

The Changing Landscape of Neonatal Diabetes Mellitus in Italy Between 2003 and 2022

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Abstract

Context: In the last decade the Sanger method of DNA sequencing has been replaced by next-generation sequencing (NGS). NGS is valuable in conditions characterized by high genetic heterogeneity such as neonatal diabetes mellitus (NDM).

Objective: To compare results of genetic analysis of patients with NDM and congenital severe insulin resistance (c.SIR) identified in Italy in 2003-2012 (Sanger) vs 2013-2022 (NGS).

Methods: We reviewed clinical and genetic records of 104 cases with diabetes onset before 6 months of age (NDM + c.SIR) of the Italian dataset.

Results: Fifty-five patients (50 NDM + 5 c.SIR) were identified during 2003-2012 and 49 (46 NDM + 3 c.SIR) in 2013-2022. Twenty-year incidence was 1:103 340 (NDM) and 1:1 240 082 (c.SIR) live births. Frequent NDM/c.SIR genetic defects (*KCNJ11*, *INS*, *ABCC8*, 6q24, *INSR*) were detected in 41 and 34 probands during 2003-2012 and 2013-2022, respectively. We identified a pathogenic variant in rare genes in a single proband (*GATA4*) (1/42 or 2.4%) during 2003-2012 and in 8 infants (*RFX6*, *PDX1*, *GATA6*, *HNF1B*, *FOXP3*, *IL2RA*, *LRBA*, *BSCL2*) during 2013-2022 (8/42 or 19%, $P = .034$ vs 2003-2012). Notably, among rare genes 5 were recessive. Swift and accurate genetic diagnosis led to appropriate treatment: patients with autoimmune NDM (*FOXP3*, *IL2RA*, *LRBA*) were subjected to bone marrow transplant; patients with pancreas agenesis/hypoplasia (*RFX6*, *PDX1*) were supplemented with pancreatic enzymes, and the individual with lipodystrophy caused by *BSCL2* was started on metreleptin.

Conclusion: NGS substantially improved diagnosis and precision therapy of monogenic forms of neonatal diabetes and c.SIR in Italy.

Key Words: monogenic diabetes, neonatal diabetes mellitus, congenital severe insulin resistance, autoimmune neonatal diabetes mellitus, molecular genetics

Abbreviations: c.SIR, congenital severe insulin resistance; DKA, diabetic ketoacidosis; NDM, neonatal diabetes mellitus; NGS, next-generation sequencing; PNDM, permanent NDM; TNDM, transient NDM.

Neonatal diabetes mellitus (NDM) is a subtype of monogenic diabetes with onset within 6 months of age (1). NDM may be isolated or syndromic, depending on the single gene involved and its mode of inheritance can be autosomal dominant, autosomal recessive, or X-linked. In addition, most cases of NDM transient subtype (TNDM), which remits within weeks/months from diabetes onset, are associated with aberrations of chromosome 6q24 (1). NDM genes affect pancreatic beta cell development and/or function that cause severe insulin deficiency, with patients often times presenting with diabetic ketoacidosis (1). Individuals with congenital severe insulin resistance (c.SIR) show syndromic features such as lipodystrophy, acanthosis nigricans, and hirsutism and present with nonketotic diabetes at birth, frequently alternating with hypoglycemia (2). Fasting insulin concentrations $>25 \mu\text{U/mL}$ ($>150 \text{ pmol/L}$) are considered to be a sign of SIR in lean children/adolescents (1), but in neonates with c.SIR insulin levels in the triple/quadruple-digit range ($>100\text{--}1000 \mu\text{U/mL}$) (2) are often observed at random sampling (neonates are fed every 2-4 hours), along with high C-peptide levels. c.SIR is caused by biallelic, usually recessive, variants in genes that impair insulin action like *INSR* (2, 3).

NDM is not frequent, with an estimated incidence in Europe of about 1:100 000 live births (4), while c.SIR is considered exceedingly rare (an estimated 1:4 000 000 live births for Donohue and Rabson–Mendenhall syndrome) (2). According to the International Society for Pediatric and Adolescent guidelines 2022, all cases with a clinical diagnosis of NDM and c.SIR deserve genetic testing (1).

Until 2013, a relatively short list of genes/chromosomal defects was used to investigate NDM and c.SIR in Europe and the United States, namely, *KCNJ11*, *INS*, *ABCC8* (both permanent NDM and TNDM), 6q24 (TNDM), and *INSR* (c.SIR), while some rare, recessive permanent NDM (PNDM) genes (eg, *EIF2AK3*, *GCK*, *PDX1*, *PTF1A*, *GLIS3*, others) were screened “on demand,” according to the proband’s phenotype. By screening the 4 main genes in 27 subjects with NDM identified between 2005 and 2010, we were able to reach a genetic diagnosis in 18 (66%) (4). In the last 15 years, however, the list of genes causing nonautoimmune PNDM has been constantly increasing and now exceeds 25. New genes of autoimmune PNDM have been also discovered,

adding to the prototype of this subgroup (ie, *FOXP3*) (1). In contrast, just 3 genetic defects (6q24, *ABCC8*, *KCNJ11*) still account for about 90% of cases with TNDM (1, 5). Nevertheless, a few new TNDM genes have been found in recent years (1).

In the last decade, new techniques of DNA sequencing, collectively called “next-generation sequencing” or NGS, progressively replaced the Sanger method in all fields of genetics, including NDM (6, 7). By allowing parallel massive DNA sequencing of thousands genes, NGS has proven to be the ideal method to investigate diseases with high genetic heterogeneity, accelerating the identification of pathogenic variants in rare loci.

In this paper we evaluated the impact of NGS on NDM and c.SIR by comparing the 2003-2012 period, when Sanger was the only method in use in Italy, and 2013-2022 period, when NGS progressively became the method of choice.

Materials and Methods

We utilized the definition of NDM and c.SIR described earlier and in (1). NDM was diagnosed in a patient presenting with plasma glucose in the diabetes range within 6 months of age, along with low/undetectable insulin/C-peptide levels. c.SIR was suspected in neonates with diabetes, high insulin ($>25 \mu\text{U/mL}$), and/or C-peptide levels.

Sanger DNA sequencing of frequent NDM/c.SIR genes (*KCNJ11*, *INS*, *ABCC8*, and *INSR*) and methods utilized to investigate 6q24 defects (TNDM) were performed as previously described (5, 8, 9). For NGS, clinical exome sequencing (Twist Custom Panel kit, 8245 genes; Twist Bioscience, San Francisco, CA, USA) was performed at Bambino Gesù Children’s Hospital on a NovaSeq6000 platform (Illumina, San Diego, CA, USA).

The following genes were filtered out for analysis: nonautoimmune NDM: *ABCC8*, *CISD2*, *CNOT1*, *EIF2B1*, *EIF2S3*, *EIF2AK3*, *GATA4*, *GATA6*, *GCK*, *GLIS3*, *HNF1B*, *IER3IP1*, *INS*, *KCNJ11*, *KCNMA1*, *MNX1*, *NEUROD1*, *NEUROG3*, *NKX2-2*, *PAX6*, *PDX1*, *PTF1A*, *RFX6*, *SLC2A2*, *SLC19A2*, *WFS1*, *YIPF5*, and *ZFP57*; when necessary, sequencing of *INS* promoter (both for PNDM and TNDM) and *PTF1A* distal enhancer (cases with suspected

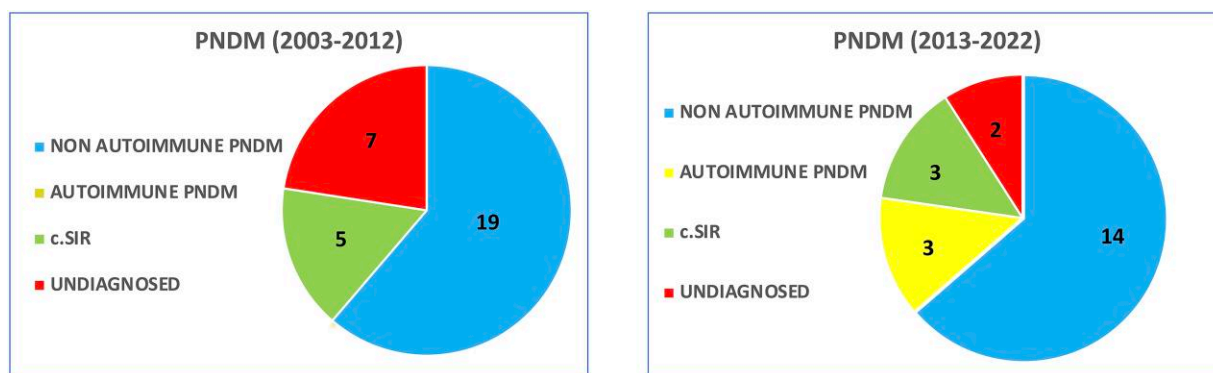


Figure 1. Genetic etiology of PNDM in the period 2003-2012 compared with the period 2013-2022.

pancreas agenesis with no extrapancreatic features) were performed; in addition *ONECUT1*, a recently identified gene was examined in 2 cases identified during 2013-2022 and negative to the initial screening; autoimmune NDM: *CTLA4*, *FOXP3*, *IL2RA*, *ITCH*, *LRBA*, and *STAT3*; congenital SIR: *AGPAT2*, *BSC12*, *CAV1*, *CAVIN1*, and *INSR*. Other Italian laboratories involved in NDM/c.SIR genetic screening based in Milan (San Raffaele Hospital), Naples (CEINGE), San Giovanni Rotondo (IRCCS Casa Sollievo della Sofferenza), and Novara (University of Eastern Piedmont) utilized slightly different NGS methods and lists of genes.

Statistics

Count and rate have been reported for each group. Chi-square analysis was used to compare the subgroups. Statistical significance was set at $P < .05$.

Informed consent to perform genetic studies for diagnostic purpose was obtained by parents or guardians of the probands. Ethics committee approval was not requested, since the General Authorization to Process Personal Data for Scientific Research Purposes (authorization no. 9/2014) declared that retrospective archive studies that use identifier codes, preventing the data from being traced back directly to the data subject, do not need ethics approval.

Results

During 2003-2022 we observed 96 cases with NDM (PNDM: 45; TNDM: 51). Fifty probands were identified between 2003 and 2012 (PNDM: 26; TNDM: 24) and 46 between 2013 and 2022 (PNDM: 19; TNDM: 27). c.SIR cases were 5 during 2003-2012 and 3 between 2013 and 2022.

Permanent NDM

Genetic etiology of PNDM was established in 19 individuals (73%) from 2003-2012 and in 17 cases (89.5%) from 2013-2022 (Fig. 1). The main cause of PNDM in both periods was *KCNJ11* (20 patients) followed by *INS* (8 patients) (Table 1). *KCNJ11*, *ABCC8*, and *INS* variants identified have been previously described by our group or by others (8, 10-13) (Table 2). All but 1 persons with PNDM caused by *KCNJ11* or *ABCC8* pathogenic variants were successfully switched from insulin to sulfonylureas (Table 2).

Between 2003 and 2012 a single neonate with PNDM associated with a rare gene (*GATA4*) was identified (14) (Table 1). In contrast, during 2013-2022 we identified 3 infants with

Table 1. Genetics causes of PNDM in Italy 2003-2022

	2003-2012 no. of cases per locus	2013-2022 no. of cases per locus	Consanguinity of parents
<i>KCNJ11</i>	12	8	n.a.
<i>INS</i>	6	2	n.a.
<i>ABCC8</i>	—	1	n.a.
<i>GATA4</i>	1	—	n.a.
<i>GATA6</i>	—	1	n.a.
<i>PDX1</i>	—	1	no
<i>RFX6</i>	—	1	yes
<i>FOXP3</i>	—	1	n.a.
<i>IL2RA</i>	—	1	?
<i>LRBA</i>	—	1	no
Diagnosed	19 (73%)	17 (89.5%)	1
Unknown	7	2	2
Total	26	19	3

Comparison of % of case diagnosed 2003-2012 vs 2013-2022. $P = \text{n.s.}$
 Comparison of % of rare forms of PNDM 2003-2012 (1 out 19; 5.2%) vs 2013-2022 (6 out 17; 35.2%): $P = .023$. Rare genes/forms: *GATA4*, *GATA6*, *PDX1*, *RFX6*, *FOXP3*, *IL2RA*, *LRBA*.
 Abbreviations: ?, unknown; n.a., not applicable; PNDM, permanent neonatal diabetes mellitus.

rare syndromic PNDM caused by genes encoding for transcription factors *RFX6* (homozygous) (15), *PDX1* (compound heterozygous) (16), *GATA6* (NM_005257.6: c.1502C>G; p.Ser501Ter, heterozygous, novel variant), and 3 cases with autoimmune PNDM caused by *FOXP3* (17), *LRBA* (compound heterozygous) (18), and the novel homozygous variant c.227G>A; p.Trp76Ter of *IL2RA* gene (NM_000417.2) (Fig. 1, Table 1 and Table 3). Rare forms of PNDM, either dominant, recessive, or X-linked were identified more frequently in 2013-2022 than 2003-2012 ($P = .023$) (Table 1). Autoimmune PNDM was genetically diagnosed in 15.8% of neonates (3 out of 19) during 2013 and in none between 2003 and 2012.

No genetic diagnosis was obtained in 7 infants with PNDM during 2003-2012 (27%) and in 2 during 2013-2022 (10.5%) (Fig. 1 and Table 1). Six out of 9 were syndromic (4 diagnosed during 2003-2012): 1 with IPEX-like features (*FOXP3*, *NEUROG3* negative, deceased), 1 with heart malformation and clinical and laboratory findings compatible with pancreas and gallbladder agenesis (*PDX1*, *GATA6* negative, deceased), 1 with severe prematurity and multicystic kidney (lost at

Table 2. Variants identified in common PNDM/TNDM genes *KCNJ11*, *INS*, and *ABCC8*

	<i>KCNJ11</i>	<i>INS</i>	<i>ABCC8</i>
PNDM	His46Tyr, Gly53Val, Val59Ala, Val59Met (2), Arg201Ser, Arg201Cys (4), Arg201His (6), Glu322Lys, Tyr330His ^a	Leu30Pro, Leu30Val, Gly32Ser, Tyr50Cys, Arg89Cys (2), Cys96Tyr, Leu105Pro	Glu1141Lys
TNDM	Cys42Arg, Arg50Gln (4), Glu179Lys, Ser225Arg ^b , Glu227Leu, Glu229Lys (2), Thr293Ser		Leu213Pro, Leu225Pro ^b , Ser459Arg, Thr540Ile ^b +4415-13G>A, Arg825Trp, Arg1182Gln, Arg1380Cys (3) Arg1380His (2) Val1523Met

Abbreviations: PNDM, permanent neonatal diabetes mellitus; TNDM, neonatal diabetes mellitus transient subtype.

^aNot responsive to sulfonylureas.

^bNovel variants.

Table 3. Clinical features of *FOXP3*, *IL2RA*, and *LRBA* autoimmune permanent neonatal diabetes mellitus cases (2012-2022)

	<i>FOXP3</i>	<i>IL2RA</i>	<i>LRBA</i>
Age at onset	Day 1	Day 8	6 months
Blood glucose, mg/dL	400	1504 (severe DKA)	700 (DKA)
C-peptide	<0.3 ng/mL	n.a.	n.a.
Type 1 diabetes autoantibodies	ICA	ICA	IA-2A, IAA
Other autoantibodies	Antihormonin, antivillin	No	No
Persistent diarrhea	Yes at 1 month	No	Yes at 9 months
Bone marrow transplant	Yes	Yes (at 5 m)	Yes (at 16 months)
Birth weight	2000	3380	3950

Abbreviations: DKA, diabetic ketoacidosis; n.a., not available.

follow-up), 1 with cleidocranial dysplasia (lost at follow up), 1 with severe epilepsy not responsive to drugs (deceased), 1 with intestinal malformation carrying a heterozygous *RFX6* missense variant (data not shown). Among the 3 subjects with nonsyndromic PNDM without a genetic diagnosis, 1 carried a *ONECUT1* variant in heterozygous state (19).

NDM transient subtype

A genetic diagnosis was reached in 18 (75%) and 22 (81.5%) infants with TNDM during 2003-2012 and 2013-2022, respectively (Fig. 2). During 2003-2012 pathogenic variants in *ABCC8*, *KCNJ11*, and 6q24 aberrations were identified in 6, 6, and 6 cases (Table 4) (5). All 6 cases without a genetic diagnosis were lost to follow-up. Four *ABCC8/KCNJ11*-negative subjects did not perform a 6q24 test and 1 did not perform any genetic test. Another neonate with heart and kidney malformation was negative for 6q24 but DNA sequencing of other TNDM genes was not performed.

In the period 2013-2022 we identified 9 probands with 6q24 defects, 7 and 5 with pathogenic variants in *ABCC8* and *KCNJ11*, and 1 with a rare *HNF1B* mutation (NM_000458.4:c.1045+1G>A, p.?) (20) (Fig. 2 and Table 4). Most of the *ABCC8*- and *KCNJ11*-TNDM pathogenic or likely pathogenic variants identified during the whole period 2003-2022 have been previously described, while 2 were novel. A likely pathogenic *KCNJ11* de novo variant (NM_000525.4: c.675C>A,

p.Ser225Arg) (Table 2) was identified in a patient who remitted to normal glycemia without any insulin therapy 1 week after diabetes onset. In 2 brothers with TNDM diagnosed with diabetes in 2010 (first observation period) and 2017 (second observation period) compound heterozygous variants of *ABCC8* (NM_000352.6: c.4412-13G>A, p. ?; c.1619C>T, p.Thr540Ile) (Table 2) were detected. The variant c.4412-13G>A, maternally inherited, was functionally tested by others (21) (where the variant is denoted as c.4415-13G>A). This study showed that c.4412-13G>A impacts on splicing by inducing retention of 11 nucleotides from intron 37 and also a new 3' splice site within the intron. Thus, we classified the variant as pathogenic, loss of function (American College of Medical Genetics and Genomics, class 5) (22). The paternally inherited variant Thr540Ile (previously identified by Sanger) is not present in gnomAD database and has been considered to be likely pathogenic per American College of Medical Genetics and Genomics rules (22) because it is in trans with a pathogenic variant of the same gene. Both parents of these 2 patients were normoglycemic at the oral glucose tolerance test.

In 2 out of 6 patients with TNDM negative for 6q24 from 2013-2022, *KCNJ11* and the rare TNDM genes *SLC2A2* and *HNF1B*, an *ABCC8* variant of uncertain significance was identified. In 1 patient the variant of uncertain significance was spontaneous (c.2959T>C, p.Ser987Pro), while in the other (c.157A>T, p.Ser53Cys) it was inherited from the normoglycemic mother. These 2 subjects were included in the TNDM group of unknown etiology (Fig. 2). The remaining 4 infants with TNDM (2013-2022) were negative for *ABCC8* and *KCNJ11* and lost to follow-up without being tested for 6q24.

NDM and Mode of Inheritance

Most variants in frequent NDM genes (*KCNJ11*, *INS*, *ABCC8*) are dominant and in many patients occur spontaneously (Table 5, second column from the left); however, familial cases with typical vertical transmission are not infrequent (Table 5, third column). Interestingly, in patients with TNDM due to *KCNJ11* or *ABCC8* mutations, it is not rare to observe a different age at diabetes onset (adolescence, adulthood, ie, not neonatal) in the parent carrying the variant ((6), and this investigation). As already described by others (6), recessive PNDM forms (Table 5) are rarely identified in patients born to nonconsanguineous parents. In our dataset, most individuals with recessive forms of NDM carried homozygous variants, but 2 individuals bore compound heterozygous variants of *PDX1* (pancreas hypoplasia) and *LRBA* (autoimmune case PNDM).

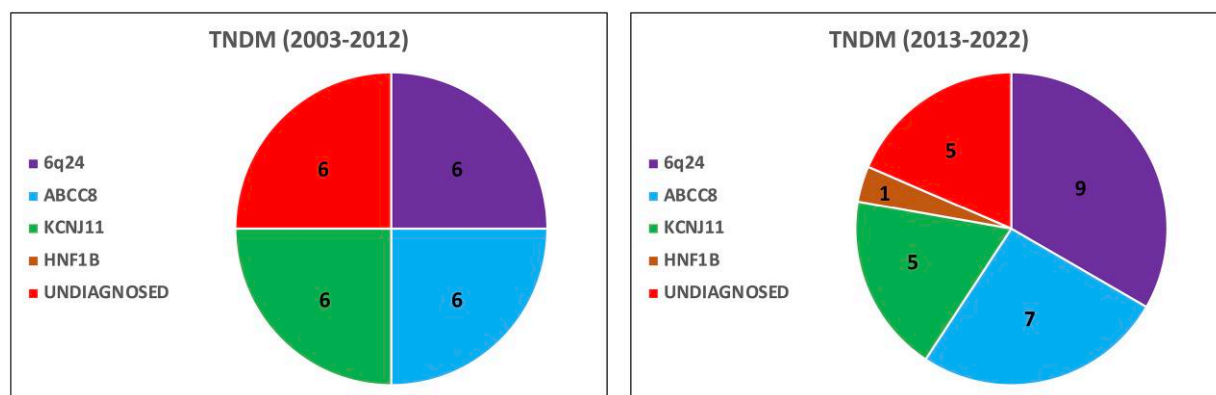


Figure 2. Genetic etiology of TNDM in the period 2003-2012 compared with the period 2013-2022.

Table 4. Genetic causes of neonatal diabetes mellitus transient subtype in Italy 2003-2022

	2003-2012 no. of cases per locus	2013-2022 no. of cases per locus
<i>KCNJ11</i>	6	5
<i>ABCC8</i>	6	7
6q24	6	9
<i>HNF1B</i>	—	1
Diagnosed	18	22
Unknown/incomplete	6	5
Total	24	27

NDM and Prematurity

When diagnosing NDM, prematurity (<37 weeks of gestational age) may pose a challenge because premature neonates can present with transient hyperglycemia (23). Among infants with established genetic diagnosis during the whole period of observation (2003-2022), 3 PNDM (out of 36, 8.3%) and 6 TNDM (out of 39, 15.3%) were premature. Among neonates with TNDM, the most frequent genetic findings were 6q24 and *ABCC8* (Table 6). However, prematurity was more frequent in patients (PNDM + TNDM, 2003-2022) without diagnosis (8 out of 21 or 38%) compared with those with a genetic diagnosis (9 out of 75, or 12%) ($P = .005625$). Notably in 3 out of 4 premature patients with TNDM, 6q24 testing was not performed, while all 4 premature PNDM without a genetic diagnosis (born between 2008 and 2012) were syndromic.

Congenital SIR

Five neonates with biallelic *INSR* variants were diagnosed with c.SIR in the period 2003-2012 (9). During 2013-2022, 2 apparently unrelated cases with c.SIR associated with the same homozygous *INSR* mutation (NM_000208.4; c.3289C>T; p.Gln1097Ter) and 1 with the novel homozygous *BSCL2* variant (NM_001122955.4; c.832-833delGA; p.Asn278GlnfsTer18) (Table 7) were identified.

Six out of 7 *INSR* patients (2003-2022) had classical c.SIR features (intrauterine growth restriction, hirsutism, acanthosis nigricans, facial dysmorphisms) and could be divided in Donohue syndrome (4 patients) and Rabson-Mendenhall syndrome (3 patients) according to survival (4 patients with

Table 5. Mode of inheritance of variants identified in patients with PNDM, TNDM, and c.SIR

Gene	De novo, autosomal dominant	Autosomal dominant	Autosomal recessive	X-linked
<i>KCNJ11</i> -PNDM	19	1		
<i>KCNJ11</i> -TNDM	4	7	1	
<i>ABCC8</i> -PNDM		1		
<i>ABCC8</i> -TNDM	7	4	2	
<i>INS</i> -PNDM	7	1		
<i>GATA4</i> -PNDM	1			
<i>GATA6</i> -PNDM	1			
<i>RFX6</i> -PNDM			1	
<i>PDX1</i> -PNDM			1	
<i>HNF1B</i> -TNDM	1			
<i>LRBA</i> -PNDM			1	
<i>IL2RA</i> -PNDM			1	
<i>FOXP3</i>				1
<i>INSR</i>			7	
<i>BSCL2</i>			1	

Please note that 6q24 cases have not been reported in the table. Aberrations of 6q24 are usually sporadic, but some specific defects can be inherited. All patients with PNDM or TNDM caused by heterozygous, spontaneous mutations (de novo) have a 50% chance of passing the mutant allele to each child. In this table, 14 cases inherited their variant from a parent; in 2 *ABCC8* kindreds, however, the parent carrying the variant was normoglycemic, ie, nonpenetrance was observed. Abbreviations: c.SIR, congenital severe insulin resistance; PNDM, permanent neonatal diabetes mellitus; TNDM, NDM transient subtype.

Donohue syndrome died before 24 months of age). Only in a patient with Rabson-Mendenhall, now 14 years old, hirsutism and acanthosis nigricans were not evident at birth, but later in life. Moreover, 2 *INSR* patients of the period 2013-2022 presented with nephrocalcinosis, a specific feature of *INSR*-c.SIR (Table 7) (9). The single case with the *BSCL2* variant somehow resembled *INSR*-c.SIR because the case presented with hyperglycemia with episodes of hypoglycemia, hirsutism, protruding abdomen, and lipodystrophy, but differed for birth weight (normal) and triglyceride levels (very high in *BSCL2*, normal-low in *INSR*; Table 7) and absence of nephrocalcinosis.

Precise genetic diagnosis impacted on therapy of individuals with c.SIR: the patient carrying the *BSCL2* variant was started

on metreleptin (24) with excellent results for glucose and lipid metabolism (manuscript in preparation). In 2 patients with Rabson–Mendenhall syndrome (9) with diabetes of long duration (>3 years) and modest or null response to metformin and insulin therapy, the SGLT2i empagliflozin (5 mg) was introduced as an add-on treatment with good (25) to excellent results (manuscript in preparation). All patients with congenital SIR reported in this investigation bear biallelic, recessive mutations (Table 7).

NDM and c.SIR Incidence

During 2003–2012, there were 5 469 539 live births in Italy, setting the incidence of NDM to 1:109 390 (or 0.88/100 000;

Table 6. Premature (<37 weeks of gestational age) per locus

	PNDM 2003–2022 no. of cases per locus	PNDM 2003–2022 no. premature (<37 weeks)	TNDM 2003–2022 no. of cases per locus	TNDM 2003–2022 no. premature (<37 weeks)
KCNJ11	20	1	10	1
INS	8	/	/	/
ABCC8	1	/	13	2
6q24	/	/	15	2
GATA4	1	/	/	/
GATA6	1	1	/	/
PDX1	1	1	/	/
RFX6	1	/	/	/
HNF1B	/	/	1	1
FOXP3	1	/	/	/
IL2RA	1	/	/	/
LRBA	1	/	/	/
Diagnosed	36	3 (8.3%)	39	6 (15.3%)
Unknown	9	4 (44.4%)	12	4 (33.3%)
Total	45	7	51	10

2003–2022 overall: diagnosed premature = 12%; unknown premature = 38%, $P = .005625$.

Abbreviations: PNDM, permanent neonatal diabetes mellitus; TNDM, NDM transient subtype.

Table 7. Clinical features of *INSR* and *BSCL2* cases (2003–2022)

	<i>INSR-6</i>	<i>INSR-7</i>	<i>BSCL2</i>	<i>INSR-1 to 5 (2003–2012)</i>
Insulin, $\mu\text{U/mL}$ (min/max)	241/2980	553	61/84.8	54/3950
Blood glucose mg/dL (min/max)	30–320 (CGM)	undetectable–327	46–480	<30/621
Triglycerides (mg/dL)	83	n.a.	1180	115, 126, 182 (n.a. in 2 pat.)
Plasma K ⁺	Low	n.a.	Normal	Low (3 out of 5)
Hirsutism	Yes	Yes	Yes	Yes (4 out of 5)
Protruding abdomen	Yes	Yes	Yes	Yes (4 out of 5)
Abdominal ultrasound	Nephrocalcinosis	Nephrocalcinosis	Hepatomegaly	Nephrocalcinosis: 2 out of 5
Aldosterone/renin	High/high	n.d.	n.d.	high/high (2 out of 5)
Birth weight	1840	1149	3300	990–2225
Deceased <2 years of age	No	Yes	No	3 out of 5
Consanguinity of parents	Yes	Yes	Yes	1 out of 5

Abbreviations: CGM, continuous glucose monitoring; n.a., not available.

95% CI 0.59–1.16), while in the decade 2013–2022 there were 4 451 120 live births and the incidence was 1:96 763 (or 1.05/100 000; 95% CI 0.68–1.42). For the whole period 2003–2022 the incidence was 1:103 340, well in agreement with our previous estimates (4). Of interest, PNDM incidence in Europe is confirmed to be much lower than Middle Eastern countries, while for TNDM the effect of consanguinity is less evident (Fig. 3) (26–33). The incidence of c.SIR during the entire period 2003–2022 was 1:1 240 082 live births.

Discussion

Results of our comparison between 2 decades of investigation on genetic causes of NDM and congenital SIR in Italy shows that NGS made a difference. Pathogenic variants in frequent NDM genes (*KCNJ11*, *INS*, *ABCC8*, 6q24) accounted for 72% and 69.5% in the 2 periods of observation, a similar figure to our previous finding (4). In contrast, during the period 2013–2022, when NGS became increasingly available in genetic laboratories, we identified pathogenic variants in rare recessive or X-linked PNDM genes such as *RFX6*, *PDX1*, *LRBA*, *IL2RA*, and *FOXP3*, and a dominant *GATA6* variant in a case with pancreas agenesis and heart malformation. In addition, rare causes of TNDM and c.SIR (ie, *HNF1B* and *BSCL2*, respectively) were readily detected. These results cannot be attributed to newly identified NDM or c.SIR genes, because 6 of those involved in this study were discovered when Sanger sequencing was the only method used to sequence DNA in Italy, namely, *PDX1* (identified in 1997), *FOXP3* (2001), *BSCL2* (2001), *HNF1B* (2006 as cause of pancreas hypoplasia), *IL2RA* (previously known as CD25, 2007), *RFX6* (2009), and *GATA6* (2012) and only 1, namely, *LRBA*, has been identified in recent years (2017) as a cause of autoimmune PNDM. In 2 subjects, 1 with isolated diabetes (19), NGS revealed heterozygous variants in 2 recessive genes known to cause syndromic PNDM (*RFX6*, *ONECUT1*) (34, 35). These 2 cases have been considered unsolved and await further investigation to define the etiology of neonatal diabetes.

NGS, publicly available database (eg, gnomAD), and functional studies were instrumental to identify and classify pathogenic/likely pathogenic *ABCC8* variants in 2 brothers with TNDM that have not been previously resolved. The 2 brothers

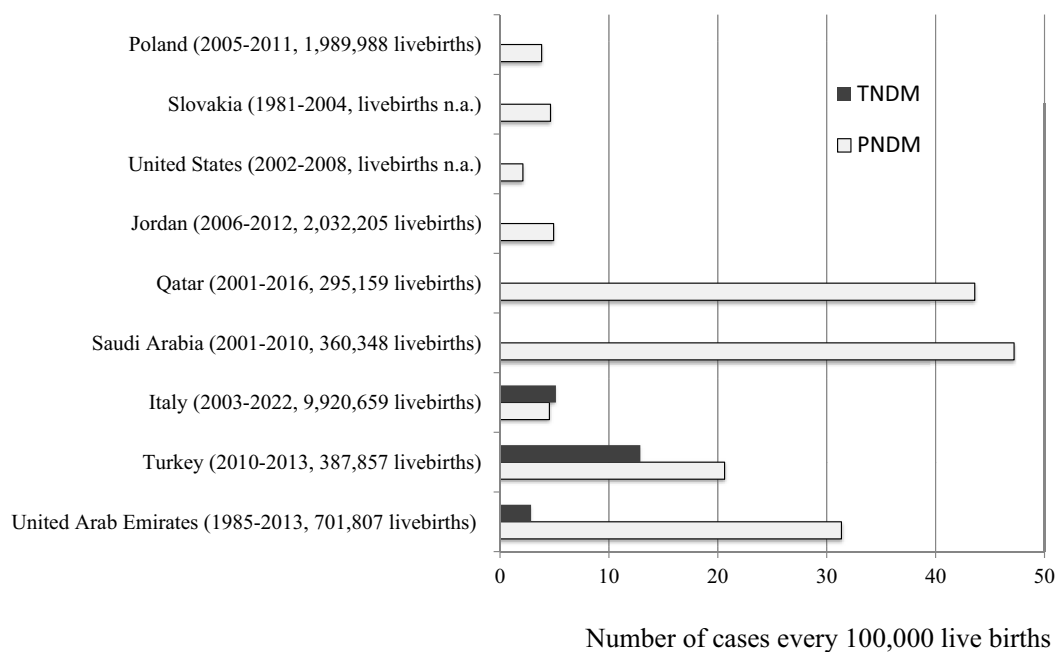


Figure 3. Estimated incidence of NDM in different countries. References concerning incidence in individual countries: Poland (28), Slovakia (29), United States (30), Jordan (31), Qatar (32), Saudi Arabia (33), Turkey (34), United Arab Emirates (35).

carried a loss of function variant in 1 allele and the missense variant Thr540Ile in the other. We reasoned that Thr540Ile has a mild activating function and causes TNDM because it is functionally hemizygous. This mechanism of disease has been previously demonstrated in *ABCC8*-PNDM (36), but to the best of our knowledge, it has never been described in TNDM.

During 2013-2022, we observed an apparent increase in TNDM, likely because screening of blood glucose at birth has become more common with better detection of mild, transient diabetes (5). Nevertheless, many TNDM cases remained unsolved, probably because of a lesser “incentive” to pursue genetic testing in nonsyndromic patients with modest, remitting hyperglycemia treated with short-lived insulin therapy or only rehydration. Of interest, a patient with *KCNJ11*-TNDM with diabetes onset in 2022 entered remission without any insulin or sulfonylurea treatment, confirming an observation we made in 2 other patients with TNDM associated with *KCNJ11* pathogenic variants (5). Therefore, caution should be exerted with the implementation of sulfonylurea therapy before the results of genetic testing because of the risk of hypoglycemia. In addition, this finding reinforces the notion that insulin treatment is an applicable but in some instances dispensable criterion for the diagnosis of TNDM.

NGS was especially useful to identify rare genes of autoimmune PNDM and c.SIR. For instance, patients with *FOXP3*, *IL2RA*, and *LRBA* PNDM may present similar extrapancreatic features (eg, enteropathies, hypothyroidism) (37). In addition, *LRBA* can cause autoimmune PNDM without syndromic symptoms/signs associated with severe immune dysregulation (38). Simultaneous screening allowed swift identification of autoimmune PNDM genes involved, that in turn lead to a specific therapy (ie, bone marrow transplant). In addition, quick identification of pathogenic variants in *RFX6* (34) and *PDX1* (previously known as *IPF1*) (39) along with signs of exocrine insufficiency in PNDM cases with probable pancreatic hypoplasia/agenesis prompted replacement therapy with

pancreatic enzymes. Importantly, this intervention was not based on ultrasound imaging of the pancreas.

Our data confirm that *INSR* mutations are a major cause of c.SIR, with NGS identifying a single patient with the *BSCL2* pathogenic variant. In this case, physical examination was puzzling, but 2 clinical features set apart the infant bearing an *BSCL2* variant from the *INSR* group: lack of intrauterine growth restriction and hypertriglyceridemia (Table 7) (40, 41). Finally, we found that c.SIR is 12-fold rarer than NDM but about 3 times more frequent than a previous estimate (2). Considering that major causes of NDM in Italy are autosomal dominant genes (*KCNJ11*, *INS*, and most of *ABCC8* cases) while c.SIR is recessive, this is not an unexpected finding.

A limitation of our study is the small number of patients involved, which does not allow robust conclusions to be drawn about the incidence of rare, recessive forms of PNDM that are common in the Middle East (Fig. 3). For instance, in Abu Dhabi, Turkey, and Qatar biallelic pathogenic variants in the *PTF1A* enhancer, *INS* promoter, and *EIF2AK3* genes account for 30% to 78% of all cases of PNDM (30, 33, 42), although we did not identify any such variant. Still, our study illustrates the advantages provided by NGS over Sanger sequencing in terms of time to and precision of genetic diagnosis. We conclude that combining results of massive parallel sequencing with pertinent clinical features of the proband allow prompt confirmation of genetic findings and timely administration of the best available treatments.

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Author Contributions

F.B. conceived the paper, researched data, wrote the paper. N.R. wrote the paper, researched data, M.D. researched data, made statistical analysis, critically revised the paper. M.M., R.R., researched data, revised data. V.C. researched data, made statistical analysis. N.T. researched data, revised manuscript. I.R., S.Z., S.C., E.P., R.S., C.B., B.P., G.M., B.P.2, N.M., R.D.P., M.G., V.G., L.R., V.T., A.S., M.C.M., G.M.2, E.P., D.T., P.C., M.C., D.I., R.B., A.N. researched clinical data. All Authors approved the final version of the manuscript. F.B. is the guarantor of the manuscript.

Disclosures

The authors have nothing to disclose

Data Availability

Original data generated and analyzed during this study are included in this published article or in the data repositories listed in References.

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