Monogenic Diabetes Accounts for 6.3% of Cases Referred to 15 Italian Pediatric Diabetes Centers During 2007 to 2012

Maurizio Delvecchio,[1] Enza Mozzillo,[2] Giuseppina Salzano,[3] Dario Iafusco,[4] Giulio Frontino,[5] Patrizia I. Patera,[6] Ivana Rabbone,[7] Valentino Cherubini,[8] Valeria Grasso,[9] Nadia Tinto,[10] Sabrina Giglio,[11] Giovanna Contreas,[12] Rosa Di Paola,[13] Alessandro Salina,[14] Vittoria Cauvin,[15] Stefano Tumini,[16] Giuseppe d'Annunzio,[14] Lorenzo Iughetti,[17] Vilma Mantovani,[18] Giulio Maltoni,[19] Sonia Toni,[20] Marco Marigliano,[12] and Fabrizio Barbetti,[9,21] for the Diabetes Study Group of the Italian Society of Pediatric Endocrinology and Diabetes (ISPED)

[1]Department of Pediatric Science and Surgery, Pediatric Hospital "Policlinico-Giovanni XXIII" of Bari, Bari 072006, Italy; [2]Department of Translational Medical Science, Section of Pediatrics, Regional Center of Pediatric Diabetology, University of Naples Federico II, Naples 80138, Italy; [3]Department of Pediatric Sciences, University of Messina, Messina 98122, Italy; [4]Regional Center for Pediatric Diabetes "G. Stoppoloni," Department of Pediatrics, Second University of Naples, Naples 80138, Italy; [5]Department of Pediatrics, Endocrine Unit, Diabetes Research Institute (OSR-DRI), Scientific Institute Hospital San Raffaele, Milan 20132, Italy; [6]Endocrinology and Diabetes Unit, University Department of Pediatric Medicine, Bambino Gesù Children Hospital, Rome 00165, Italy; [7]Department of Pediatrics, University of Turin, Regina Margherita Hospital, Turin 10126, Italy; [8]S.O.D. Pediatric Diabetes, Department of Women's and Children Health, Salesi Hospital, Ancona 60123, Italy; [9]Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome 00173, Italy; [10]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, and CEINGE, Advanced Biotechnology, Naples 80138, Italy; [11]Medical Genetics Unit, Department of Clinical and Experimental Biomedical Sciences "Mario Serio," and Meyer Children's University Hospital, University of Florence, Florence 50121, Italy; [12]Pediatric Diabetes and Metabolic Disorders Unit, Department of Surgical Science, Dentistry, Ginecology and Pediatrics, University of Verona, Verona 37129, Italy; [13]Research Unit of Diabetes and Endocrine Disease, IRCCS Casa del Sollievo della Sofferenza, San Giovanni Rotondo 71013, Italy; [14]Istituto Giannina Gaslini, Regional Center for Pediatric Diabetes, Genoa 16147, Italy; [15]Pediatric Unit, Santa Chiara Hospital, Trento 38122, Italy; [16]Center of Pediatric Diabetology, University of Chieti, Chieti 66100, Italy; [17]Pediatric Unit, Department of Medical and Surgical Sciences for Mothers, Children and Adults, University of Modena and Reggio Emilia, Modena 41122, Italy; [18]Center for Applied Biomedical Research (CRBA) and Medical Genetics Unit, S. Orsola University Hospital, Bologna 40138, Italy; [19]Department of Pediatrics, S. Orsola-Malpighi University Hospital, Bologna 40138, Italy; [20]Juvenile Diabetes Center, Meyer Children's Hospital, Florence 50139, Italy; and [21]Bambino Gesù Children Hospital, IRCCS, Rome 00165, Italy

Context: An etiologic diagnosis of diabetes can affect the therapeutic strategy and prognosis of chronic complications.

Objective: The aim of the present study was to establish the relative percentage of different diabetes subtypes in patients attending Italian pediatric diabetes centers and the influence of an etiologic diagnosis on therapy.

Design, Setting, and Patients: This was a retrospective study. The clinical records of 3781 consecutive patients (age, 0 to 18 years) referred to 15 pediatric diabetes clinics with a diagnosis of diabetes or impaired fasting glucose from January 1, 2007 to December 31, 2012 were examined. The clinical

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 27 June 2016. Accepted 7 February 2017.
First Published Online 16 February 2017

Abbreviations: HbA1c, hemoglobin A1c; IFG, impaired fasting glucose; MODY, maturity onset diabetes of the young; NDM, neonatal diabetes mellitus; T1D, type 1 diabetes; TNDM, transient neonatal diabetes mellitus.

characteristics of the patients at their first referral to the centers, type 1 diabetes-related autoantibodies, molecular genetics records, and C-peptide measurements, if requested for the etiologic diagnosis, were acquired.

Main Outcome Measures: The primary outcome was to assess the percentage of each diabetes subtype in our sample.

Results: Type 1 diabetes represented the main cause (92.4%) of diabetes in this group of patients, followed by monogenic diabetes, which accounted for 6.3% of cases [maturity onset diabetes of the young (MODY), 5.5%; neonatal diabetes mellitus, 0.6%, genetic syndromes, 0.2%]. A genetic diagnosis prompted the transfer from insulin to sulphonylureas in 12 patients bearing mutations in the *HNF1A* or *KCNJ11* genes. Type 2 diabetes was diagnosed in 1% of the patients.

Conclusions: Monogenic diabetes is highly prevalent in patients referred to Italian pediatric diabetes centers. A genetic diagnosis guided the therapeutic decisions, allowed the formulation of a prognosis regarding chronic diabetic complications for a relevant number of patients (*i.e., GCK*/MODY), and helped to provide genetic counseling. (***J Clin Endocrinol Metab* 102: 1826–1834, 2017**)

Type 1 diabetes (T1D) is the most prevalent cause of diabetes in the youth in North America and Europe (1). In contrast, in other continents and countries (*e.g.*, China), a much lower annual incidence has been reported. However, the relative percentage in the Western world of other forms of diabetes in children and adolescents, such as type 2 diabetes and monogenic diabetes, seems to vary greatly (2–5); this might be because of a number of reasons, including errors in clinical diagnosis (6). The identification of the exact etiologic cause of diabetes is important, because it can direct therapeutic decisions and influence genetic counseling (7).

The aim of the present study was to assess the prevalence of the different etiologies of diabetes mellitus in a large group of patients aged <18 years at diagnosis and referred to tertiary diabetes centers representative of peninsular Italy. Our data have shown that monogenic diabetes is the second prevailing cause of diabetes after T1D in Italian youth and that the correct etiologic diagnosis greatly affects the treatment and likely the prognosis of diabetic complications (8–11).

Methods

Data were collected from 3781 patients consecutively diagnosed with diabetes or impaired fasting glucose (IFG) during a 6-year period from January 1, 2007 to December 31, 2012. All the patients were aged <18 years at the diagnosis of diabetes or IFG. The patients had attended the pediatric diabetes clinics of 15 Italian centers based in Ancona, Bologna, Chieti, Florence, Genoa, Messina, Milan, Modena, Naples (two centers), Rome, San Giovanni Rotondo, Trento, Turin, and Verona. These centers are scattered throughout Italy from northernmost part (Trento) to southernmost region of Sicily (Messina). For all patients, the following data were gathered: date of birth, gender, age at diagnosis, ethnicity, and T1D autoantibodies (ICA, GADA, IA-2A, IAA, and ZnT8A). For patients with a clinical diagnosis of monogenic diabetes confirmed by genetic testing, the mutations identified were obtained and checked for novelty using HGMDpro (Qiagen Bioinformatics, Aarhus, Denmark). In addition, details on therapy before and after the genetic diagnosis were acquired. Genetic analysis of the common maturity onset diabetes of the young (MODY) genes (*i.e., GCK*, *HNF1A*, *HNF4A*) was performed in different laboratories located in Bologna, Florence, Genoa, Naples, Rome, San Giovanni Rotondo, and Verona. All centers adopted a "metabolic phenotype" strategy of genetic screening, starting with the *GCK* gene in all individuals with IFG or stable fasting hyperglycemia not exceeding 150 mg/dL (8.3 mol/L) and negative to T1D autoantibodies. If a mutation in the *GCK* gene was identified, the screening was stopped. If no mutations were found in the *GCK* gene, the screening continued with the analysis of *HNF1A* and, subsequently, *HNF4A*. Patients presenting with severe, progressive hyperglycemia but who were negative for T1D autoantibodies were directly screened for *HNF1A* and then *HNF4A*. In patients with defects of the urogenital tract, *HNF1B* was analyzed first in five laboratories (Bologna, Florence, Genoa, Rome, and San Giovanni Rotondo), and multiplex ligation-dependent probe amplification was used to assess deletions. Rare MODY genes (*i.e.*, PDX1, NEUROD1, INS, ABCC8, KCNJ11) were investigated last. Most cases of neonatal diabetes mellitus (NDM) and all cases of severe insulin resistance were analyzed in Rome using sequential DNA sequencing of *KCNJ11*, *INS*, *ABCC8*, and *GATA6* for NDM and *INSR* for severe insulin resistance. Defects of chromosome 6 in patients with transient NDM were tested for in laboratories in Catanzaro and Milan. Analysis to determine the presence of Wolfram syndrome was performed in Messina, Genoa, and Bologna.

Patients with a clinical diagnosis of type 2 diabetes belonged to two groups: those referred to the clinic with symptoms of diabetes and those referred to the obesity clinic. For both groups, we gathered data on blood pressure, hemoglobin A1c (HbA1c), C-peptide, total cholesterol, and triglycerides. The present study was performed in accordance with the Declaration of Helsinki. The parents of each patient provided written informed consent.

Figure 1 synthesizes, in the form of a flowchart, the consensus reached among the centers involved in the present study regarding the clinical and laboratory steps that guided the genetic testing and, in general, the etiologic diagnosis.

Statistical analysis

Statistical analysis was performed using SPSS, version 20.0, for Mac OS (IBM Corp., Armonk, NY). $P < 0.05$ was considered statistically significant. Data are presented as the mean

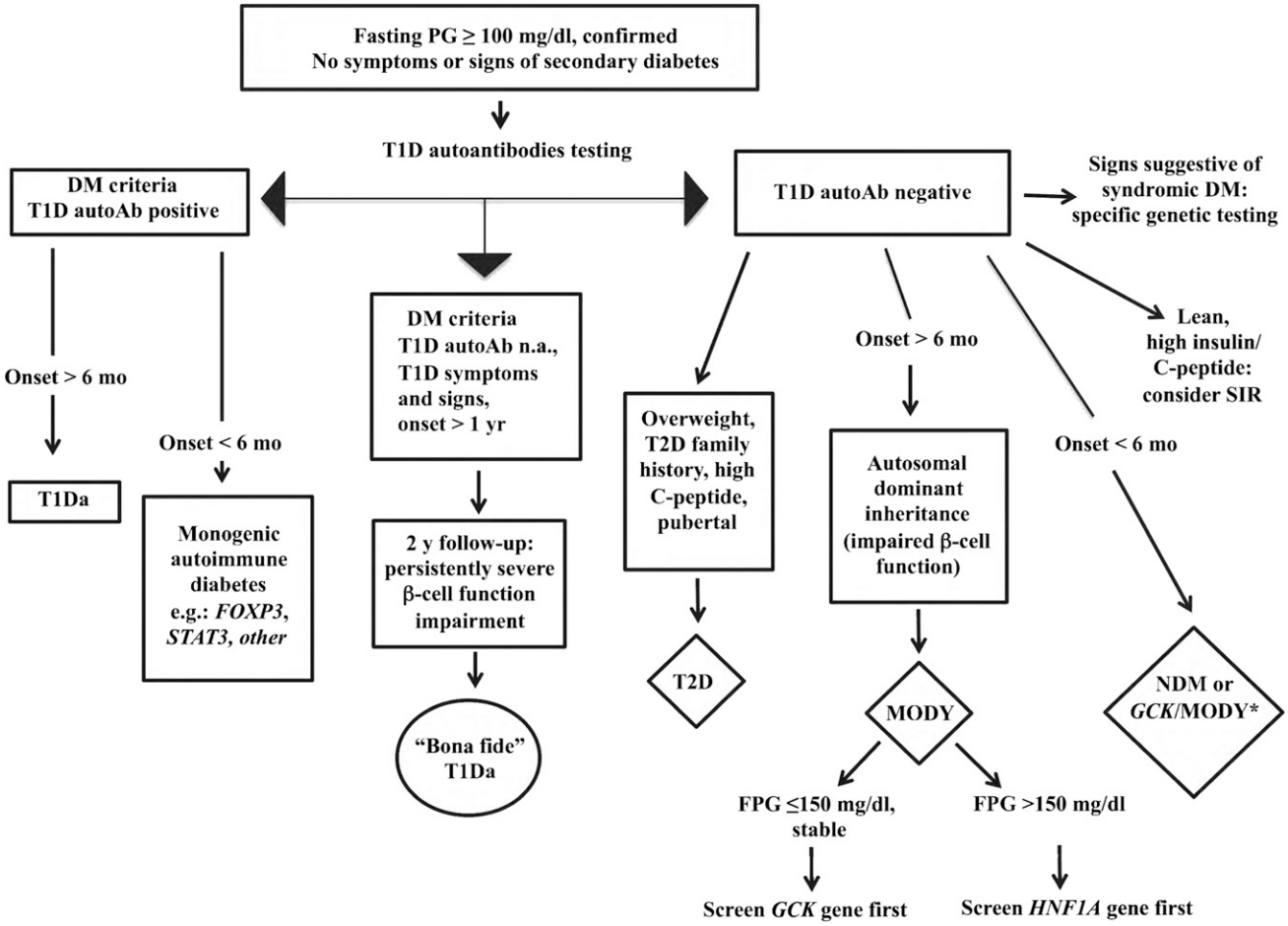

Figure 1. Stepwise flowchart of etiologic diagnoses of children's hyperglycemia. autoAb, autoantibody; DM, diabetes mellitus; FPG, fasting plasma glucose; MODY, maturity onset diabetes of the young; NDM, neonatal diabetes mellitus; n.a., not available; PG, plasma glucose; T1D, type 1 diabetes; T2D, type 2 diabetes. *Present study and Prisco *et al* (12).

± standard deviation and frequencies. Analysis of variance was used to compare the mean values between groups, and the Wilcoxon test was used to longitudinally compare the HbA1c values. The χ^2 test was used to assess the statistically significant differences between categorical variables. Paired data were analyzed using the Wilcoxon test.

Results

Type 1 diabetes

Of the 3781 patients, 3495 (92.4% of the entire data set) were clinically diagnosed with type 1 diabetes (52.3% males; Table 1). Of these 3495 patients, 3283 (93.9%) had at least one T1D autoantibody assayed, with 13.1% tested for two antibodies, 42.7% for three antibodies, 30.9% for four antibodies, and 1.2% for five antibodies. GADA were the most tested [3027 patients (86.6%)] and the most frequently positive (72%) antibodies, followed by IA-2A (positive in 65.7% of 2541 patients tested), anti-insulin antibodies (51.9% of 2646), and ICA, which were tested for less but showed greater positivity than IAA (58.6% of 1461). ZnT8A was assayed in 394 patients with a clinical diagnosis of T1D (any age) but with negative results for GADA, IA-2A, IAA, and ICA. Among those tested, 67% had positive results. As expected, when we analyzed for age at diabetes onset, we found a positive IAA test result for 67.7% of patients with diabetes diagnosed at <5 years of age. Overall, 2932 patients (90.7% of those tested) had positive results for at least one antibody. Some patients referred to the diabetes centers because of IFG had positive autoantibody test results and subsequently developed full-blown diabetes (Table 1).

Fifteen patients with negative test results for all five antibodies were provisionally classified as having T1Db (idiopathic) based on the following considerations: the cases were sporadic (*i.e.*, no family history of diabetes) and the patients had a lean body habitus and a mode of presentation typical of T1D with reduction of the insulin dose only during "honeymoon" period. Although some of these patients might bear a spontaneous MODY mutation, such as HNF1A, HNF4A, INS, KCNJ11, or ABCC8, none has undergone genetic analysis at the last follow-up point. Therefore, they were listed with the T1D patients in Table 1.

Table 1. Frequency of the Different Causes of Diabetes in 3781 Patients Referred to 15 Italian Pediatric Diabetes Clinics

Variable	T1D	T2D	MODY	NDM	Genetic Syndromes	Other	Total
Patients, n (%)	3495 (92.4)	37 (1.0)	210 (5.5)	21 (0.6)	9 (0.24)	9 (0.24)	3781
IFG/DM, %	0.2/98.8	19/81	73/27	NA	NA	NA	NA
Mean ± SD age at referral, y	8.4 ± 4.2	13.8 ± 2.4[a]	9.4 ± 4.0[b]	<6 mo	NA	NA	NA
Northern Italy	1289 (36.9)	27 (73.0)	69 (33.0)	12	3	4	1404 (37.1)
Central Italy	1102 (31.5)	3 (8.1)	59 (27.8)	6	0	1	1170 (30.9)
Southern Italy	1104 (31.6)	7 (18.9)	82 (39.2)	3	6	4	1207 (31.9)

Abbreviations: NA, not applicable; SD, standard deviation; T2D, type 2 diabetes.

The relative percentage of IFG/DM was calculated using data from 166 MODY patients; the relative percentage of patients identified in each Italian macroregion within homogeneous diagnostic groups is given in parentheses.

[a]Compared with T1D, $P < 0.001$.

[b]Compared with T1D, $P = 0.003$; MODY vs T2D, $P < 0.001$.

Ethnicity was available for 2881 patients: 88.6% were born of Italian parents, 4.2% of another white ethnicity, 3.4% of North African parents, and 1.3% of other African countries. The remaining 2.5% were born of parents from other minorities.

Type 2 diabetes

Thirty-seven patients (1%; female/male ratio, 1:2) were clinically classified as having type 2 diabetes (Table 1). All 37 patients were overweight or obese according to the body mass index z-score, with negative results for T1D-related autoantibodies. The mean fasting C-peptide (available for 29 patients) was 3.77 ng/mL (interquartile range, 1.9 to 4.8). Of the 32 patients whose liver ultrasound imaging and liver enzyme test results were available, 21 showed signs of nonalcoholic fatty liver disease. Twenty-two (0.58% of the total) presented with diabetes symptoms. Among those without symptoms, 7 presented with IFG and were classified as having diabetes on oral glucose tolerance testing (OGTT; Table 1). As expected, the mean HbA1c value was higher in the symptomatic patients [10.4% (90 mmol/mol) vs 7.2% (55 mmol/mol); $P < 0.001$]. These patients were also older (mean age, 14.6 years vs 12.7 years; $P < 0.01$). No difference was found in C-peptide, total cholesterol, triglycerides, or blood pressure between the two groups.

Monogenic diabetes

For all cases, mutation was confirmed by Sanger sequencing in both strands, and segregation of the mutation was ascertained in the probands' parents. A total of 240 patients (MODY plus NDM plus genetic syndromes; *i.e.*, 6.3% of our sample) had a genetic mutation (Table 1).

GCK/MODY, HNF1A/MODY, HNF4A/MODY, HNF1B/MODY

Most patients with a final genetic diagnosis of MODY (all genes) had presented with IFG at referral (73%; Table 1). The most common form of monogenic diabetes was GCK/MODY, with 181 mutations (4.7% of the entire data set), 21 of which were novel according to HGDMpro (Table 2), followed by *HNF1A*/MODY (16 mutations; 2 novel), *HNF4A* (6 mutations), and *HNF1B* (3 mutations). Three probands with *HNF4A*/MODY carried novel mutations, two of which will be presented in a separate report. Of the 3 patients diagnosed with *HNF1B*/MODY, 1 had total deletion of one allele and 2 carried known mutations. Patient age at referral to the diabetes centers for those with *GCK*/MODY, *HNF1A*/MODY, and *HNF4A*/MODY was 9.0 ± 4 years (range, 0.1 to 17.95), 13.0 ± 2.8 years (range, 6.8 to 17.5), and 10.8 ± 2.3 years (range, 7.2 to 15.2), respectively. Patient age at the molecular diagnosis was 9.5 (*GCK*), 13.4 (*HNF1A*), and 11.9 (*HNF4A*) years. Not surprisingly, individuals with a GCK mutation often presented with IFG (117 of 146 for whom data were available; 80%), and 75% (12 of 16) of those with HNF1A had plasma glucose values greater than the diabetic threshold.

PDX1/MODY, INS/MODY, ABCC8/MODY

Two novel *PDX1* variants were considered pathogenic and will be reported separately. A new INS/MODY mutation (c.125C>T, p.Val42Ala) identified in the proband and three family members with diabetes has been recently reported (13). The index case was classified as IFG according to the fasting glucose values and HbA1c but OGTT showed diabetes (13). A patient carried an already described *ABCC8* mutation (*ABCC8*/G1479R) in a heterozygous state, previously found to be associated with hyperinsulinemic hypoglycemia (14). The proband presented with diabetes at 12 years of age. His mother, who carried the mutation, was diagnosed with gestational diabetes mellitus during her first pregnancy and had persisting hyperglycemia after delivery and during each of two subsequent pregnancies. The elder brother of the proband, a mutation carrier, was classified as having diabetes on OGTT at the age of 24 years but was initially tested for hypoglycemia.

Table 2. Novel Mutations in *GCK*, *HNF1A*, *HNF4A*, and *WFS1* Genes (According to HGDMpro)

Gene/Location	Mutation Type	Nucleotide Change (HGVS)	Predicted Protein Change	HGVS	Phenotype
GCK/Exon 2	Deletion	c.48_50delAGA	p.E17del	p.Glu17fs	MODY
GCK/Exon 2	Missense	c.167A>G	p.Lys56Arg	K56R	MODY
GCK/IVS2	Splice	c.208+1G>T	NA		MODY
GCK/Exon 4	Missense	c.457C>T	p.Pro153Ser	P153S	MODY
GCK/Exon 4	Missense	c.466C>A	p.His156Asn	H156N	MODY
GCK/Exon 4	Missense	c.475A>T	p.Ile159Phe	I159F	MODY
GCK/Exon 7	Missense	c.685G>T	p.Gly229Cys	G229C	MODY
GCK/Exon 7	Missense	c.688T>G	p.Cys230Gly	C230G	MODY
GCK/Exon 7	Missense	c.763A>C	p.Thr255Pro	T255P	MODY
GCK/Exon 7	Deletion	c.775_777delGCC	p. p.Ala259del	p.Gly258_Phe260del	MODY
GCK/Exon 7	Stop	c.859C>T	p. Gln287Ter	Q287*	MODY
GCK/Exon 8	Missense	c.925C>G	p.Leu309Val	L309V	MODY
GCK/Exon 8	Deletion	c.960_970del	p.Ala320del	p.Glu319_fs	MODY
GCK/Exon 8	Missense	c.1019G>A	p.Ser340Asn	S340N	MODY
GCK/Exon 9	Missense	c.1180C>A	p.Arg394Ser	R349S	MODY
GCK/Exon 9	Insertion	c.1182insA	p. p.R394ins	p.Glu395fs	MODY
GCK/Exon 9	Missense	c.1222G>A	p.Val408Met	V408M	MODY
GCK/Exon 9	Missense	c.1228C>G	p.Gly410Arg	G410R	MODY
GCK/Exon 10	Missense	c.1310C>T	p.Thr437Ile	T437I	MODY
GCK/Exon 10	Missense	c.1318G>A	p.Glu440Lys	E440K	MODY
GCK/Exon 10	Insertion/duplication	c.1332_1333dupGC	p.Gly444delins	p.Gly444fs	MODY
HNF1A	Missense	c.226G>A	p.Asp76Asn	D76N	MODY
HNF1A	Insertion	c.1182insA	p.P394ins	p.Pro394fs	MODY
HNF4A	Splice site	c.426+1G>A	NA		MODY
WFS1	Insertion/duplication	c.2155_2168dup14	Phe725fs (+ Gly702Ser)	p.F725fs	Wolfram

Abbreviations: HGFV, Human Genome Variation Society; NA, not applicable.

Neonatal diabetes mellitus

NDM was initially diagnosed in 22 patients, 13 with transient NDM (TNDM). TNDM was caused in four cases by defects in chromosome 6 and in four by previously described mutations of genes encoding for the potassium adenosine triphosphate channel *ABCC8* (R1380C; V1523M) or *KCNJ11* (R50Q; E229K). In two patients, genetic screening of common TNDM genes was incomplete (*i.e.*, investigation of *ABCC8*, *KCNJ11*, or UDP6 and methylation defects were missing). In the other two patients, the results of screening of known genetic causes of TNDM (including *INS* gene promoter mutations) were negative, and the origin of TNDM remained elusive. In 1 case, a previously described heterozygous mutation of GCK was identified, and the clinical diagnosis was modified to *GCK*/MODY. Thus, the final count of the NDM cases was 21 (Table 1).

Among the patients with permanent NDM, six carried a *KCNJ11* mutation (H46Y, V59M, R201C, R201H, E322K; one novel mutation will be reported separately) and one carried the previously described *INS* mutation R89C. One patient with pancreatic agenesis (deceased at 1 month old) had negative results for *PDX1* and *GATA6* mutations, and one patient with syndromic NDM (multicystic kidney, choanal atresia) had negative results in the search of mutations in *HNF1B*. Two patients carrying a *KCNJ11* mutation were not of Italian ancestry (one Chinese and one from North Africa).

Other forms of monogenic diabetes

Wolfram syndrome. Wolfram syndrome was diagnosed in four patients. Of these four patients, three carried homozygous or compound heterozygous *WFS1* gene mutations (1 novel heterozygous mutation; Table 2) and one, a *CISD2* mutation, leading to Wolfram syndrome 2, which has been reported recently (15).

Thiamine-responsive megaloblastic anemia. In 1 patient with a diagnosis of anemia, deafness, and diabetes, a compound heterozygous mutation of *SLC19A2* was identified and reported previously (16).

Severe insulin resistance syndromes. Four patients presenting with congenital, severe insulin resistance (Donohue syndrome, Rabson-Mendenhall syndrome) bore biallelic mutations of the *INSR* gene; two of these patients were of North African origin. All these cases have been previously reported (17).

Non-T1D, unclassified; other

In a small number of patients clinically classified as having MODY, genetic screening elicited inconclusive

results, and these patients were included in the column of "Other" in Table 1.

Effect of Molecular Diagnosis on Therapeutic Aspects

GCK/MODY

Of the 136 patients with GCK/MODY for whom treatment data were available, 126 (92.6%) were not receiving therapy and 7 (5.1%) were consuming a specific diet. Only three patients were taking insulin, with one patient taking insulin plus metformin. After the molecular diagnosis, the patients with no therapy increased to 131, 2 patients were consuming a specific diet, and 2 patients continued insulin therapy (refusal of the parents to stop insulin); 1 patient was lost to follow-up. The mean HbA1c was 6.4% (46 mmol/mol) before and 6.2% (44 mmol/mol) 6 months after the molecular diagnosis.

HNF1A/MODY

Data were available for all 16 patients with an HNF1A mutation. Before the genetic diagnosis, six patients had had no therapy, one was receiving dietary therapy, three were taking oral hypoglycemic agents [one, sulfonylurea (SU); two, metformin], and six were taking insulin. After the diagnosis of *HNF1A*/MODY, five of the patients taking insulin were successfully transferred to either SUs (four patients) or repaglinide, and one could not reach optimal control with SU and was switched back to insulin. Another two patients, one with no therapy and one taking metformin, started SU, and one patient taking metformin stopped the drug. Four patients remained free of therapy. The mean HbA1c of patients without therapy or dietary therapy was 6.5% (48 mmol/mol) before and 6% (42 mmol/mol) 6 months after molecular diagnosis. For those taking insulin at the moment of genetic testing was 8.2% (66 mmol/mol) and 6.7% (50 mmol/mol; P = 0.028; Wilcoxon test) after 6 months (four patients taking SU).

HNF4A/MODY

At the clinical diagnosis, only one of the six patients with HNF4A/MODY was taking insulin and one was receiving dietary therapy. After mutation identification, five patients had no treatment, and one continued insulin. The HbA1c level was 5.9% (41 mmol/mol) at presentation and had not changed 6 months after the genetic diagnosis.

ABCC8/MODY

An attempt to switch the proband with ABCC8/MODY and his mother to SUs failed.

Permanent NDM

All six patients with *KCNJ11* mutations associated with the permanent form of NDM, including the carrier of the novel mutation, were successfully transferred from insulin to SUs.

Discussion

The present results show that monogenic diabetes accounts for ≥6.3% of all patients presenting to pediatric diabetes clinics for diabetes or IFG. Also, MODY alone, at 5.5%, represents the second prevailing cause of hyperglycemia after T1D in Italian youth. The prevalence of MODY, the most common cause of monogenic diabetes, has currently been estimated at 1% to 2% of diabetes cases (18). If we exclude those patients classified with IFG, the MODY mutations represented about 1.85% of our data set, a percentage in line with the calculation of the MODY quota in patients with diabetes by Fajans and Bell (18). Nevertheless, it should be remembered that patients carrying GCK mutations, even if their fasting plasma glucose exceeds the threshold for diabetes, are almost invariably asymptomatic. Thus, decision making about genetic testing should not only rely on symptoms or signs of diabetes but also on careful clinical evaluation of any infant with a fasting plasma glucose level chronically >100 mg/dL (5.5 mol/mol).

In this context, the usefulness of T1D-related autoantibodies as a first step in the diagnostic process is shown by the results from the present study. The patients in our study presenting with IFG had no positive results from autoantibody testing (Table 1). These findings have confirmed previous findings from the Italian Society of Pediatric Endocrinology and Diabetology diabetes study group showing that in a group of 748 patients with incidental hyperglycemia (>100 mg/dL; twice), 10%, 4.6%, and 4.9% tested positive for ICA, GADA, and IA-2A, respectively, some of whom developed full-blown T1D within 42 months (19). Thus, the application of T1D autoantibody testing, in addition to the clinical criteria for an etiologic diagnosis of T1D, might in part explain why our results outweigh the percentage of MODY mutations found (0.65%) in a large cohort of German/Austrian patients with a diagnosis of diabetes aged <20 years (3), a number that did not change much even after "reclassification" as MODY for patients initially categorized as type 2 diabetes (20). The very same conclusion can be drawn from a comparison of our results with those of SEARCH: after analysis of three MODY genes (*GCK*, *HNF1A*, and *HNF4A*) in patients selected for negativity of T1D autoantibodies and C-peptide levels of ≥0.8 ng/mL, the estimated prevalence was 1.2% (21). In the SEARCH study, a *GCK* mutation was identified in 14 of

47 patients with MODY mutations (29%; the proportion of *HNF1A* mutations found was 55%). In contrast, *GCK* mutations accounted for 86% of MODY cases (180 of 209) of our study and *HNF1A* for only 7.6%. We do not have an evidenced-based explanation for this difference. However, remembering the metabolic phenotype of patients carrying heterozygous, loss-of-function GCK mutations, we can hypothesize that the capillary presence in the Italian national territory of family pediatricians specifically following up individuals from birth to 14 years of age maximizes the referral of children with slightly supranormal (*i.e.,* >100 mg/dL) fasting plasma glucose levels to diabetes pediatric clinics. This interpretation seems to be supported by our finding that the mean age of MODY patients at presentation (9.4 years; Table 1) was 2 full years less than that reported by the SEARCH investigators (11.5 years) (21). Another interesting observation was the quite similar repartition of patients carrying MODY mutations among Northern, Central, and Southern Italy (33%, 27.8%, and 39.2% of the total number of MODY patients, respectively), a result that seems at odds with that of the United Kingdom (22), where referrals decreased according to the distance from the center offering genetic testing. We believe that this result is likely linked to the even distribution of MODY molecular genetics laboratories that serve as "hubs" for our three macroregions, allowing easy referral and access to genetic testing for the most common form of monogenic diabetes in youth (*i.e.,* GCK, HNF1A, HNF4A). In addition, and of note, only three GCK patients were taking insulin before genetic testing, a result that confirms the clinical savviness of Italian pediatric diabetologists. Moreover, the even distribution of MODY subtypes throughout Italy suggests that the greater prevalence of GCK mutations resulted from a recruitment "bias" rather than a different genetic background.

MODY/NDM patients carrying mutations in specific genes (*e.g., HNF1A*, *HNF4A*, *KCNJ11*, and *ABCC8*) can respond to SUs or mitiglinides. The switch to SUs was successful in all patients with *HNF1A* mutations identified in the present study, except for one. This result seems pertinent, considering that the guidelines from the International Society for Pediatric and Adolescent Diabetes (ISPAD) promoting the switch from insulin to SU or mitiglinides in patients diagnosed with HNF1A/MODY (23) are not always carefully followed (24). No patient with *KCNJ11*/NDM presented with epilepsy and developmental delay [DEND (developmental delay, epilepsy, and NDM) syndrome], a combination sometimes bound to SU primary failure (25), and all were easily switched from insulin to glyburide. In contrast, a trial with SUs could not control hyperglycemia in the proband with *ABCC8* dominant-negative mutation or in his mother.

Another interesting aspect of our results was the low prevalence of type 2 diabetes, which accounted for only 1% of the whole data set (with symptomatic patients a mere 0.58%), a percentage similar to that obtained in the DPV-Wiss (3, 20). Also, this result seems robust, because the data used to support each clinical diagnosis, including T1D-related autoantibodies as recommended by the ISPAD guidelines (26), were collected for all patients, which should reduce the margin of error.

Our study had limitations. First, we mainly included tertiary centers for pediatric diabetes that were mostly based in university hospitals and treating a large number of patients. A recent survey on the organization and regional distribution of pediatric diabetes centers in the Italian territory (27) identified a total number of 68 centers caring for 15,563 children and adolescents with diabetes. Therefore, our sample might not represent the "real world," having excluded in part the diabetes clinics treating a small number of patients and with reduced access to T1D-related autoantibody determination and genetic testing. In addition, we did not include centers located in Sardinia—the region with the greatest incidence of T1D in Italy and ranking second in the Western world—which are reported to monitor 2610 patients with autoimmune diabetes (27).

A second limitation was that even in the privileged setting of the 15 centers examined, a relevant number of patients clinically classified as having T1D had no autoantibody performed to confirm the diagnosis. This might have hampered the discovery of sporadic cases carrying mutations in MODY genes such as HNF1A, which can mimic T1D at onset. It has been shown that these mutations can be identified in patients without high-risk HLA haplotypes and negative to T1D autoantibodies (28). This is especially true if one considers that the current ISPAD consensus states that a positive single antibody is sufficient to confirm the diagnosis (29). Type 1 diabetes is characterized by a profound defect of insulin secretion (29) that may temporarily remit (honeymoon period) during the first year after disease onset (30). Thus, the observation after 2 years of diagnosis (*i.e.,* well beyond typical honeymoon) of a continued insulin treatment at full dose, combined with low/undetectable C-peptide, is usually considered a realiable clinical surrogate of autoantibodies and warrants a diagnosis of type 1 diabetes. The National Institute for Health and Care Excellence recommends considering autoantibody testing if it can serve as guidance for genetic testing (31).

In conclusion, in this large sample of patients referred to tertiary pediatric diabetes clinics scattered throughout Italy, the diagnosis of monogenic diabetes cases

confirmed by genetic testing reached 6.3%, two full percentage points beyond the highest (estimated) data published to date (32).

Acknowledgments

List of contributors: Lucia Ferrito, Antonio Iannilli (Ancona), Stefano Zucchini (Bologna), Gabriella Levantini, Paola Cipriano (Chieti), Barbara Piccini (Florence), Nicola Minuto (Genoa), Fortunato Lombardo, Claudia Ventrici (Messina), Riccardo Bonfanti, Andrea Rigamonti (Milan) Barbara Predieri (Modena), Adriana Franzese, Pietro Buono (Naples, Federico II), Angela Zanfardino, Santino Confetto, (Naples, Second University), Marco Cappa, Danila Benevento (Rome), Maria Giovanna Ignaccolo, Davide Tinti (Turin), Roberto Franceschi (Trento), and Claudio Maffeis (Verona).

Address all correspondence and requests for reprints to: Fabrizio Barbetti, MD, PhD, Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Via Montpellier 1, Rome 00133, Italy. E-mail: fabrizio.barbetti@uniroma2.it

Author contributions: all authors and contributors researched the data; F.B. wrote and critically revised the manuscript and performed statistical analyses; E.M., M.M., D.I., V.C., and L.I. critically revised the manuscript; and E.M. and M.M. edited the manuscript.

Disclosure Summary: The authors have nothing to disclose.

References

1. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. *Endocrinol Metab Clin North Am*. 2010;**39**(3):481–497.
2. 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.
3. 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 and. 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 multicentre database. *Diabet Med*. 2009;**26**(5):466–473.
4. Pettitt DJ, Talton J, Dabelea D, Divers J, Imperatore G, Lawrence JM, Liese AD, Linder B, Mayer-Davis EJ, Pihoker C, Saydah SH, Standiford DA, Hamman RF; SEARCH for Diabetes in Youth Study Group. Prevalence of diabetes in U.S. youth in 2009: the SEARCH for diabetes in youth study. *Diabetes Care*. 2014;**37**(2):402–408.
5. Mozzillo E, Salzano G, Barbetti F, Maffeis 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.
6. Carmody D, Naylor RN, Bell CD, Berry S, Montgomery JT, Tadie EC, Hwang JL, Greeley SA, Philipson LH. GCK-MODY in the US National Monogenic Diabetes Registry: frequently misdiagnosed and unnecessarily treated. *Acta Diabetol*. 2016;**53**(5):703–708.
7. Thanabalasingham G, Pal A, Selwood MP, Dudley C, Fisher K, Bingley PJ, Ellard S, Farmer AJ, McCarthy MI, Owen KR. Systematic assessment of etiology in adults with a clinical diagnosis of young-onset type 2 diabetes is a successful strategy for identifying maturity-onset diabetes of the young. *Diabetes Care*. 2012;**35**(6):1206–1212.
8. Velho G, Vaxillaire M, Boccio V, Charpentier G, Froguel P. Diabetes complications in NIDDM kindreds linked to the MODY3 locus on chromosome 12q. *Diabetes Care*. 1996;**19**(9):915–919.
9. Bacon S, Kyithar MP, Rizvi SR, Donnelly E, McCarthy A, Burke M, Colclough K, Ellard S, Byrne MM. Successful maintenance on sulphonylurea therapy and low diabetes complication rates in a HNF1A-MODY cohort. *Diabet Med*. 2016;**33**(7):976–984.
10. Steele AM, Shields BM, Wensley KJ, Colclough K, Ellard S, Hattersley AT. Prevalence of vascular complications among patients with glucokinase mutations and prolonged, mild hyperglycemia. *JAMA*. 2014;**311**(3):279–286.
11. Iafusco D, Salardi S, Chiari G, Toni S, Rabbone I, Pesavento R, Pasquino B, de Benedictis A, Maltoni G, Colombo C, Russo L, Massa O, Sudano M, Cadario F, Porta M, Barbetti F; Early Onset Diabetes Study Group of the Italian Society of Pediatric Endocrinology and Diabetology (ISPED). No sign of proliferative retinopathy in 15 patients with permanent neonatal diabetes with a median diabetes duration of 24 years. *Diabetes Care*. 2014;**37**(8):e181–e182.
12. Prisco F, Iafusco D, Franzese A, Sulli N, Barbetti F. MODY 2 presenting as neonatal hyperglycemia: a need to reshape the definition of "neonatal diabetes"? *Diabetologia*. 2000;**43**(10):1331–1332.
13. Piccini B, Artuso R, Lenzi L, Guasti M, Braccesi G, Barni F, Casalini E, Giglio S, Toni S. Clinical and molecular characterization of a novel INS mutation identified in patients with MODY phenotype. *Eur J Med Genet*. 2016;**59**(11):590–595.
14. Pinney SE, MacMullen C, Becker S, Lin Y-W, Hanna C, Thornton P, Ganguly A, Shyng S-L, Stanley CA. Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant K_{ATP} channel mutations. *J Clin Invest*. 2008;**118**(8):2877–2886.
15. Mozzillo E, Delvecchio M, Carella M, Grandone E, Palumbo P, Salina A, Aloi C, Buono P, Izzo A, D'Annunzio G, Vecchione G, Orrico A, Genesio R, Simonelli F, Franzese A. A novel CISD2 intragenic deletion, optic neuropathy and platelet aggregation defect in Wolfram syndrome type 2. *BMC Med Genet*. 2014;**15**:88.
16. Mozzillo E, Melis D, Falco M, Fattorusso V, Taurisano R, Flanagan SE, Ellard S, Franzese A. Thiamine responsive megaloblastic anemia: a novel SLC19A2 compound heterozygous mutation in two siblings. *Pediatr Diabetes*. 2013;**14**(5):384–387.
17. Grasso V, Colombo C, Favalli V, Galderisi A, Rabbone I, Gombos S, Bonora E, Massa O, Meschi F, Cerutti F, Iafusco D, Bonfanti R, Monciotti C, Barbetti F. Six cases with severe insulin resistance (SIR) associated with mutations of insulin receptor: is a Bartter-like syndrome a feature of congenital SIR? *Acta Diabetol*. 2013;**50**(6):951–957.
18. Fajans SS, Bell GI. MODY: history, genetics, pathophysiology, and clinical decision making. *Diabetes Care*. 2011;**34**(8):1878–1884.
19. Lorini R, Alibrandi A, Vitali L, Klersy C, Martinetti M, Betterle C, d'Annunzio G, Bonifacio E; Pediatric Italian Study Group of Prediabetes. Risk of type 1 diabetes development in children with incidental hyperglycemia: a multicenter Italian study. *Diabetes Care*. 2001;**24**(7):1210–1216.
20. 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.
21. Pihoker C, Gilliam LK, 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; SEARCH for Diabetes in Youth Study Group. 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**:4055–4062.
22. 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.
23. Rubio-Cabezas O, Hattersley AT, Njolstad PR, Mlynarski W, Ellard S, White N, Chi DV, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2014 Compendium. The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*. 2014;**15**(Suppl. 20):47–64.
24. Raile K, Schober E, Konrad K, Thon A, Grulich-Henn J, Meissner T, Wölfle 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.
25. Hattersley AT, Ashcroft FM. Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. *Diabetes*. 2005;**54**(9):2503–2513.
26. Zeitler P, Fu J, Tandon N, Nadeau K, Urakami T, Bartlett T, Maahs D; International Society for Pediatric and Adolescent Diabetes. ISPAD Clinical Practice Consensus Guidelines 2014. Type 2 diabetes in the child and adolescent. *Pediatr Diabetes*. 2014;**15**(Suppl 20):26–46.
27. Giorgetti C, Ferrito L, Zallocco F, Iannilli A, Cherubini V; Study Group for Diabetes of ISPED. Organization and regional distribution of centers for the management of children and adolescents with diabetes in Italy. *Ital J Pediatr*. 2015;**41**:74.
28. Lambert AP, Ellard S, Allen LIS, Gallen IW, Gillespie KM, Bingley PJ, Hattersley AT. Identifying hepatic nuclear factor 1α mutations in children and young adults with a clinical diagnosis of type 1 diabetes. *Diabetes Care*. 2003;**26**(2):333–337.
29. 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.
30. Karges B, Meissner T, Icks A, Kapellen T, Holl RW. Management of diabetes mellitus in infants. *Nat Rev Endocrinol*. 2011;**8**(4):201–211.
31. Hammond P. Improving the detection and management of type 1 diabetes. *Practitioner*. 2016;**260**(1789):21–26, 3.
32. 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.