Monogenic diabetes accounts for 6.3% of cases referred to 15 Italian pediatric diabetes centers during 2007 to 2012

Monogenic Diabetes accounts for 6.3% of cases referred to 15 Italian pediatric diabetes Centers during 2007-2012.

Maurizio Delvecchio, Enza Mozzillo, Giuseppina Salzano, Dario Iafusco, Giulio Frontino, Patrizia I Patera, Ivana Rabbone, Valentino Cherubini, Valeria Grasso, Nadia Tinto, Sabrina Giglio, Giovanna Contreas, Rosa Di Paola, Alessandro Salina, Vittoria Cauvin, Stefano Tumini, Giuseppe d'Annunzio, Lorenzo Iughetti, Vilma Mantovani, Giulio Maltoni, Sonia Toni, Marco Marigliano, Fabrizio Barbetti, and the Diabetes Study Group of the Italian Society of Pediatric Endocrinology and Diabetes (ISPED).

The Journal of Clinical Endocrinology & Metabolism
Endocrine Society

Submitted: June 27, 2016
Accepted: February 07, 2017
First Online: February 16, 2017

The Journal of Clinical Endocrinology & Metabolism; Copyright 2017 DOI: 10.1210/jc.2016-2490

Monogenic Diabetes accounts for 6.3% of cases referred to 15 Italian pediatric diabetes Centers during 2007-2012.

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], Fabrizio Barbetti[9,21], and 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, Italy

2. Department of Translational Medical Science, Section of Pediatrics. Regional Center of Pediatric Diabetology, University of Naples Federico II, Naples, Italy

3. Department of Pediatric Sciences, University of Messina, Messina, Italy

4. Regional Center for Pediatric Di San Raffabetes "G. Stoppoloni", Department of Pediatrics, Second University of Naples, Naples, Italy

5. Department of Pediatrics, Endocrine Unit, Diabetes Research Institute (OSR-DRI), Scientific Institute Hospital San Raffaele, Milan, Italy

6. Endocrinology and Diabetes Unit, University Department of Pediatric Medicine, Bambino Gesù Children Hospital, Rome, Italy

7. Department of Pediatrics, University of Turin, Regina Margherita Hospital, Turin, Italy

8. S.O.D. Pediatric Diabetes, Department of Women's and Children Health, Salesi Hospital, Ancona, Italy

9. Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy

10. Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, and CEINGE, Advanced Biotechnology, Naples, Italy

11. Medical Genetics Unit, Department of Clinical and Experimental Biomedical Sciences "Mario Serio", and Meyer Children's University Hospital, University of Florence, Florence, Italy

12. Pediatric Diabetes and Metabolic Disorders Unit, Department of Surgical Science, Dentistry, Ginecology and Pediatrics, University of Verona, Verona, Italy

13. Research Unit of Diabetes and Endocrine Disease, IRCCS Casa del Sollievo della Sofferenza, S. Giovanni Rotondo, Italy

14. Istituto Giannina Gaslini, Regional Center for Pediatric Diabetes, Genoa, Italy

15. Pediatric Unit, S. Chiara Hospital, Trento, Italy

16. Center of Pediatric Diabetology, University of Chieti, Chieti, Italy

17. Pediatric Unit, Department of Medical and Surgical Sciences for Mothers, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy

18. Center for Applied Biomedical Research (CRBA) and Medical Genetics Unit, S. Orsola University Hospital, Bologna, Italy

19. Department of Pediatrics, S. Orsola-Malpighi University Hospital, Bologna, Italy

20. Juvenile Diabetes Center, Meyer Children's Hospital, Florence, Italy

21. Bambino Gesù Children Hospital, IRCCS, Rome, Italy

Received 27 June 2016. Accepted 07 February 2017.

Context: Etiologic diagnosis of diabetes may impact on therapeutic strategy, and prognosis of chronic complications.
Objective: The aim of the study was to establish the relative percentage of different diabetes subtypes in patients attending Italian pediatric diabetes centers, and the influence of etiologic diagnosis on therapy.
Design, Setting and Patients: This was a retrospective study. Clinical records of 3781 consecutive patients (age: 0-18 years) referred to fifteen pediatric diabetes clinics and diagnosed with diabetes or IFG between Jan/1/2007 and Dec/31/2012 were examined. Clinical characteristics of patients at the first time of referral to Centers, type 1 diabetes-related autoantibodies, molecular genetics records, and C-peptide measurement, if requested for etiologic diagnosis, were acquired.
Main outcome measures: The primary outcome was to assess the percentage of each diabetes subtype of our sample.
Results: Type 1 diabetes represents the main cause (92.4%) of diabetes of this group of patients, followed by monogenic diabetes, that accounts for 6.3 % of cases (Maturity Onset Diabetes of the Young; MODY: 5.5%, Neonatal Diabetes Mellitus**;** NDM: 0.6%, genetic syndromes: 0.2%). Of interest, genetic diagnosis prompted the transfer from insulin to sulphonylureas in twelve patients bearing mutations in *HNF1A* or *KCNJ11* genes. Type 2 diabetes was diagnosed in 1% of patients.
Conclusions. Monogenic diabetes is highly prevalent in patients referred to Italian pediatric diabetes centers. Genetic diagnosis guided therapeutic decisions, allowed to formulate a prognosis on chronic diabetic complications in a relevant number of patients (i.e. *GCK*/MODY), and helped to provide genetic counseling.

PRECIS: We evaluated diabetes etiology in 3781 pediatric patients and found that genetic mutations are the second cause of hyperglycemia after type 1 diabetes, while type 2 diabetes is relatively rare.

INTRODUCTION

Type 1 diabetes is the most prevalent cause of diabetes of youth in North America and Europe (1), while in other continents/countries (e.g. China) much lower incidence per year is reported. Differently, 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 due to a number of reasons, including errors in clinical diagnosis (6). The identification of the exact etiologic cause of diabetes is important, because can direct therapeutic decisions and influence genetic counseling (7).

Aim of the present study is to assess the prevalence of the different etiologies of diabetes mellitus in a large group of patients with age at diagnosis <18 years referred to tertiary diabetes Centers representative of peninsular Italy. Our data show that monogenic diabetes is the second prevailing cause of diabetes after type 1 diabetes in Italian youth, and that the correct etiologic diagnosis greatly impacts on treatment, and likely on prognosis of diabetic complications (8-11).

METHODS.

Data on 3,781 patients consecutively diagnosed with diabetes or impaired fasting glucose during a 6-year period from January 1st 2007 to December 31st 2012, and age at diagnosis of diabetes/IFG <18 years were collected from 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 type 1 diabetes autoantibodies (ICA, GADA, IA-2A, IAA and ZnT8A). For patients with a clinical diagnosis of monogenic diabetes

confirmed by genetic testing, the mutation(s) identified was obtained and checked for novelty utilizing HGMDpro®; in addition, details on therapy before and after genetic diagnosis were acquired. Genetic analysis of common Maturity Onset Diabetes of the Young (MODY) genes (i.e. *GCK*, *HNF1A*, *HNF4A*) has been 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 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 was identified, the screening was stopped. Instead the screening continued with the analysis of HNF1A and subsequently HNF4A gene, if GCK resulted negative. Patients presenting with severe, progressive hyperglycemia, but negative for T1D autoantibodies were directly screened for HNF1A and then HNF4A. In patients with defects of uro-genital tract, HNF1B was analyzed first in five laboratories (Bologna, Florence, Genoa, Rome and San Giovanni Rotondo), and multiplex ligation-dependent probe amplification (MLPA) was used to assess deletions. Rare MODY genes (i.e. PDX1, NEUROD1, INS, ABCC8, KCNJ11) were investigated lastly. Most cases of neonatal diabetes mellitus (NDM) and all cases with severe insulin resistance (SIR) were analyzed in Rome by sequential DNA sequencing of *KCNJ11*, *INS*, *ABCC8* and *GATA6* for NDM, and of *INSR* for SIR. Defects of chromosome 6 in patients with transient NDM were performed in laboratories in Catanzaro and Milan. Analysis of Wolfram syndrome was performed in Messina, Genoa and Bologna.

Patients with a clinical diagnosis of type 2 diabetes belonged to two groups: those who were referred to the clinic with symptoms of diabetes and those referred to the obesity clinic; for both groups we gathered data on blood pressure, HbA1c, C-peptide, total cholesterol and triglycerides. The study was conducted according to the Declaration of Helsinki. Parents of each patient provided written informed consent.

Figure 1 synthesizes in the form of flow-chart a consensus reached among the Centers involved in the present study on the clinical/laboratory steps that guided to genetic testing and, in general, to etiologic diagnosis.

Statistics.

The statistical analysis was run with SPSS version 20.0 for Mac OS. A p value < 0.05 was considered statistically significant. Data are presented as mean ± standard deviation (SD) and frequencies. Analysis of variance (ANOVA) test was used to compare mean values between groups, the Wilcoxon test to compare longitudinally HbA1c. The Chi-square test was used to assess the statistical difference between categorical variables. Paired data were analyzed by Wilcoxon test.

RESULTS

Type 1 diabetes.

Three-thousands four-hundred ninety-five patients, 92.4% of the entire data set, were clinically diagnosed with type 1 diabetes (52.3% males) (Table 1), 93.9% of which (3,283 patients) had at least one type 1 diabetes autoantibody assayed, with 13.1% tested for 2 antibodies, 42.7% for 3 antibodies, 30.9% for 4 antibodies and 1.2% for 5 antibodies. GADA were the most tested (3,027 patients, 86.6%) and the most frequently positive (72%) antibodies, followed by IA-2A (positive in 65.7% out of 2,541 patients tested), anti-insulin antibodies (51.9% out of 2,646), and ICA, that were the less tested but showed higher positivity than IAA (58.6% out of 1,461). ZnT8A was assayed in 394 patients clinically diagnosed with type 1 diabetes (any age), but negative to GADA, IA-2A, IAA and ICA; among those tested, 67% resulted positive**.** As expected, when we analyzed for age at diabetes onset, we found a positive IAA test in 67.7% of patients with diabetes diagnosed below five years of age. Overall, 2,932 patients (90.7% of those tested) were positive to at least 1 antibody. Of note, some patients referred to

diabetes Centers as IFG were autoantibody positive, and subsequently developed full-blown diabetes (Table 1).

Fifteen patients who resulted negative to all five antibodies were provisionally classified as type 1 diabetes b, based on the following considerations: they were sporadic cases (i.e. no family history of diabetes), had a lean body habitus, a mode of presentation typical of type 1 diabetes with reduction of insulin dose only during "honey moon" period. Though some of these patients may bear a spontaneous MODY mutation, such as HNF1A, HNF4A, INS, KCNJ11 or ABCC8, none has been subjected to genetic analysis at this time. For this reason they are listed together with the type 1 diabetes in Table 1.

Ethnicity was available for 2,881 patients: 88.6% were born from Italian parents, 4.2% from other white ethnicity, 3.4% from North African parents, and 1.3% from other African Countries. The remaining 2.5% were born from parents of other minorities.

Type 2 diabetes.

Thirty-seven patients (1%; female to male ratio 1.2) were clinically classified as type 2 diabetes (Table 1)**;** all were overweight or obese according to BMI z-score, and negative to T1D-related autoantibodies. Mean fasting C-peptide (available for 29 patients) was 3.77 ng/ml (interquartile range 1.9-4.8 ng/ml). Twenty-one patients (out of 32 whose liver ultrasound imaging and liver enzymes were available) showed signs of non-alcoholic fatty liver disease. Twenty-two (0.58% of the total) presented with diabetes symptoms. Among those without symptoms, 7 presented as IFG and were classified diabetic at OGTT (Table 1). As expected, mean HbA1c value was higher in symptomatic patients (10.4% [90 mmol/mol] vs 7.2% [55 mmol/mol], $p < 0.001$), who were also older (mean age 14.6 years vs 12.7, $p<0.01$); no difference in C-peptide, total cholesterol, triglycerides or blood pressure was found 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 probands' parents. A total of 240 patients (MODY+NDM+genetic syndromes), i.e. 6.3% of our sample, carried a genetic mutation (Table 1).

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

Most patients with final genetic diagnosis of MODY (all genes) presented as 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 resulted novel according to HGDM professional (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 reported in a separate paper. Of the 3 patients diagnosed with *HNF1B*/MODY, one had total deletion of one allele and the other two carried known mutations. Age at referral to diabetes Centers of *GCK*/MODY, *HNF1A*/MODY and *HNF4A*/MODY was 9.0 ±4 (0.1-17.95), 13.0 ±2.8 (6.8-17.5) and 10.8 ± 2.3 years (7.2-15.2) respectively, and age at molecular diagnosis was 9.5 (*GCK)*, 13.4 (*HNF1A*), and 11.9 (*HNF4A*) years. Not surprisingly, individuals with GCK mutation often presented as IFG (117 out of 146 whose data were available; 80%), while 75% (12 out of 16) of HNF1A had plasma glucose values above 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 3 family members with diabetes has been published recently (12). Interestingly, the index case was classified as IFG according to fasting glucose values and HbA1c, but resulted diabetic at OGTT (12). A patient carried an already described *ABCC8* mutation (*ABCC8*/G1479R) in heterozygous state, previously found associated with hyperinsulinemic hypoglycemia (13). The proband presented with diabetes when 12 years old. His

mother, who bears the mutation, was diagnosed with GDM 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, has been classified as diabetic at OGTT at the age of 24, but was initially investigated for hypoglycemia.

NDM

Twenty-two patients were initially diagnosed with neonatal diabetes, thirteen of which with the transient form. TNDM was caused in 4 cases by defects of chromosome 6, and in 4 by already described mutations of genes encoding for the K_{ATP} channel *ABCC8* (R1380C; V1523M) or *KCNJ11* (R50Q; E229K). In 2 cases genetic screening of common TNDM genes was incomplete (i.e. either investigation of *ABCC8*, *KCNJ11* or UDP6 and methilation defects were missing), while in the other 2 the screening of known genetic causes of TNDM (including *INS* gene promoter mutations) was negative and the origin of transient diabetes remained elusive. In one case an already described heterozygous mutation of GCK was identified, and the clinical diagnosis modified to *GCK*/MODY. Thus, final counting of NDM cases was 21 (Table 1).

Among patients with PNDM, 6 carried a *KCNJ11* mutation (H46Y, V59M, R201C, R201H, E322K; one novel mutation will be reported elsewhere) and one the already described *INS* mutation R89C. One patient with pancreatic agenesis (deceased at one month of age) was negative for *PDX1* and *GATA6* mutations, while one patient with syndromic NDM (multicystic kidney, choanal atresia) was negative to the search of mutations in *HNF1B*. Two patients carrying a *KCNJ11* mutation were not of Italian ancestry (1 Chinese, 1 from North Africa).

OTHER FORMS OF MONOGENIC DIABETES

Wolfram syndrome.

Four patients were diagnosed with Wolfram's syndrome. Three carried homozygous or compound heterozygous *WFS1* gene mutations (1 novel heterozygous mutation) (Table 3) and one a *CISD2* mutation, leading to WFS2, that has been reported recently (14).

Thiamine-responsive megaloblastic anemia (TRMA).

In a patient diagnosed with anemia, deafness and diabetes, a compound heterozygous mutation of *SLC19A2* was identified and published (15).

Severe insulin resistance syndromes.

Four patients presenting with congenital, severe insulin resistance (Donohue syndrome, Rabson-Mendenhall syndrome) bore biallelic mutations of *INSR* gene; two of these were of North-African origin. All these cases have been previously reported (16).

Non-type 1 diabetes, unclassified; other.

In a small number of patients clinically classified as MODY, genetic screening elicited inconclusive results and were included in the column "Other" of Table 1.

IMPACT OF MOLECULAR DIAGNOSIS ON THERAPEUTIC ASPECTS.

GCK/MODY.

Among 136 patients whose data on treatment were available, 126 (92.6%) had no therapy and 7 (5.1%) were on a diet. Only 3 patients were on insulin, with one on insulin plus metformin. After molecular diagnosis, patients with no therapy increased to 131, patients on a diet were 2, and other two continued insulin therapy (refusal of the parents to stop insulin); one patient was lost at follow up. Mean HbA1c was 6.4% (46 mmol/mol) before and 6.2% (44 mmol/mol) after 6 months from molecular diagnosis.

HNF1A/MODY.

Data were available for all 16 patients with HNF1A mutation. Before genetic diagnosis six patients had no therapy, one was on a diet, 3 on oral hypoglycemic agents (1 on sulfonylurea, 2 on metformin), and

six were on insulin. After diagnosis of *HNF1A*/MODY, five of the patients on insulin have been successfully transferred to either sulfonylureas (SU; four patients), or repaglanide, while one failed to reach optimal control on SU and was switched back to insulin. Other two patients, one with no therapy and the other on metformin, were started on SU, while the other on metformin stopped the drug. Four patients remained free of therapy. Mean HbA1c of patients without therapy or on a diet was 6.5% (48 mmol/mol) before and 6% (42 mmol/mol) after six months from molecular diagnosis, while for those on 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 six months (four on SU).

HNF4A/MODY.
At clinical diagnosis, only one out of six patients was on insulin and another on diet. After mutations identification, five had no treatment, while one continued insulin; HbA1c was 5.9%, (41 mmol/mol) at presentation and did not change 6 months after genetic diagnosis.

ABCC8/MODY.
A first attempt to switch the proband and his mother to sulfonylureas failed.

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

DISCUSSION

In this paper we present evidence that monogenic diabetes can account for at least 6.3% of all cases presenting in pediatric diabetes clinic for diabetes or impaired fasting glucose, and that MODY alone, with 5.5%, represents the second prevailing cause of hyperglycemia after type 1 diabetes in Italian youth. Prevalence of MODY, the commonest cause of monogenic diabetes, is currently estimated at 1-2% of diabetes (17). If we exclude patients who were classified as IFG, MODY mutations represent about 1.85% of our data set, a figure which is in line with Fajans and Bell's calculation of MODY quota in patients with diabetes (17). Nevertheless, it must been taken into account that patients carrying GCK mutations, even if their fasting plasma glucose exceeds the threshold for diabetes, are almost invariably asymptomatic. As a consequence, decision making about genetic testing can not only rely on symptoms/signs of diabetes, but on careful clinical evaluation of any infant with fasting plasma glucose chronically exceeding 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 stressed by the fact that patients of this study presenting as IFG resulted positive to autontibodies testing (Table 1). This result confirmed previous findings from ISPED 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 respectively to ICA, GADA and IA-2A, some of which developed full-blown T1D within 42 months (18). Thus, the application of T1D autoantibody testing in addition to clinical criteria for etiologic diagnosis of type 1 diabetes may in part explain why our result outweighs the percentage of MODY mutations found (0.65%) in a large cohort of German/Austrian patients diagnosed with diabetes < 20 years of age (19), a number that did not change much even after "reclassification" as MODY of patients initially categorized as type 2 diabetes (20). The very same conclusion can be drawn from the comparison our results with those of SEARCH, that after analysis of three MODY genes (*GCK*, *HNF1A* and *HNF4A*) in patients selected for negativity of type 1 diabetes autoantibodies and C-peptide levels of 0.8 ng/ml or greater, obtained an estimated prevalence of 1.2% (21). Interestingly, in the SEARCH study a *GCK* mutation was identified in 14 cases out of 47 patients with MODY mutations, or 29% (*HNF1A* mutations found were 55%). In contrast, *GCK* mutations account for 86% of MODY cases (180/209) of our study and *HNF1A* for only 7.6%. We do not have an evidenced-based

explanation for this difference, but bearing in mind the metabolic phenotype of patients carrying heterozygous, loss-of-function GCK mutations, we can hypothesize that the capillary presence on the Italian national territory of family pediatricians specifically following individuals from birth to 14 years of age maximizes the referral of children with slightly supranormal (i.e. > 100 mg/dl) fasting plasma glucose to diabetes pediatric clinics. This interpretation seems to be supported by our finding that mean age of MODY patients at presentation (9.4 years, Table 1) is two full years below the one reported by SEARCH investigators (11.5 years) (21). Another interesting observation is the quite similar repartition of patients carrying MODY mutations between 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 UK (22), where referrals drop 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 the three macro-regions, allowing an easy referral and access to genetic testing for the commonest form of monogenic diabetes in youth (i.e. GCK, HNF1A, HNF4A). In addition, and of note, only three GCK patients were on 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 higher prevalence of GCK mutations is due to recruitment "bias" rather than different genetic background.

MODY/NDM patients carrying mutations in specific genes, e.g. *HNF1A*, *HNF4A*, *KCNJ11* and *ABCC8* can respond to sulphonylureas (SU) or metiglinides. Noteworthy, switch to SU was successful in all patients with *HNF1A* mutations identified in this study, but one. This result seems valuable, considering that ISPAD's guidelines promoting the switch from insulin to SU or metiglitinides 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 syndrome), a combination that is usually bound to SU primary failure (25), and all were easily switched from insulin to glyburide. In contrast, a first trial with sulfonylureas could not control hyperglycemia in the proband with *ABCC8* dominant-negative mutation and in his mother.

Another interesting aspect of our results is the low prevalence of type 2 diabetes, which accounts for only 1% of the whole data set (with symptomatic patients being a mere 0.58%), a percentage similar to that obtained in DPV-Wiss (19,20). Also this result seems robust, because data used to support each clinical diagnosis, including T1D-related autoantibodies as reccomended by ISPAD guidelines (26) were collected in all patients, and this should reduce the margin of error.

Our study has limitations. The first one is that it included mainly tertiary Centers for pediatric diabetes, mostly based in University hospitals and dealing with a large number of patients. A recent survey on the organization and regional distribution of pediatric diabetes centers on the Italian territory (27) identified a total number of 68 centers taking care to 15,563 children and adolescents with diabetes. Therefore our sample may not represent the "real world", having excluded in part diabetes clinics handling a small number of patients and with reduced access to type 1 diabetes-related autoantibodies determination and genetic testing. In addition, we did not include centers located in Sardinia –the region with highest incidence of type 1 diabetes in Italy and ranking second in the Western world- that are reported to follow 2,610 patients with autoimmune diabetes (27).

A second limitation is that -even in the privileged setting of the 15 centers examined- a relevant number of patients clinically classified as affected by type 1 diabetes had no autoantibody performed to confirm the diagnosis. This may have hampered the discovery of sporadic cases carrying mutations in MODY genes such as HNF1A that can mimic type 1 diabetes at onset. It has been shown that these mutations can be identified in patients with no high-risk HLA-haplotypes and negative to type 1 diabetes autoantibodies (28). This is especially true if one consider that the current ISPAD consensus states that a positive single antibody is sufficient to confirm the diagnosis (29). However, the

observation of perduring insulin treatment at full dose combined with low fasting C-peptide levels within 2 years of diagnosis, i.e. well beyond the time of onset of temporary remission of hyperglycemia one may notice in some patients with type 1 diabetes (so called "honey moon") (30), can confirm (or not) the profound defect of insulin secretion typical of autoimmune diabetes (29) and can be used as clinical surrogate of autoantibodies. Interestingly, National Institute for Health and Care Excellence (NICE) recommends to consider autoantibody testing if this 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 reaches 6.3%, two full percent points beyond the highest (estimated) figure published so far (32).

ACKNOWLEDGEMENTS

Corresponding Author and person to whom reprints requests should be addressed: Prof. Fabrizio Barbetti, MD, PhD, Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Via Montpellier 1, 00133, Rome ITALY, Phone: +39 06-2090-0672, Fax: +39 06-2090-0674, email: fabrizio.barbetti@uniroma2.it

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), Claudio Maffeis (Verona)

Author contributions:
All Authors and contributors researched data. FB wrote and critically revised the manuscript, MD performed statistics, MD, EM, MM, DI, VC, LI critically revised the manuscript, MD, EM, MM edited the manuscript.

Conflict of interest.
Authors have no conflict of interest to declare.

Disclosure Statement:
The Authors have nothing to disclose

REFERENCES

1. Maahs DM, West NA, Lawrence JM, Mayer-Davies EJ. Epidemiology of type 1 diabetes. Endocrinol Metab Clin North Am 2010;39:481-497
2. Irgens HU, Molnes J, Johansson BB, Ringdal M, Skrivarhaug T, Undlien DE, Sovik O, Joner G, Molven A, Njolstad PR. Prevalence of monogenic diabetes in the population-based Norwegian Childhood Diabetes Registry. Diabetologia 2013;56:1512–1519
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. 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;26:466–473
4. Pettitt DJ, Talton J, Dabelea D, Divers J, Imperatore G, Lawrence JM, Liese AD, Linder B, Mayer-Davis AJ, Pihoker C, Saydah SH, Standorf DA, Hamman RF; SEARCH for Diabetes in Youth

Study Group. Prevalence of Diabetes Mellitus in U.S. Youth in 2009: The SEARCH for Diabetes in Youth Study. Diabetes Care 2014;37:402-408.
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:e15-e18
6. Carmody D, Naylor RN, Bell CD, Berry S, Montgomery JT, Tadie EC, Hwang JL, Greely SAW, Philipson LH. GCK-MODY in the US monogenic diabetes registry: frequently misdiagnosed and unnecessarily treated. Acta Diabetol 2016;53:703-708
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 clinical diagnosis of yung-onset type 2 diabetes is a successful strategy for identyfing maturity-onset diabetes of the young. Diabetes Care 2012; 35:1206-1212.
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;19:915-919
9. Bacon S, Kyithar MP, Rizvi SR, Donnelly E, McCarthy A, Burke M, Colclough, Ellard S, Byrne MM. Successful maintenance on sulphonylurea therapy and low diabetes complication rates in a HNF1A-MODY cohort. Diabetic Med 2016;33:976-984
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;311:279-286
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 and the Early Onset Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetology (ISPED). No sign of proliferative retinopathy in 15 patients with Permanent Neonatal Diabetes Mellitus with a median diabetes duration of 24 years. Diabetes Care 2014;37:e181-e182
12. Piccini B, Artuso R, Lenzi L, Guasti M, Braccesi G, Barni F, Casalini E, Giglio S, Toni S. Clinical and molecular chracterization of a novel INS mutation identified in patients with a MODY phenotype. Eur J Med Genet 2016;59:590-595
13. 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;118:2877-86
14. 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
15. 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 Dabetes 2013;14:384-387
16. Grasso V, Colombo C, Favalli V, Galderisi A, Rabbone I, Gombos S, Bonora E, 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 it a Bartter-like syndrome a feature of congenital SIR. Acta Diabetol 2013;50:951-957
17. Fajans SS, Bell GI. History, genetics, pathophisiology, and clinical decision making. Diabetes Care 2011;34:1878-1884
18. Lorini R, Alibrandi A, Vitali L, Klersy C, Martinetti M, Betterle C, D'Annuzio G, Bonifacio E. Risk of type 1 diabetes development in children with incidental hyperglycemia. Diabete Care 2001;24:1210-1216

19. Schober E, Rami B, Grabert M, Thon A, Kapellen T, Reiner T, Holl RW. 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;26:466-473
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 clasified as type 2 diabetes mellitus: 15 years follow-up usung routine data from the German/Austrina DPV database. Diabetes Res Clin Pract 2011;94:463-467
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. 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;53:2504-2508
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 Diab 2014;15 (Suppl. 20):47-64
24. Raile K, Schober E, Konrad K, Thon A, Grulich-Henn J, Meissner T, Wolfle J, Scheuing N, Holl RW. Treatment of young patients with HNF1A mutations (HNF1A-MODY). Diabet Med 2015;32:526-530
25. Hattersley AT, Ashcroft FM. Activating mutations in Kir6.2 and neonatal diabetes. Diabetes 2005;54:2503-2513
26. Zeitler P, Fu J, Tandon N, Nadeau K, Urakami T, Bartlett T, Maahs D. Type 2 diabetes in the child and adolescnts. Pediatr Diab 2014;15(Suppl. 20):26-46
27. Giorgetti C, Ferrito L, Zallocco F, Iannilli A, Cherubini V. Organization and regional distribution of ecnters for the management of children and adolescents with diabetes in Italy. Ital J Pediatr 2015;41:74
28. Lambert PA, 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;26:333-337
29. Craig ME, Jefferies C, Dabelea D, Balde N, Seth A, Donaghue KC. Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diad 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 2012;8:201-211
31. Hammond P. Improving the detection and management of type 1 diabetes. Practitioner 2016;260:21-26
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;55:2631-2635

Figure 1. Step-wise flow chart to etiologic diagnosis of children's hyperglycemia. PG: plasma glucose; DM: diabetes mellitus; T1D: type 1 diabetes; T2D: type 2 diabetes; MODY: maturity onset diabetes of the young; NDM: neonatal diabetes mellitus; yr: year; mo: month; n.a.: not available; *present study and Prisco F et al., MODY 2 presenting as neonatal hyperglycemia: a need to reshape the definition of "neonatal diabetes"? Diabetologia 2000; 43:1331-1332.

Table 1. Frequency of the different cause of diabetes in 3,781 patients referred to 15 Italian pediatric diabetes clinics. Type 1: type 1 diabetes; Type 2: type 2 diabetes; MODY: Maturity Onset Diabetes of the Young; NDM: Neonatal Diabetes Mellitus. n.a.=not applicable. Relative percentage of IFG/DM has been calculated on data from 166 MODY patients. Relative percentage of patients identified in each Italian macroregion within homogeneous diagnostic groups is given in parenthesis. *vs type 1 diabetes: p< 0.001; §vs type 1 diabetes p = 0.003; MODY vs type 2 diabetes: p <0.001.

	Type 1	*Type 2*	*MODY*	*NDM*	*Genetic syndromes*	*Other*	*Total*
Number of patients	3,495 (92.4%)	37 (1.0%)	210 (5.5%)	21 (0.6%)	9 (0.24%)	9 (0.24%)	3,781
IFG/DM %	0.2/98.8	19/81	73/27	n.a.	n.a.	n.a.	n.a.
Age at referral, years (M ± SD)	8.4 ± 4.2	13.8 ± 2.4*	9.4 ± 4.0§	<6 months	n.a.	n.a.	n.a.
Northern Italy	1,289 (36.9%)	27 (73.0%)	69 (33.0%)	12	3	4	1,404 (37.1%)
Central Italy	1,102 (31.5%)	3 (8.1%)	59 (27.8%)	6	0	1	1,170 (30.9%)
Southern Italy	1,104 (31.6%)	7 (18.9%)	82 (39.2%)	3	6	4	1,207 (31.9%)

Table 2. Novel mutations in *GCK*, *HNF1A*, *HNF4A*, and *WFS1* genes (HGDM professional). NA: not available.

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/ dupl	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

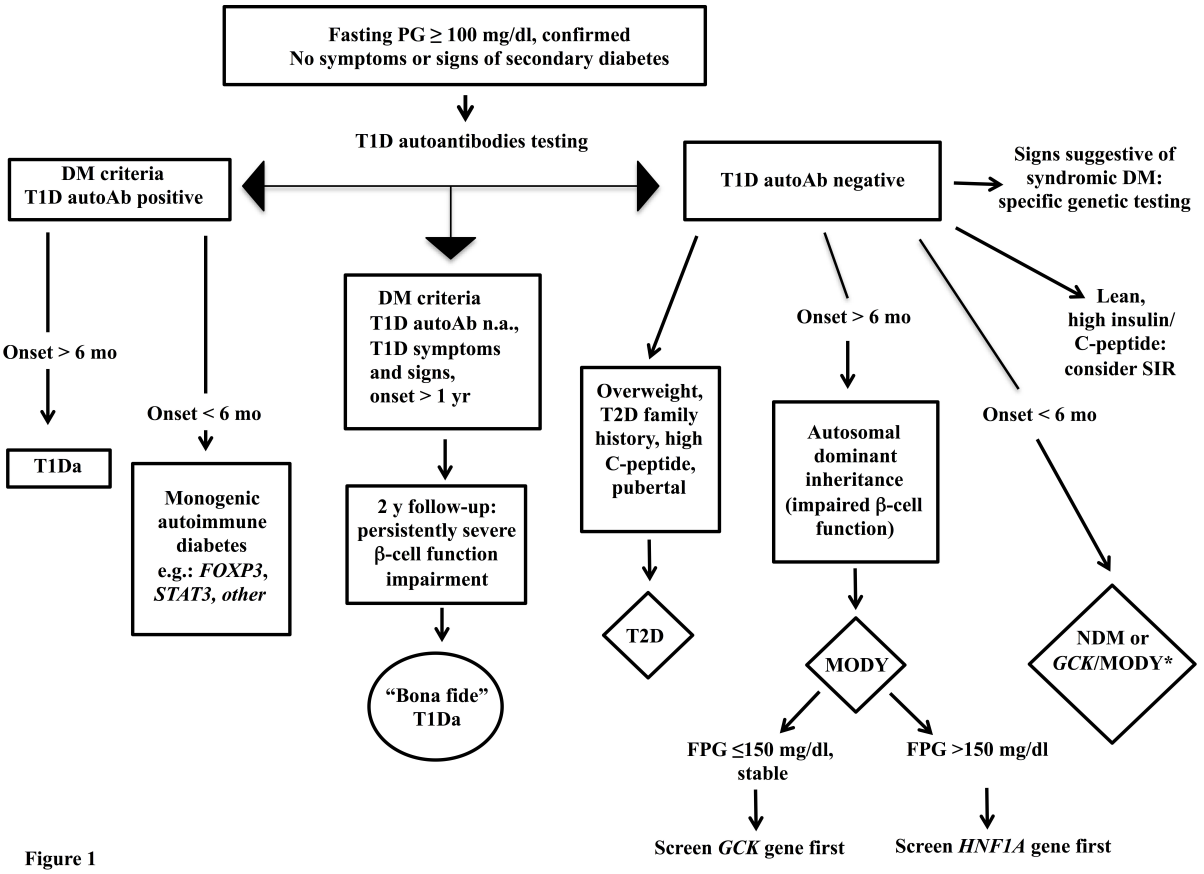

Figure 1