1B    | 15.6 (10–22)                     | 57.3 ± 17.6                      | 21.15 ± 4.4 (18–27.7)    | 60 (3/5)         | 0/4                           | 1 OHA (SU) 4 insulin       | 1 N           |
| HNF1A    | 12.3 (10–14)                     | 46.5 ± 6.4                       | 23.6 ± 7.7 (16–32)       | 100 (3/3)        | 0/2                           | 1 OHA (Met) 1 Insulin 1 OHA (SU) | 1 R           |
| HNF4A    | 24                               | 48                               | 18.28                    | 100 (1/1)        | 1/1                           | 1 OHA (SU+Met)           | 0             |
| KCNJ11   | 8.1 (0.03–16)                    | NA                               | 22.9 ± 0.7 (22.2–23.6)   | 100 (2/2)        | 0/1                           | 1 OHA (SU) 1 Insulin       | 1 R + N + P    |
| PDX1     | 0.03                             | NA                               | 14                       | 100 (1/1)        | 0/1                           | Insulin + Exocrine enzymes | 0             |

HbA1c = glycosylated haemoglobin; BMI = body mass index; NA = not available; Mt D = mitochondrial diabetes; MD = clinically diagnosed monogenic diabetes; Family Hx = family history; OHA = oral hypoglycaemic agent; Met = metformin; SU = Sulfonylurea; Pio = pioglitazone; DPP-4 = dipeptidyl peptidase-4. Microvascular complications: R = retinopathy; N = nephropathy; P = polyneuropathy. Data are presented as the mean (range) ± SD. Since information for diabetic antibodies and for the family history was missing in several patients, we reported the data obtained for the total number of patients. In the GCK group, 1 subject had positive IA-2 autoantibodies. In the HNF4A group, 1 subject had positive GAD autoantibodies.

#### Table 2b: Subject characteristics for mitochondrial diabetes and clinical diagnosis of MD.

| Genotype | Age at diabetes diagnosis (years) | HbA1c at diagnosis (mmol/mol) (%) | BMI at last visit (kg/m²) | Family Hx (%) (n) | Auto-antibodies positive/ tested | Treatment at last visit | Complications |
|----------|----------------------------------|----------------------------------|--------------------------|------------------|-------------------------------|-------------------------|---------------|
| Mt D     | 36.6 (29–34)                     | 92.4 ± 50.5 10.6 ± 4.6           | 22.36 (16–24.8)          | 100 (8/8)        | 0/5                           | 1 OHA (SU+ Pio+ DPP-4) 5 Insulin 2 OHA (Met) + Insulin | 2 N P     |
| Clinical MD | 25.1 (10–48)                  | 65.2 ± 34.7 8.1 ± 3.2            | 23.01 ± 4.2 (15.5–31)    | 73.7 (28/38)     | 1/23                          | 14 OHA 14 Insulin 1 OHA + Insulin 10 Diet only 1 NA | 2 R P |

NA = not available; Mt D = mitochondrial diabetes; clinical MD = clinically diagnosed monogenic diabetes; Family Hx = family history; OHA = oral hypoglycaemic agent; Met = metformin; SU = sulfonylurea; Pio = pioglitazone; DPP-4 = dipeptidyl peptidase-4. Microvascular complications: R = retinopathy; N = nephropathy; P = polyneuropathy. Data are presented as the mean (range) ± SD. Since information for diabetic antibodies and for the family history was missing in several patients, we reported the data obtained for the total number of patients. One subject with a clinical diagnosis had positive GAD and IA-2 antibodies.
Diabetes complications
Microvascular complications were found in 15.4% (4 out of 26) of MODY patients, in 62.5% (5 out of 8 subjects) of patients with mitochondrial diabetes, and in 22.5% (9 out of 40) with a clinical suspicion of MD (tables 2a and 2b).

The likelihood of MD estimated by the MODY probability calculator
There is then the question of which selection criteria should be used for the genetic analysis. Recently, an algorithm called the MODY probability calculator has been proposed to estimate the likelihood of MD for subjects with diabetes onset before the age of 35 years [10]. The authors recommend the use of a positive predictive value of >20% as an indicator for MODY testing (http://www.diabetesgenes.org/content/mody-probability-calculator). All the genetically confirmed MODY subjects in this study showed a positive predictive value of >20%, except for 7 subjects in whom we did not perform the calculation because diabetes was diagnosed after 35 years of age (table 3).

To further characterise the clinically diagnosed MD group, we used the MODY probability calculator to determine the positive predictive value for MD in each subject, except for 5 subjects whose detailed information was missing (table S1 in appendix 1). In the clinically diagnosed group younger than 35 years of age, 73.3% (22/30) had a positive predictive value >20%, and 26.7% (8/30) a positive predictive value <20%. In our survey, 19 subjects (25.7%) developed diabetes at a later age. We therefore reported the results according to the age of diabetes onset to include all subjects. For the patients of 35 years or older at diagnosis, an upper age limit of 35 years was put into the MODY probability calculator to obtain the positive predictive value. In the older age group, 40% (2/5) had a positive predictive value >20%, and 60% (3/5) a positive predictive value <20% (table S1).

Mitochondrial diabetes was diagnosed after 35 years of age in 50% of the patients. For the mitochondrial diabetes group, diagnosed younger than 35 years of age, 50% (2/4) had a positive predictive value >20%, but of those diagnosed at 35 years or older, only 25% (1/4) had a positive predictive value >20% (table S1).

Developing a novel NGS panel to diagnose MD
To date, more than 40 genes that cause MD have been identified, and every year new genes are discovered [12]. Our goal was to create an innovative diagnostic instrument that takes advantage of the power of NGS to offer a rapid and comprehensive analysis of patients with a suspected form of MD. In our first gene panel, we included 42 genes that have been reported to cause diabetes (table 4). We included all known MODY genes at that time, genes that cause neonatal diabetes, and genes that cause monogenic autoimmune and syndromic diabetes. Known enhancer regions and introns associated with diabetes were also included in the panel [3, 13, 14].

To offer the most robust sequencing tool in a clinical setting, we favoured the approach of custom-designed NGS restricted to 42 genes rather than performing whole exome sequencing using a commercial catalogue design that often harbours uncaptured regions. We optimised the efficiency of the probe design with the help of the capture kit provider to ultimately obtain a set of probes that covered 99.89% of the targets (table 4).

The validation of this assay was done in a blind manner as proposed by the national guidelines. Independent DNA samples, which were previously analysed by Sanger sequencing, were tested from nine patients with defects in six different genes, and all anomalies were properly identified. A mutation in the KCNJ11 gene required post-hoc analysis, followed by an adjustment of the pipeline for deletions. No mutations were identified in the negative control DNA. All 465 genomic regions corresponding to the 42 genes were thoroughly covered at an average of 500-fold in our setting (nine patient samples were loaded on a 316 chip, Ion Torrent PGM). In addition to identifying point variants (missense and nonsense), deletions or insertions/duplications of a few nucleotides, this assay also allows for the detection of larger deletions of one or even several exons as shown in four patients with large deletions (table 5).

Another advantage of this approach is that the mutation search can be restricted to a subset of genes based on the clinical phenotype. If no mutation is identified, the investigation can be extended to more or even all 42 genes, since the raw sequencing data are securely conserved and can be reopened at any time for analysis.

We now propose the following selection criteria for genetic screening depicted in figure 2. Too strict criteria can miss a large proportion of people with MD [34–37]. The counts in figure 2 reflect the number of patients from the survey with a genetically confirmed diagnosis of MD. The proposed flowchart is feasible with the documented cases of monogenic diabetes in this survey. An advanced genetic analysis will also contribute to the elucidation of even more complex forms of diabetes due to digenic or oligogenic defects. The knowledge gained will lead to novel drug development for specific mutations, further refining precision medicine in diabetes.

Table 3: Positive predictive values calculated by the MODY probability calculator in subjects with a genetic diagnosis of MODY.

| Gene defect | PP >20% | PP ≤20% |
|-------------|---------|---------|
| GCK         | 7       | 0       |
| HNF1B       | 5       | 0       |
| HNF1A       | 3       | 0       |
| HNF4A       | 1       | 0       |
| KCNJ11      | 1       | 0       |
| Total       | 17      | 0       |

PP = positive predictive value. Data are number of patients (n). The probability for MODY was calculated for each patient with genetically confirmed MODY diabetes using the MODY probability calculator. The cut-off value for the positive predictive of 20% was used as an indicator for genetic testing (http://www.diabetesgenes.org/content/mody-probability-calculator). The calculator was developed for subjects with diabetes onset <35 years of age, which is why the results are depicted according to the age of diabetes onset.
Custom-designed gene panel with 42 diabetes genes and known enhancer regions and introns with coverage of 99.89% of the targets. Chromosome: Chr. 1.

Table 4: Gene panel for the diagnosis of monogenic diabetes.

| Gene name   | RefSeq accession number (GenBank) | Chromosome location | Theoretical coverage (%) | Missing nucleotides (n) | Non-covered (%) |
|-------------|-----------------------------------|---------------------|--------------------------|------------------------|-----------------|
| HNF4A       | NM_000457                         | Chr.20              | 100                      |                        |                 |
| GCK         | NM_000162                         | Chr.7               | 100                      |                        |                 |
| HNF1A       | NM_000545                         | Chr.12              | 100                      |                        |                 |
| PDX1        | NM_000209                         | Chr.13              | 100                      |                        |                 |
| HNF1B       | NM_000458                         | Chr.17              | 100                      |                        |                 |
| NEUROD1     | NM_002500                         | Chr.2               | 100                      |                        |                 |
| KLF11       | NM_003597                         | Chr.2               | 100                      |                        |                 |
| CEL         | NM_001807                         | Chr.9               | 100                      |                        |                 |
| PAX4        | NM_006193                         | Chr.7               | 100                      |                        |                 |
| INS         | NM_000207                         | Chr.11              | 100                      |                        |                 |
| BLK         | NM_001715                         | Chr.8               | 100                      |                        |                 |
| ABCC8       | NM_000352                         | Chr.11              | 100                      |                        |                 |
| KCNJ11      | NM_000525                         | Chr.11              | 100                      |                        |                 |
| SLC19A2     | NM_006996                         | Chr.1               | 100                      |                        |                 |
| DNAJC3      | NM_006280                         | Chr.13              | 100                      |                        |                 |
| PLAGL1      | NM_001809654                      | Chr.6               | 100                      |                        |                 |
| GATA6       | NM_005257                         | Chr.18              | 100                      |                        |                 |
| GATA4       | NM_002052                         | Chr.8               | 100                      |                        |                 |
| SLC2A2      | NM_000340                         | Chr.3               | 100                      |                        |                 |
| NKX2-2      | NM_002509                         | Chr.20              | 100                      |                        |                 |
| NEUROG3     | NM_020999                         | Chr.10              | 100                      |                        |                 |
| GLIS3       | NM_152629                         | Chr.9               | 99.7                     | 9                      | 0.30%           |
| RFX6        | NM_173560                         | Chr.6               | 100                      |                        |                 |
| MNK1        | NM_005515                         | Chr.7               | 100                      |                        |                 |
| EIF2AK3     | NM_004836                         | Chr.2               | 100                      |                        |                 |
| WFS1        | NM_006005                         | Chr.4               | 100                      |                        |                 |
| IER3P1      | NM_016097                         | Chr.18              | 100                      |                        |                 |
| PAX6        | NM_000280                         | Chr.11              | 100                      |                        |                 |
| FOXP3       | NM_014009                         | Chr. X              | 100                      |                        |                 |
| STAT3       | NM_139276                         | Chr.17              | 100                      |                        |                 |
| PCDH1       | NM_000281                         | Chr.10              | 100                      |                        |                 |
| SIRT1       | NM_012238                         | Chr.10              | 100                      |                        |                 |
| LRB4        | NM_001199282                      | Chr.4               | 99.98                    | 2                      | 0.02%           |
| ZPFS57      | NM_001109809                      | Chr.6               | 100                      |                        |                 |
| PTF1A enhancer| hg19                             | Chr.10              | 96.6                     | 25                     | 3.40%           |
| INS intron  | hg19                              | Chr.11              | 100                      |                        |                 |
| PPP1R15B    | NM_032833                         | Chr.1               | 100                      |                        |                 |
| TMRT10A     | NM_152292                         | Chr.4               | 100                      |                        |                 |
| KMT2D       | NM_003482                         | Chr.12              | 98.87                    | ~200                   | 1.13%           |
| KDM6A       | NM_021140                         | Chr. X              | 100                      |                        |                 |
| RAP1A       | NM_001010935                      | Chr.1               | 100                      |                        |                 |
| RAP1B       | NM_015646                         | Chr.12              | 100                      |                        |                 |
| CISD2       | NM_001008388                      | Chr.4               | 100                      |                        |                 |
| PTF1A       | NM_178161                         | Chr.10              | 100                      |                        |                 |

Discussion

In Switzerland, as in most countries, MD remains underdiagnosed due to its clinical heterogeneity and the lack of comprehensive genetic analysis. In this survey, the classical Sanger method was the only method used for the genetic testing of MD. Traditionally, genetic testing for MD has focused on a few genes depending on the patient’s phenotype, but in our new NGS-based diagnostic tool, multiple genes (n = 42) are sequenced in parallel. Novel genes that are involved in the pathogenesis of MD will be incorporated into subsequent designs. Such methods have already been proposed by several research groups in the UK, Poland, France, Norway and the USA [7, 16–21].

Over the last several years, many countries in Europe and across the globe have launched a new concept for the diagnosis and treatment of rare diseases (also called orphan diseases, http://www.orpha.net) and personalised medicine. Since MD belongs to this category, approval from health insurance for genetic testing will hopefully be obtained more easily. In Switzerland, a specific form is available in “documents” on the webpage of the Swiss Society of Medical Genetics (SGMG). The request for the genetic analysis for monogenic diabetes is available at the following website: http://www.hug-ge.ch/sites/interhug/files/structures/gr-demande-analyse/diagnol-std_e.pdf. The
Figure 2: An updated pathway for clinical decision-making for monogenic diabetes screening. The MODY probability calculator will use clinical features from the three different groups with either presumed MODY or type 1 or type 2 diabetes to calculate the probability for monogenic diabetes and therefore the indication for genetic testing. The calculator was developed for people with diabetes onset <35 years of age and should be used accordingly [10]. A direct molecular analysis is indicated for cases of neonatal and syndromic diabetes. For presumed autoantibody negative type 1 diabetes cases, an additional indicator for genetic screening are persisting C-peptide levels after the honeymoon period of >200 pmol/l with glucose >8 mmol/l, to avoid suppression of C-peptide levels by hypoglycemia [21–33]. We show the numbers of the genetically confirmed diabetes cases from the survey (total number 34) in the different categories: Type 1 diabetes: T1D; type 2 diabetes: T2D. Number of patients: N.

Table 5: DNA used for the validation of the diagnostic gene panel.

| Patient | Gene name | Reference sequence | Gene defect | Protein effect | Pathogenicity classified according Richards ** |
|---------|-----------|--------------------|-------------|---------------|-----------------------------------------------|
| 1       | HNF4A     | NM_000457.4        | c.724G>A    | p.Val242Met   | pathogenic                                    |
| 2       | GCK       | NM_000628.3        | c.608T>C    | p.Val203Ala   | pathogenic                                    |
| 3       | HNF1A     | NM_000545.6        | c.166G>T    | p.Glu56*      | pathogenic                                    |
| 4       | HNF1B     | NM_000458.3        | c.1-7,+,*7del | p.          | pathogenic                                    |
| 5       | KCNJ11    | NM_000525.3        | c.96_96delinsCTG | p.Gln30fs  | pathogenic                                    |
| 6       | EIF2AK3   | NM_004836.6        | c.2707C>T   | p.Arg903*     | pathogenic                                    |
| 7       | Neg.      | -                  | No pathogenic variant | p.      | -                                            |
| 8       | HNF1A     | NM_000545.6        | c.327-?526+7del | p.       | pathogenic                                    |
| 9       | HNF1A     | NM_000545.6        | c.1-7,+,*7del | p.       | pathogenic                                    |
| 10      | HNF1B     | NM_000458.3        | c.1-7_1045+7del | p.      | pathogenic                                    |

**Richards et al. [19]. We used the following analyses for the assessment of pathogenicity, 1. exonic silent variants, if not located in the first or in the last codon of an exon, were discarded; 2. all missense variants were evaluated according to their frequency in the general population (absent or very rare in the databases ExAC and gnomAD); 3. the pathogenic prediction was evaluated by different bioinformatics tools (SIFT, PolyPhen-2 and MutationTester); 4. the status regarding the pathogenicity according to ClinVar was sought; 5. the conservation score according to GERP was considered; 6. the literature was checked to ascertain whether the identified variants had been reported. Deletions/insertions: delins. Protein sequence: p. Coding DNA sequence: c.

costs of the NGS analysis are based on the different national billing guidelines and the cost-effectiveness of testing for MD will improve as genetic testing becomes rapidly cheaper [22–24]. For the time being, careful selection of patients is essential. Studies evaluating diagnostic strategies for MD are on-going [25]. In this survey, we identified 34 subjects with a genetically confirmed diagnosis of MD and 40 subjects with potential MD, but this number (74) represents only a fraction of the estimated number of subjects with MD. Furthermore, even when clinicians have identified subjects with a high clinical suspicion of MD, a genetic analysis was not conducted in 54% of the cases. These patients miss out on treatment optimisation with potentially increased metabolic control and decreased long-term complications and family counselling [26]. So far, the most commonly recognised mutations are located in the GCK gene, followed by mitochondrial and HNF1A and HNF1B mutations. HNF1B diabetes seems to be overrepresented in this study in comparison to internationally published data, where only 1–2% present with this form of diabetes [27]. The difference could be explained by the clinically easily recognisable renal phenotype, which may explain the increasing requests for genetic analysis for this diabetes subtype. Furthermore, the
low number of reported cases may be leading to an over-estimation in the results. Many subjects in the group with a genetic confirmation of MD were not treated according to international guidelines, and many patients still receive unnecessary treatment [26]. Patients with GCK mutations do not require pharmacological treatment, but 14.3% were receiving insulin treatment, and an additional 35.7% were getting OHA [28]. In the early course of HNF1A and HNF4A diabetes, glinides or low doses of sulfonylureas are more appropriate than insulin therapy [9, 29]. Most patients were given insulin in addition to OHA, and sulfonylureas were offered after molecular diagnosis in only a few cases. Mitochondrial diabetes usually requires insulin treatment, which was administered to 87.5% of the patients.

In clinical practice, the MODY probability calculator represents a useful tool for the selection of patients who should undergo genetic testing. The use of the MODY calculator in our study was very helpful for the majority of patients since all genetically confirmed MODY diabetes cases had a positive predictive value >20%. However, this method does not allow for a distinction between the different forms of MD. In the clinically diagnosed MD group, the positive predictive value was >20% in 73.3% of the subjects younger than 35 years of age, suggesting that genetic testing would be indicated. This probability calculator has not been developed for mitochondrial diabetes or patients older than 35 years at diabetes onset and should not be used. Increasing the cut-off of the positive predictive value could increase pick-up rate and increase cost-effectiveness. Another useful parameter for discriminating between type 1 and MODY diabetes is the urinary C-peptide/creatinine ratio (≥0.2 nmol/mmol), which has not yet been used [30].

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No potential conflict of interest relevant to this article was reported.

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Appendix 1

Supplemental data

Table S1: Probability for MODY each patient with mitochondrial diabetes and in the group with clinical diagnosis.

|                      | PP >20% <35 years (n) | PP ≤20% <35 years (n) | PP >20% ≥35 years (n) | PP ≤20% ≥35 years (n) |
|----------------------|------------------------|------------------------|------------------------|------------------------|
| Mitochondrial Diabetes | 2                      | 2                      | 1                      | 3                      |
| Clinical MD           | 22                     | 8                      | 2                      | 3                      |

PP = positive predictive value Data are number of patients (n). In the clinical group data, 5 patients could not be analysed because of missing data. The probability for MODY was calculated for each patient with mitochondrial diabetes and in the group with clinical diagnosis using only the probability calculator. The cut-off value for the positive prediction of 20% was used as an indicator for genetic testing (http://www.diabetesgenes.org/content/mody-probability-calculator). The calculator was developed for subjects with diabetes onset <35 years of age, which is why the results are depicted according to the age of diabetes onset. To get a positive predictive value for the subjects with age of onset ≥35 years of age, we put 35 years of age into the calculator.

Figure S1: Geographical distribution of the medical centres that responded to the survey. The geographical locations of the twelve medical centres and hospitals responding to the survey are depicted.