Heliyon 10 (2024) e36006

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

MODY calculator applied in patients with clinical diagnosis of type 1 diabetes mellitus: Is a higher cutoff needed?

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ARTICLE INFO

Keywords:
MODY
Monogenic diabetes
Clinic prediction model
Genetic sequencing

ABSTRACT

Aim: This study aimed to evaluate the mean post-test probability (PTP) of the Maturity-onset diabetes of the young (MODY) calculator in a multiethnic cohort of patients previously diagnosed with type 1 diabetes (T1DM).

Materials and methods: The MODY probability calculator proposed by Shields and colleagues (2012) was applied to 117 patients from a T1DM outpatient clinic at a tertiary hospital in Brazil. Additionally, two exons of the *HNF1A* gene were sequenced in eight patients who hadn't received insulin treatment within six months after the diagnosis.

Results: 17.1 % of patients achieved PTP >10 %; 11.1 % achieved PTP >25 % (and all patients >30 %), and 7.7 % achieved PTP >40 %. Among the patients who were selected for genetic sequencing, 100 % presented PTP >30 %, with 66.6 % achieving PTP >40 % and 41.6 % achieving PTP >75 %. These cutoffs are as suggested for the Brazilian population, according to previous investigations. No mutation was observed in the sequenced exons.

Conclusion: Considering that only around 10 % of the evaluated cases achieved PTP >30 %, it is highly probable that the most suitable cutoff to select patients for genetic sequencing in a Brazilian cohort of T1DM is higher than the cutoff used in Caucasian populations.

1. Introduction

Maturity-onset diabetes of the young (MODY) corresponds to a heterogeneous group of monogenic forms of diabetes. MODY is generally characterized by mutations in genes involved in the physiology of insulin secretion and can affect up to 4 % of the diabetic population [1,2]. So far, mutations in 11 different genes have been correlated with MODY [3]. Mutations in hepatocyte nuclear factor 1A (*HNF1A*) and glucokinase gene (*GCK*) represent the most prevalent causes of MODY, corresponding to 30–60 % and 30–50 % of all

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https://doi.org/10.1016/j.heliyon.2024.e36006
Received 13 March 2024; Received in revised form 14 June 2024; Accepted 7 August 2024
Available online 8 August 2024

Table 1
Main characteristics of patients who achieved PTP >25 %.

Patient	Age at diagnosis	Gender	Treatment with insulin/ OHA	BMI (kg/ m^2)	HbA1c (%)	Current age	Family history of diabetes	Ethnicity	PTP (%)
1	19	F	Yes	38.1	8	37	Yes	W	35.8
2	25	M	Yes	22.4	9	54	Yes	W	35.8
3	16	M	Yes	25.7	11.9	25	Yes	NW	62.4
4	12	F	Yes	31.2	7.6	43	No	W	75.5
5	18	F	No	22.4	7.4	24	Yes	NW	75.5
6	25	M	No	29.7	7.5	41	Yes	W	45.5
7	28	F	Yes	15.3	10.4	32	No	W	32.9
8	31	F	No	27.1	5.7	33	No	W	32.9
9	18	F	Yes	23.4	10.7	36	Yes	W	75.5
10	30	F	Yes	28.3	5.2	32	Yes	W	49.4
11	11	F	Yes	27.5	10.7	31	Yes	W	75.5
12	12	M	Yes	23.5	6.4	37	Yes	W	75.5

OHA = oral hypoglycemic agent. BMI = body mass index. F = female. M = male. W = white. NW = Non-white. HbA1c = glycated hemoglobin. PTP = post-test probability.

cases, respectively; mutations in hepatocyte nuclear factor 4A (*HNF4A*) and hepatocyte nuclear factor 1B (*HNF1B*) account for 5–10 % and less than 5 % of cases, respectively. The other subtypes of MODY are extremely rare and have been reported in a few families; also, there is scarce data regarding these subtypes reported in the literature, and they collectively account for less than 1 % of all causes of MODY [4]. A list of the genes associated with MODY can be seen in Frame 1 [5].

To the present day, the diagnosis of MODY represents a challenge to clinical practice, since the confirmation usually relies on genetic tests of high cost, and the forwarding of patients to genetic testing depends on medical suspicion based on clinical characteristics, not satisfactorily patterned. At the same time, the assertive diagnosis is of utmost importance, considering that the treatment of the main MODY subtypes differs from the treatment of classic forms of diabetes mellitus (DM), such as type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). In addition, incorrect treatment may compromise the quality of life of patients [6,7].

Patients with mutations in *GCK-MODY* – one of the most prevalent mutations associated with MODY – present mild and stable fasting hyperglycemia, and slight increases in the oral glucose tolerance test (<54 mg/dL). In addition, glycated hemoglobin (HbA1c) rarely surpasses 7.6 % (60 mmol/mol) and the risk of micro- and macrovascular complications is low. This phenotype differs drastically from T1DM [8,9]. On the other hand, the clinical presentation of MODY caused by mutations in hepatocyte nuclear factors (*HNF1A* and *HNF4A*), also quite common, is characterized by progressive hyperglycemia, and as years pass by, the risk of micro- and macrovascular complications is comparable to T1DM (5). These mutations occur more frequently in exons 2 and 4 of *HNF1A* (0.33 and 0.28 mutations per nucleotide, respectively) [10]. These comparisons between the types of MODY only reaffirm how the group is heterogeneous, and the clinical outcomes may differ drastically.

Shields and colleagues (2012) [11] developed a MODY probability calculator through logistic regression, which has shown better sensitivity and specificity in discriminating MODY from T1DM and T2DM when compared to criteria previously established. The calculator only demands a few clinic data, generally available in most health services, and can be accessed at no cost at https://www.diabetesgenes.org. The clinical data necessary in order to fill in the online MODY probability calculator are gender, use of insulin or other oral hypoglycemic agents (OHA) at the moment of data collection, time of insulin treatment, body mass index (BMI), HbA1c, current age, family history of DM, and ethnicity. All these data are included directly on the website, and it provides the post-test probability (PTP) for MODY.

In the United Kingdom, the suggested cutoff for molecular genetic tests of MODY is set at a post-test probability (PTP) > 25 % in patients who haven't been under insulin treatment in the six-month post-diagnosis period, and >10 % in patients who have undergone insulin treatment within the six-month post-diagnosis period. In the latter case, it is suggested that additional C-peptide and islet autoantibody tests are conducted before genetic testing. The use of stimulation c-peptide testing 90 min after a mixed meal, with values ≥ 0.2 nmol/L (≥0.6 ng/L), is considered a reliable marker for significant endogenous release of insulin. An alternative to serum C-peptide testing in hospital conditions is the use of the C-peptide/creatinine ratio (UCPCR), which is stable at room temperature for up to three days. UCPCR ≥0.53 nmol/mmol stimulated 120 min after a mixed-meal tolerance test (MMTT) indicates a high correlation

Table 2
Main characteristics of patients who achieved a PTP >10 %.

Patient	Age at diagnosis	Gender	Treatment with insulin/ OHA	BMI (kg/ m^2)	HbA1c (%)	Current age	Family history of diabetes	Ethnicity	PTP (%)
13	12	M	Yes	23.5	6.4	37	Yes	W	75.5
14	19	F	Yes	21.5	9.1	24	Yes	W	12.6
15	29	F	Yes	28.1	8	45	Yes	NW	12.6
16	11	F	Yes	25.6	6.4	34	Yes	W	12.6
17	30	F	Yes	28.3	5.2	32	Yes	W	49.4

OHA = oral hypoglycemic agent. BMI = body mass index. F = female. M = male. W = white. NW = Non-white. HbA1c = glycated hemoglobin. PTP = post-test probability.

Table 3
Frequency of significative results of qualitative variables.

Variable	Predominance	Relative Frequency (%)	p-value
Gender	Female	61.5	0.045
Treatment with insulin or OHA	Yes	97.4	0.015
Start of insulin treatment	<6 months post diagnosis	83.7	0.042
Family history of DM	No	61.5	0.045
Ethnicity	White	84.6	0.033

OHA = oral hypoglycemic agent. DM = diabetes mellitus.

Table 4
Descriptive measurements of quantitative variables.

Variable	Mean	PD	VC (%)
Age at diagnosis	16.6	8.4	49.7
BMI (kg/m^2)	26.5	5.2	19.6
HbA1c (%)	9.0	2.1	23.3
Current age	33.9	10.7	31.6
PTP (%)	9.1	18.0	197.8

PD = pattern deviation. VC = variation coefficient. BMI = body mass index. PTP = post-test probability.

to serum C-peptide. Thus, a positive C-peptide test and a negative pancreatic autoantibodies test increase the probability of MODY over T1DM [12,13]. However, it must be stated that the calculator has been developed based on a Caucasian European cohort and must thus be evaluated in multiethnic populations, in which the cutoff might be different [11].

In Brazil, two large studies have investigated the performance of the Shields et al. (2012) [11] calculator. In the first one, conducted by Tarantino and colleagues (2020), 34 patients who had been previously diagnosed with T1DM, T2DM, or gestational diabetes mellitus (GDM), were genetically sequenced for *HNF1A-MODY* and *GCK-MODY* after demonstrating suggestive criteria for MODY, such as diagnosis before 35 years of age, body mass index (BMI) < 30 kg/m^2, negative autoantibodies, and familiar history of DM in, at least, two generations. After sequencing, 11 mutations were found: 6 in *GCK* and 5 in *HNF1A*. The PTP of these 11 patients, according to the MODY probability calculator, was >75 % [14]. In the second study, the MODY calculator performance was retrospectively investigated based on data from 391 patients – 231 of them diagnosed with T1DM, 46 with T2DM, and 114 with MODY - acquired from 1953 to 2020 in the diabetes ambulatory of a quaternary hospital in the city of São Paulo. The cutoff of 40 % presented maximum sensitivity, while the cutoff of 60 % showed maximum specificity [15].

It is important to mention that the performance of the MODY probability calculator has not been investigated in many cohorts with patients previously diagnosed with T1DM in Brazil. Patients who were diagnosed with DM in the early stages of life are easily classified as T1DM and are prescribed insulin in the first few days after diagnosis. Nevertheless, patients with latent diabetes of the adult (LADA), an autoimmune form of diabetes which develops during adult age, may be classified as T2DM, and remain for prolonged periods under therapy using oral antidiabetics (OADs). These factors might increase or decrease the value of PTP, culminating in selection errors. The prevalence of monogenic diabetes among pediatric diabetes patients is not negligible and may correspond to 6.5 % [16]. A correct diagnosis is necessary for the most suitable course of treatment, and to guarantee the quality of life of patients.

The present investigation aimed to identify the MODY calculator cutoff in a multiethnic population of patients previously diagnosed with T1DM. As most health services in Brazil lack financial resources for genetic sequencing, and in multiple cases laboratory data such as C-peptide and islet autoantibodies are scarce, this potential alternative low-cost approach may be valuable for the diagnosis of MODY. The present screening aimed at enriching the patient data of the two previous Brazilian studies which investigated the cutoff among patients with T1DM, including the genetic sequencing of two exons of the *HNF1A* gene, which are more prevalently related to MODY.

2. Materials and methods

All patients from a T1DM outpatient clinic in a tertiary hospital in Brazil were invited to participate in the study. This T1DM outpatient clinic receives, on average, 180 regular T1DM-diagnosed patients of all age groups. Medical care takes place once a week, in the afternoon, and each patient returns every four months. As inclusion criteria, the patient had to present a T1DM diagnosis by our healthcare team or by primary health services belonging to the Unified Health System (SUS) in nearby cities before referral to our outpatient clinic. In our outpatient clinic, the T1DM diagnosis is confirmed by a positive autoantibody (GAD and/or IA2) and/or non-functional serum C-peptide tests (<1,1 ng/mL). The diagnosis process in primary health services is not completely known and does not follow a pattern; in most cases, once patients are directed to the outpatient clinic, they are already under intensive insulin treatment. The only exclusion criterion adopted was diagnosis after 35 years of age since the proposition of the calculator depends on a diagnosis age of under 35 years. Also, during the screening and the testing, all patients had the possibility of opting out of the study, and in that case, they were also excluded from the investigation.

The MODY probability was calculated using the MODY calculator developed by Shields et al. (2012) (11). The data from patients, including weight and height, were acquired in the ambulatory and originated the BMI. The more recent HbA1c value registered in the

Table 5
Linear association between PTP and age (at diagnosis and current), BMI, and HbA1c.

Association	Correlation coefficient	p-value
PTP x Age at diagnosis	0.188	0.044
PTP x BMI	−0.015	0.87
PTP x Current age	0.053	0.57
PTP x HbA1c	−0.148	0.115

BMI = body mass index. HbA1c = glycated hemoglobin. PTP = post-test probability. Pearson correlation was used.

electronic medical record of the patients was considered, but only if the test method had been certified by the National Glycohemoglobin Standardization Program (NGSP). Moreover, a Sanger sequencing of two exons of the gene most correspondent to the phenotype of the selected patients was conducted. To be considered eligible for genetic sequencing, the patient had to achieve a PTP >25 % and not have received insulin treatment in the first 6 months after diagnosis.

- Blood collection

Peripheral blood samples (5 mL) were collected by venipuncture into collection tubes containing EDTA as an anticoagulant.

- DNA extraction

Genomic DNA was extracted from total blood using a protocol patterned in our Institution. The method separates the leucocytes by centrifugation, sample digestion with proteinase K, and DNA precipitation with ethanol and sodium chlorate. Isolated DNA concentration and quality were investigated using the NanoDrop 2000 spectrophotometer (Thermo Scientific), by measuring their absorbance at 260 nm, and A260/A280 ratio, which was around 1.8, as expected.

- PCR amplification of Exons

The gene exons were amplified using Platinum Taq DNA polymerase (Invitrogen), 1.5 μM of MgCl2, 200 μM of dNTP, 200 nM of each primer, and around 100 ng of total DNA. The reaction was conducted using the following cycling: 2min 94 °C/40 × (30s 94 °C/30s 60 °C/72 °C 1min)/72 °C 10min. Amplification was verified by agarose gel electrophoresis. Amplified samples were purified from primers and residual nucleotides by incubation with exonuclease I and alkaline phosphatase, and then subjected to a sequencing reaction.

- Sanger sequencing

The purified PCR products were subjected to linear amplification by the Sanger method, using the BigDye Terminator v3.1 kit (Applied Biosystems), as suggested by the manufacturer. Minor adjustments were conducted, and the solution was obtained using 0.5 μL of ready reaction mix, 1.75 μL of buffer solution, 300 ng of DNA, and 1 μM of primer. The reaction was conducted using the following cycling: 5min 95 °C/40 × (15s 95 °C/-1 °C/s until 50 °C/10s 50 °C/4min 60 °C).

The product of this reaction was purified by precipitation, according to the manufacturer protocol, denaturized in formamide, and sequenced in the 3500 Genetic Analyzer (Applied Biosystems). The software was adjusted to accept QV ≥ 20, and heterozygous nucleotides were detected by the presence of overlapping peaks in which the lower was ≥30 % of the height of the higher peak.

Exons 2 and 4 of gene *HNF1A* were sequenced since they represent the most prevalent mutating exons of this gene. *HNF1A* was selected due to the phenotypical characteristics of patients selected for genetic sequencing [10], and due to the proximity of clinical outcomes with T1DM.

- Statistical analysis

All quantitative data was verified for normal distribution using the Shapiro-Wilks test. The profile of the case series was presented using descriptive statistics with frequency distribution and descriptive measurements of position and variability. The association between PTP and the quantitative variables of age (at diagnosis and current), BMI, and HbA1c was established by the linear Pearson correlation coefficient [17].

Table 6
Linear association between PTP and age (at diagnosis and current), BMI, and HbA1c.

Association	Correlation coefficient	p-value
Ethnicity x family history of DM	−0.052	0.574
Ethnicity x Start of insulin treatment	0.085	0.364
Ethnicity x Treatment with insulin or OHA	0.081	0.387

OHA = oral hypoglycemic agent. DM = diabetes mellitus. Pearson correlation was used.

The association between PTP and gender, current treatment with insulin, family history of diabetes mellitus, and ethnicity were tested using Mann-Whitney, while PTP and time of treatment were evaluated by Kruskal-Wallis followed by Dunn's posthoc test. The level of significance considered was 5 % [17].

3. Results

A MODY probability calculator was applied to 117 patients from a T1DM outpatient clinic at a quaternary hospital in Brazil. Among the 117 patients, 20 (17.1 %) achieved PTP >10 %, 13 (11.1 %) achieved PTP >30 %, and 9 (7.7 %) achieved PTP >40 %. Considering all patients who hadn't received insulin treatment in the 6-month post-diagnosis period, 12 patients (10.3 %) achieved PTP >25 % and were selected for genetic sequencing (Table 1). It is important to mention that 100 % of these 12 patients presented PTP >30 %. However, during the blood collection phase, four patients decided to drop out of the study, and the genetic sequencing was conducted in eight patients. Of the patients who had received insulin in the 6 months after the diagnosis, 5 (4.3 %) achieved PTP >10 % (Table 2). The patients who hadn't received insulin treatment and were selected for the genetic sequencing achieved PTP >30 %, with 66.6 % achieving PTP >40 %, and 41.6 % achieving PTP >75 %.

There was a predominance of female patients over male patients; of patients under pharmacological treatment for diabetes; of patients who started the treatment within six months after the diagnosis; of patients whose parents were not diagnosed with diabetes; and of patients of white ethnicity. The frequency of the qualitative variables can be observed in Table 3.

The average age at the time of diagnosis and the average current age of the patients were 16,6 and 33,9 years old, respectively. Also, the average BMI was 26,5 kg/m^2 (reference values ranging from 18.5 to 24.9) while the average HbA1c was 9.0 % (71 mmol/mol) (reference values ranging from 4.0 to 5.6 % - 20–38 mmol/mol). Finally, the average PTP was 9.1 %. All quantitative variables are compiled in Table 4. Because our cohort presents over 15 % of non-white patients, and we were interested in investigating the possible differences between Caucasian populations and multiethnic populations, we conducted Mann-Whitney U tests to compare age at diagnosis, current age, BMI, HbA1C and PTP according to the ethnicity: white and non-white. From these parameters, the only one that presented a significant difference between groups was age at diagnosis ($p = 0.016$), with the white patients being diagnosed at an earlier age than non-white patients. Even though there was no significant difference regarding PTP between groups ($p = 0,889$), the average of white patients was lower than the average of non-white (8.8 % vs. 10.5 %). Considering the correlation between PTP and the variables, there was a positive correlation between PTP and age at diagnosis. The linear association between PTP and all variables is presented in Table 5. The sequencing of exons 2 and 4 did not show any genetic variants associated with MODY. Considering the impact of ethnicity on the variables, there was no verified influence, and the results can be seen in Table 6.

4. Discussion

The present data enabled the design of a patient profile, considering the patient cohort at the T1DM outpatient clinic of a quaternary hospital in Brazil. The screening of patients confirmed the predominance of female, Caucasian, with no family history of diabetes, and under pharmacological treatment. Within the group that used insulin, most patients had started the treatment within six months after the diagnosis, as expected considering a cohort of patients diagnosed with T1DM.

There was a positive correlation between PTP and age at diabetes diagnosis; the lower the age at diagnosis, the higher the probability of MODY. The PTP was significantly higher among women, patients not under pharmacological treatment, patients who did not start insulin treatment within six months post-diagnosis, and patients with a family history of diabetes. These data are in accordance with the cohort evaluated by Shields and colleagues (2012). No correlation was observed between PTP and ethnicity, although Shields et al. (2012) reported that white patients presented an increased probability of presenting MODY [11]. This fact can be correlated with the number of patients who self-declared themselves as white, which was consistently higher in comparison with patients who self-declared as non-white (84.6 % and 15.4 %, respectively).

In our cohort, all patients selected for genetic sequencing achieved PTP >30 %, and 7.7 % achieved PTP >40 %, in accordance with previous Brazilian investigations. In the first Brazilian study, 100 % of patients with positive tests for MODY achieved PTP >75 % [14]. In the second, maximum sensitivity was observed with a cutoff of 40 % and maximum specificity with a cutoff of 60 % [15]. This finding is pertinent since it strongly suggests that the cutoff to select patients for the MODY testing in a multiethnic population, such as the Brazilian population, must be higher than the cutoff originally proposed by Shields and colleagues (2012), which used a cohort of Caucasian Europeans [11]. In our investigation, there was no difference in PTP between white and non-white patients, although the average of non-white patients was slightly higher. This indicates that further investigations with multiethnic populations and with a greater N are mandatory in order to fully comprehend if non-white diabetic patients present particular features regarding MODY. In addition, the age at diagnosis was higher in non-white patients compared to Caucasians, indicating that this group took around 5 more years to be diagnosed with DM. This finding could indicate a vast range of possibilities, from differences in access to medical care, negligence of symptoms, or onset of symptoms. Considering the size of our cohort, we estimate that further investigations, consisting of larger samples, must be conducted to further elucidate this population. Also, considering the validation of the MODY calculator tor Caucasians, further investigations aiming at non-Caucasian populations is mandatory so that its predictor efficiency is validated.

In the original MODY calculator proposition, the absence of treatment with insulin or oral hypoglycemic agents (OHA) increases PTP in favor of MODY compared to the other forms of DM [11]. However, the diagnosis of DM amongst children and young adults is frequently followed by insulin prescription, due to the assumption that it is a case of autoimmune DM. In a subsequent study, the authors admit that the MODY calculator has limited accuracy in identifying patients with MODY among insulinized patients [18]. Our findings agree with this statement, given that the mean PTP in our cohort was low (9.1 %).

An interesting alternative to applying the MODY calculator in cohorts with patients classified as T1DM might be the employment of biomarkers. Shephard and co-workers (2016) demonstrated a screening based on biomarkers using a sample of 808 patients over 20 years old. Initially, C-peptide samples were measured using the urinary C-peptide and creatinine ratio (UCPCR), considering that endogenous insulin production measured by C-peptide drops considerably after the honeymoon phase. Patients who presented a UCPCR $\geq$2 nmol/mmol were further tested for two islet autoantibodies (GAD and IA2). Finally, patients with low UCPCR and negative autoantibodies were tested for monogenic diabetes. The screening pathway was able to identify 20 patients with monogenic diabetes among the 808 included in the study (1 in 4 patients). Out of the 20 patients who were identified with monogenic diabetes, 19 had MODY [19].

A similar screening was conducted by Shields et al. (2017), using an even larger cohort (n = 1,407) of patients who had been diagnosed with DM less than 30 years ago and were younger than 50 years of age. A minimum prevalence of 3.6 % of monogenic diabetes was found, and the positive predictive value (PPV) was set at 20 %, meaning that the uptake rate was 1 in every 5 patients. The screening route outperformed the MODY calculator, which presented a higher PPV (40.4 %) but missed 55 % of all MODY cases when compared to the screening route which did not miss any MODY cases [18].

In a more recent investigation, Carlsson and colleagues (2020) dosed four autoantibodies (GAD, IA2, ZnT8A, and IAA) in a large pediatric cohort (n = 3,933) at the time of the diabetes diagnosis. Subsequently, all patients were sequenced for MODY, and 46 mutations were found. Among the 303 patients who were negative for the four autoantibodies, 46 presented MODY, representing a detection rate of 15 %. Nevertheless, in patients with negative results for the four autoantibodies and with HbA1c $<$ 7.5 % (59 mmol/mol), the detection rate was much higher (49 %) [20].

In the present patient cohort, C-peptide and autoantibody data were limited and were available for only a few patients at the time of the investigation. Regarding C-peptide, in the case of values available in medical records, there was no specification of how long the sample was collected after diagnosis. Although in our outpatient clinic the measurement of autoantibodies is carried out at the time of diagnosis, many patients are referred from neighboring areas after years of treatment in Basic Health Units (BHU), and laboratory data from these periods are scarce. This reality is observed in multiple health units in Brazil, in which laboratory data, easily available in developed countries, is frequently unavailable. Therefore, screenings based on biomarkers, though promising, might be inaccessible in poor countries. Other types of diagnosis, as reliable as biomarker testing, would be of major help, and further investigation aiming at validating these alternatives is mandatory. In this aspect, the present investigation comes close to the reality of health services in developing countries, in which data on C-peptide and autoantibodies are often not available. In addition, the use of C-peptide testing is better in discriminating MODY of T1DM after 3 years of diagnosis, after the honeymoon phase, and this reality may limit the use of C-peptide in the case of patients with less than 3 years of diagnosis [19], a limiting factor not observed with the use of the MODY calculator.

Finally, the Sanger sequencing of exons 2 and 4 of the *HNF1A* gene was performed. These exons were selected based on previous studies, which indicate that mutations in this gene are the most prevalent for the development of MODY, in particular the mutation p291fsinsC in exon 4. In this case, there is a frameshift started by the insertion of a cytosine around codon 290, which leads to the synthesis of a protein without the transactivation domain [21]. The phenotype of the selected patients was decisive for the determination of sequencing *HNF1A* due to the HbA1c values (8.3 % - 65 mmol/mol), which rarely surpasses 7.6 % (60 mmol/mol) in patients with *GCK-MODY* [9].

Additionally, it is important to mention that the MODY calculator proposed by Shields et al. (2012) was developed to capture mutations in the three main genes correlated with MODY (*HNF4A*, *GCK,* and *HNF1A*) [11]. The *HNF4A* gene was not sequenced in the present investigation due to the pathogenic – or potentially pathogenic – mutations occurring in this gene being much less prevalent than *GCK* and *HNF1A* [14]. However, no mutation in the sequenced exons was observed in the present investigation. It is possible that genetic sequencing of other genes involved with MODY could have shown significant results, and this is a limitation of the present study. Still, given the reality of the patients from our cohort and the resources available, the sequencing of the *HNF4A* gene was a viable decision.

The present investigation has some significant limitations. Considering that a vast part of our cohort is from patients forwarded to the outpatient clinic from surrounding regions, several clinical and laboratory data were not available for all of our patients. In this regard, detailed information about pharmacological treatments was unavailable and was not investigated in the present study. Not all genes associated with MODY were analyzed in the genetic sequencing – we only investigated two exons of the most frequently mutated gene –, which would have significantly contributed to the cutoff validation for the Brazilian population. Also, not all patients presented C-peptide and autoantibodies data, which could have refined the patient selection for the genetic testing. Nevertheless, our study also presents several strengths, such as the cohort size of patients diagnosed with T1DM, and that this is the third investigation that aimed to evaluate the MODY calculator performance among T1DM-diagnosed patients in the Brazilian population. In addition, this study comes close to the reality of most health services in the country, which lack financial resources, and sets up an attempt to optimize the MODY calculator use in this scenario, from the clinic perspective. Passanisi and colleagues (2021) warned about the differences between health systems around the world, and how this factor can alter epidemiological data regarding the prevalence of monogenic forms of diabetes. In the case of countries where access to tertiary health services is facilitated, the diagnosis of even milder forms of MODY, such as GCK, may not go unnoticed, unlike what happens in countries where access to healthcare is limited. Predominantly through health plans [22]. Although this study was conducted in a quaternary center, resources for genetic sequencing are still scarce; therefore, the use of a predictive model, based on clinical data, which indicates which patients have a greater chance of being carriers of MODY, is of great value.

5. Conclusion

Based on the values identified by the PTP, a non-negligible portion of the patients of this investigation would be indicated for genetic testing. In this cohort of patients with T1DM, 17.1 % achieved a PTP >10 %; 11.1 % achieved a PTP >25 % (all >30 %), and 7.7 % achieved PTP >40 %. Among the patients selected for genetic sequencing, 100 % presented PTP >30 %, with 66.6 % achieving PTP >40 % and 41.6 % achieving PTP >75 % - cutoffs suggested for the Brazilian population by previous important investigations with patients with T1DM. Considering that a little more than 10 % of all cases achieved PTP >30 %, these numbers may indicate that the most appropriate cutoff to select patients for genetic testing in a Brazilian cohort of patients diagnosed with T1DM should be higher than the one proposed for Caucasians. We emphasize the importance of studying the cutoff point of the MODY calculator among patients with a previous diagnosis of T1DM, in particular in non-Caucasian patients, since as previously reported, the prevalence of monogenic diabetes in this population tends to be high compared to the diabetic population in general, and more information relating to non-white populations is mandatory.

Ethics approval: This study was reviewed and approved by the FMB/UNESP Research Ethics Committee with the approval number: 37367820.0.0000.5411, dated November 11st 2020.

Funding statement

This study was funded by Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Data availability statement

All data associated with this study is deposited in a publicly available repository (UNESP Institutional Repository), partially available until 12/16/2024. After this date, the data will be made publicly available in full (URI: http://hdl.handle.net/11449/239386).

CRediT authorship contribution statement

Vinícius Vigliazzi Peghinelli: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Teresa de Sibio:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Conceptualization. **Igor de Carvalho Depra:** Methodology, Investigation, Conceptualization. **Milena Gurgel Teles Bezerra:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Conceptualization. **Marna Eliana Sakalem:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Adriano Francisco De Marchi Júnior:** Methodology, Conceptualization. **Paula Barreto da Rocha:** Writing – review & editing, Writing – original draft, Visualization. **Helena Paim Tilli:** Conceptualization. **Bianca Mariani Gonçalves:** Conceptualization. **Ester Mariane Vieira:** Conceptualization. **Mariana Menezes Lourenço:** Conceptualization. **Célia Regina Nogueira:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Nothing to declare.

Frame 1. MODY-associated genes

HNF4A	Hepatocyte Nuclear Factor 4A
GCK	Glucokinase
HNF1A	Hepatocyte Nuclear Factor 1A
PDX1	Pancreatic-Duodenal Homeobox 1
HNF1B	Hepatocyte Nuclear Factor 1B
NEUROD1	Neural Differentiation 1
CEL	Carboxyl Ester Lipase
INS	Insulin
ABCC8	ATP-Binding Cassette, subfamily C, member 8
KCNJ11	Potassium inwardly-rectifying Channel, subfamily J, member 11
APPL1	Adaptor protein containing a PH domain, PTB domain, and Leucine zipper motif 1

Source: Adapted from Nkonge al., 2020 [5].

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