Original Article

Genetic Heterogeneity and Challenges in the Management of Permanent Neonatal Diabetes Mellitus: A Single-Centre Study from South India

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Abstract

Aim and Objectives: 1. To study the clinical outcome, growth and glycaemic control, 2. To study the frequency and type of genetic mutations. **Methods:** This is a retrospective study with a review of data of medical records from 2008 till date. **Results:** Twelve patients (six males) with neonatal diabetes mellitus (NDM) were identified. Median (interquartile range – (IQR)) age at diagnosis was 72 (31–95) days with a history of consanguinity in 75%. The median birth weight (range) was 2345 (900–3300) g. Follow-up data were available for eight patients with a median age at (IQR) follow-up of 3.3 (3–5.3) years. At follow-up, the mean annual HbA1c was 8.2% at a mean insulin dose of 1.1 U/kg/d. One patient with Wolcott-Rallison syndrome (WRS) and 21α-hydroxylase deficiency had poor growth and intellectual difficulty. The rest demonstrated satisfactory growth with an increase of mean weight centile from 2nd to 13th, height centile from 6.5th to 20th and normal neuro-cognitive development. Eleven patients underwent genetic testing with a molecular diagnosis in 54% (6/11): *EIF2AK3* (n = 2) and one each in *INS*, *PDX1*, *IL2RA* and *FOXP3*. None had variants in *ABCC8* or *KCNJ11*. One with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome underwent haematopoietic stem cell transplant (HSCT) and later succumbed. **Conclusion:** Our study demonstrates good clinical outcomes among NDM patients without immune dysfunction. Molecular diagnosis was attained only in around half of the patients (54%) with a great genetic heterogeneity.

Keywords: Monogenic diabetes, Neonatal diabetes, NDM, Wollcott-Rallison syndrome, IPEX syndrome, Insulin, diabetes management

Introduction

Neonatal diabetes mellitus (NDM) is a rare genetic disorder that occurs at a frequency of 1 in 90,000[1] in European countries to 1 in 21,000[2] in the Middle-East. It is defined as the onset of diabetes mellitus within 6 months of age. There are two well-recognized forms of NDM: transient and permanent. These neonates require intensive treatment with insulin to ensure optimal glycaemic control while maintaining a fine balance to prevent hypoglycaemia which can be detrimental to their neurological outcome. Genetic analysis helps in establishing the diagnosis of permanent diabetes mellitus (PDM) and has a bearing on the mode of further management. A comprehensive genetic review noted an 80% detection rate of aetiological mutations in NDM.[3] Infants with potassium channel-related genetic defects in *KCNJ11* or *ABCC8* can be changed over to oral glibenclamide therapy easing the burden of daily insulin injections on the family. Genetic testing also helps with the screening of associated defects and determines the need for stem cell transplant in rare forms of NDM.

In developing countries, the lack of standardized genetic testing labs and funds often results in many children missing out on testing altogether. Our centre is unique in that regard

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as most of our genetic tests are done in-house and thus are fairly affordable. Paediatric endocrinology and endocrine genetic testing being under one roof does brings in many referrals to our centre from the country. Our aim is to study the clinical profile and genetic data in our cohort with NDM. Further, we would like to analyse their outcome and highlight how we overcame some of the challenges faced during management.

Methods

This is a retrospective observational study enrolling all children diagnosed or treated for NDM in our centre from 2008 till date. The study was conducted in a tertiary health care centre from South India. The study was cleared by the institutional review board and ethics committee (IRB Min No. 12739). A waiver for consent was obtained from the IRB.

All patients had been tested for anti-tyrosine phosphatase (anti-IA2) and anti-glutamic acid decarboxylase (anti-GAD) antibodies. The in-house genetic testing included analysis for 30 genes (Appendix 1) using next-generation sequencing technique and validated on Sanger sequencing as published earlier.[4] The mean annual HbA1c for each patient was calculated by taking the average of all available HbA1c values over the last year. The weight and height centiles were calculated using the World Health Organization (WHO) growth charts.

Data were collected from an online medical records portal and entered into Microsoft Excel for analysis. Normality of distribution was evaluated using the Shapiro–Wilk test. The continuous variables were expressed as mean ± SD for normally distributed variables or as median (interquartile range – (IQR)) for not normally distributed variables whereas categorical variables were expressed as absolute numbers or percentages as appropriate.

Results

A total of 12 patients (six males) were identified. A summarized table with the clinical and genetic data is attached [Table 1].

Clinical and biochemical characteristics at onset

The median age at diagnosis was 72.5 days with the earliest diagnosis at day 14 and the latest on day 180. Ten were born at term whereas two were born preterm at 33 and 34 weeks of gestation, respectively. Of the 12, three neonates were inborn. The median birth weight of our cohort was 2345 g with the lowest being 900 g. Eight (75%) were born to consanguineous parents. Five mothers had a poor obstetric history with at least one previous abortion and one family having two late neonatal deaths. The median (IQR) age at presentation to our centre was 73.5 days (31–188).

Of the 12, seven were managed with subcutaneous (SC) insulin (five of the seven outborn infants were already initiated on SC insulin prior to presentation at our centre). Insulin infusion at a low dose of 0.05 U/kg/h was used among five infants at presentation with diabetic ketoacidosis (DKA). They were shifted to SC insulin as soon as the acidosis normalized. Various regimes of SC insulin were given. In two of our patients with 880 g and 1120 g at presentation, we used rapid-acting analogues diluted in normal saline initially.[4] This required training of the nursing staff and adhering to a protocol to avoid dosage errors. In patient 2, the neonate was 970 g at presentation. For the first 5 days, intermittent intravenous (IV) boluses of regular insulin at 0.1 U/kg were given when glucometric values were > 250 mg/dl, as there was hardly any SC tissue for insulin delivery and only available access was IV. This necessitates the treating endocrinologist to take into consideration the minimal fat available for insulin administration resulting in variable absorption[5] and the feeding regime that the neonate is on. Later, diluted glargine once a day was used as reported earlier[6] and subsequently changed to undiluted glargine once daily using a pen device. Two other patients were managed with SC isophane insulin once to twice daily and regular insulin if needed, as also suggested by an earlier review.[6] Although developing countries pose a hurdle with lack of insulin pumps for an optimal basal-bolus pattern as described in the literature,[7] our data highlights that SC insulin dosing can be attempted and was successful even in extremely low birth weight neonates.

Median plasma glucose at presentation was 390 mg/dl with the highest being 1308 mg/dl. Islet tyrosine phosphatase 2 antibody (IA2 antibody) was negative in all. Antibody to glutamic acid decarboxylase (GAD antibody) was positive in the patient with *IL2RA* mutation [patient 9 in Table 1]. The median duration of in-hospital stay at presentation was 8 days (3–65).

Follow-up data

A total of 12 patients were diagnosed with NDM [Table 1]. Ten continued treatment here, one who was advised haematopoietic stem cell transplant (HSCT) did not come for further endocrine or transplant team follow-up (patient 9) and one patient got discharged against advice (patient 10). Two infants have follow-up < 6 months (patients 11 and 12) and patient 11 expired under unclear circumstances at home. Follow-up data from eight infants was analysed. The median follow-up age was 3.3 years and the longest follow-up was at age 12.1 years.

- Glycaemic status and other diabetes-related complications

  Six are on basal-bolus (with glargine and regular) and two on the split-mix regime (with regular and isophane insulin) with a mean dose of 1.1 U/kg/d. The median HbA1c is 8.8% (6.5–10.3) at the last follow-up with 1 year average of 8.2%. None are on oral glibenclamide treatment.

  Patient 1 was readmitted with DKA and pneumonia at 7 months of age when insulin infusion (0.05 U/kg/h) was instituted briefly. Patient 3 was admitted at 6 months of age with recurrent asymptomatic hypoglycaemia following a viral illness and was discharged after 3 days. Patient 7 was admitted with typhoid fever at 3 years of age. Patients 1 and 6 with a duration of diabetes > 5 years

Table 1: Summary of clinical characteristics, genotype and follow-up details

S.No	Age at diagnosis (days)	Age at presentation (days)	Gender	GA (wks)	Birth weight (grams)	HbA1c (initial)	Family history	Genetic mutation	Insulin dose (U/kg/d)	Age at last follow-up (yrs)	HbA1C (Av/yr)	Other co-morbidity	Outcome
1	14	14	F	37	1960	8.1	C G6P1L1A3MTP1	Negative, (Heterozygous HADH VOUS)	0.8	12.1	8.3	Nil	Well, DM complications screen neg
2	21	21	F	34	900	9	C G2A1 (this neonate born of a twin pregnancy- 1 twin passed away)	Negative, (2 heterozygous Variants-ZFP57 variant c. 1348G>A (p. Gly450Arg) & a WFS1 variant c. 1406C>T (p. Ser469Leu))	1.2	3.2	8	Nil	Well
3	14	14	M	33	1120	7.2	NC G3P2L2	Negative	0.4	3.2	6.6	Nil	Well
4	34	34	F	41	2130	10.2	NC G2P1L1	Not done				_	DAMA
5	110	110	M	40	2600	13.4	C G2A1	Negative	1.1	3.7	8.9	Nil	Well
6	180	210	M	40	3000	8.8	NC G1	INS gene heterozygous mutation c265C>T	0.2	3.5	7.4	Nil	Well
7	90	330	F	40	3000	7.1	C G2P1L1	EIF2AK3 homozygous variant c. 1763G>A, exon 10	1.9	9.8	8.1	CAH intellectual disability, short stature, Turner mosaicism	Well, DM complication screen neg
8	70	72	F	37	2300	11.3	C G3P2L2	Homozygous 2-bp deletion (1758_1759delAT) in the EIF2AK3 gene	0.6	3.2	8.4	Nil, no skeletal changes so far	Well
9	120	180	M	38	2390	6.5	C G2A1	Homozygous IL2RA missense variant		1		Immunodeficiency with chronic diarrhoea, poor weight	Lost to follow-up
10	90	210	M	40	3300	9	NC G5P4A2L2	Hemizygous FOXP3 missense variant c. 1150G>A	1.8	1.8	9.6	IPEX syndrome-chronic diarrhoea and hypothyroidism from 3 months of age, post HSCT	Transplant failed, passed away at home at 26 months of age
11	75	75	M	40	2500	7.6	C G1	Homozygous PDX1 c. 533A>G, exon 2					Died at home, unclear cause
12	38	38	M	40	1800	10.5	C G1	Negative					Alive, not completed 6 months follow-up

DM=Diabetes mellitus. GA=Gestational age. C=Consanguineous. NC=Non-consanguineous. DAMA=Discharged against medical advice

are negative for nephropathy and retinopathy screen. There were no other diabetes-related events requiring hospitalization.

- Growth

  At the first visit, their average weight and length centiles were 1.6 and 5.8. Patient 6 with Wolcott-Rallison syndrome (WRS) with skeletal dysplasia has poor growth with a velocity of only 3 cm/year and has a height of 105 cm at 9 years of age. Among the remaining, there was a significant increase in mean weight centile from 2nd to 13th and in mean length/height percentile from 6.5 to 20, as per WHO growth chart measurements, indicative of good growth catch-up on treatment. All of them had satisfactory age-appropriate growth velocity - 13 cm/year (at age 2–3 years) and 6 cm/year (>3 years).

- Development

  Patient 6 with WRS had a complicated medical course with an initial diagnosis of salt-losing 21α-hydroxylase deficiency at 1 week of life and later of NDM at 2 months age. She has intellectual concerns and plans to undergo a formal neuro-cognitive assessment. The remaining seven patients have normal development at follow-up. Patient 1 is currently studying in class 7 and doing well.

Genetic mutation analysis

Eleven underwent genetic testing (eight - in-house, two - Exeter, one - external lab in India). Mutation analysis confirmed diagnosis in 6/11 (54%) with pathogenic/likely pathogenic variants (*EIF2AK3-2*, one each for *INS*, *PDX 1*, *IL2RA*, *FOXP3*) [Table 2]. Variants of unknown significance were seen in 2/11 (*ZFP57* heterozygous mutation in 1 who also had *WFS1* variant of uncertain significance, *HADH* heterozygous mutation in 1); negative in 4/11. Table 2 highlights the classification of the genetic variants. In our cohort, 75% (8/12) were born to consanguineous parentage. Of these, 25% had *EIF2AK3* mutation in keeping with a higher proportion of this mutation seen with consanguinity [Figures 1 and 2].

Co-morbidity

Patient 6 has congenital adrenal hyperplasia (CAH) (homozygous I2G variant in *CYP21*) and is on glucocorticoid and mineralocorticoid replacement from early infancy. She has significant short stature. Apart from low-level Turner mosaicism (1.6%) on karyotype, she also has dysplastic changes on a skeletal survey in keeping with her diagnosis of WRS. Patient 8 with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome was diagnosed to have hypothyroidism and enteropathy from early infancy and was on treatment for same. This child had genetic testing done at Exeter which confirmed *FOX P3* mutation and he was referred to our centre at 7 months of age primarily for a bone marrow transplant. Patient 9 presented at 6 months of age with a diagnosis of *IL2RA* deficiency causing both NDM and recurrent diarrhoea. He was being worked up for a bone marrow transplant but subsequently did not follow up.

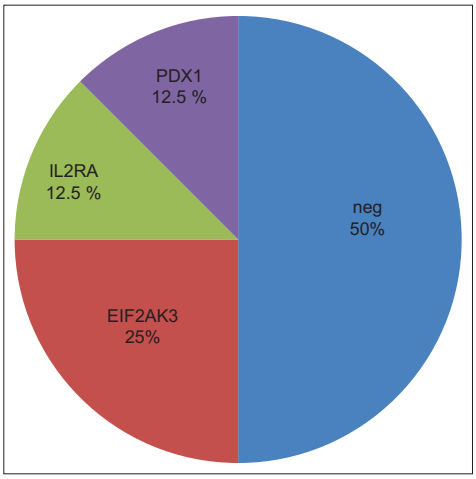

Figure 1: Genetic distribution among the consanguineous parentage

Table 2: ACMG 2015 based classification of the variants identified in the study

Patient no. from Table 1	Gene symbol	Codon change	Protein change	Genotype	Position	Effect	Novel/ reported	ACMG classification
1	HADH	c. 923C>G	Pro308Arg	Heterozygous	Exon 9	Missense	Novel	Variant with uncertain significance
2	ZFP57	c. 1348G>A	Gly450Arg	Heterozygous	Exon 5	Missense	Novel	Variant with uncertain significance
2	WFS1	c. 1406C>T	Ser469Leu	Heterozygous	Exon 8	Missense	Reported	Variant with uncertain significance (non-diabetic dad also carries same mutation)
6	INS	c. 265C>T	Arg89Cys	Heterozygous	Exon 3	Missense	Reported	Pathogenic
7	EIF2AK3	c. 1763G>A	Arg588Gln	Homozygous	Exon 10	Missense	Reported	Likely pathogenic
8	EIF2AK3	c. 1758_1759del	Ser587ThrfsTer5	Homozygous	Exon 10	Frameshift with premature truncation	Novel	Likely pathogenic
10	FOXP3	c. 1150G>A	Ala384Thr	Hemizygous	Exon 12	Missense	Reported	Likely pathogenic
11	PDX1	c. 533A>G	Glu178Gly	Homozygous	Exon 2	Missense	Reported	Pathogenic

ACMG=The American College of Medical Genetics and Genomics

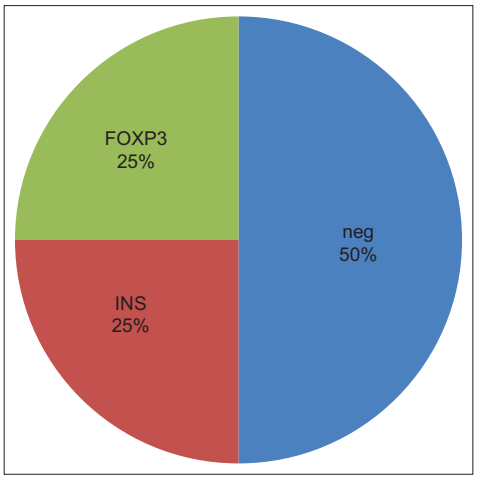

Figure 2: Genetic distribution among the non-consanguineous parentage

Discussion

Among the cohort of paediatric diabetes, NDM constitutes a minor percentage of 0.17%.[8] Permanent NDM is caused by monogenic disorders affecting a number of genes that have been comprehensively reported.[3] All infants with NDM are treated with insulin therapy initially and those with positive *KCNJ11* or *ABCC8* mutations are changed over to oral glibenclamide therapy. There is a scarcity of data with regard to the management and outcome of NDM in resource-limited settings. Our study reports a cohort of 12 patients with NDM for whom follow-up including growth and glycaemic status has been highlighted. The number of patients from a single centre is similar to other studies.[7,11,12]

Jain *et al.* have described follow-up data on 11 infants from north India.[6] The median age at presentation was 8 weeks in this cohort, and at median follow-up age of 27 months, they reported a good outcome with a mean HbA1c of 7%. The study reports a genetic mutation-positive rate of 63.6%. *KCNJ11* and *ABCC8* mutations were noted in 4/11 (33%). Another study on the south Indian cohort of 10 infants reported a genetic mutation-positive rate of 90%.[11] *KCNJ11* and *ABCC8* mutations were predominant again accounting for 30% (3/10) of their cohort and these infants have switched over to sulphonylureas accordingly. Data from other case-series also demonstrates a similar genetic distribution with regard to potassium channel defects.[12–17] Interestingly, Dalvi *et al.* also report a patient with late-onset diabetes having homozygous *ABCC8* mutation.[17] More recent literature reports *GCK, EIF2AK3* and *PTF1A* mutations at higher frequency in the middle-eastern population with high consanguinity as compared to the western population where KATP channel mutations are the predominant cause of permanent diabetes.[11,16] Excellent study by Nayak *et al.* also collated data of all previously published Indian data on NDM which demonstrates the bulk of all NDM cases to be due to potassium ATP (KATP) channel defects – *ABCC8* and then *KCNJ11*.[18] However, WRS from *EIF2AK3* mutation still remains the most frequently reported aetiology for permanent neonatal diabetes mellitus (PNDM). This study also describes a unique case of transient NDM with a homozygous *ABCC8* mutation. A similar report of a high percentage of WRS has been recently reported from Egypt as well.[19] This is in keeping with consanguineous and inbred population cohorts demonstrating the same finding.[20] Interesting is also the fact that in a recent study on monogenic diabetes from another centre in South India, all patients with WRS had a presentation at 1 year of age or later.[21] Table 3 enlists the salient features of the reported Indian literature on NDM. This highlights the degree of clinical and genetic heterogeneity that exists with regard to NDM – both the KATP channel defects as well as the syndromic forms.

Our cohort had no *KCNJ11* or *ABCC8* mutations and hence none could be changed over to oral glibenclamide. This is very different from the data published earlier where 46% of cases from consanguineous families had one of the potassium channel mutations[3] and other Indian studies which report at least 30% to have potassium channel mutations.[7,8] This could be attributed to a high degree of consanguinity in the south Indian cohort and referral bias. Perhaps with more prospective data, a better understanding of the same can be elicited.

Interestingly, two of our patients had immune dysfunction. Patient 8 [in Table 1] with IPEX syndrome was referred for HSCT and had required very high doses of insulin up to 2 U/kg/d while on immunosuppression. Unfortunately, he failed the transplant and our team was informed over the phone that he passed away at 26 months of age. Although NDM as part of IPEX is well known, there is very scarce literature on the challenges in the management of this condition. A case-series showed that among those who had immune dysfunction with *FOX P3* and NDM – all three patients succumbed within 2 years of age.[22] None of these three received HSCT. The same series also highlights three other patients with NDM without significant immune dysregulation doing fairly well till adolescence, thus depicting clinical heterogeneity within this genotype as well. This clearly highlights the need for more such patients to be reported. Patient 9 also came for an opinion regarding HSCT and had poor weight gain with diarrhoea and was diagnosed with a rare form of NDM secondary to homozygous *IL2RA* missense mutation. He was also positive for GAD antibody and this association has not been reported so far. Although this genetic mutation is reported in the literature, no case reports of the same have been described.

With premier genetic facilities being available abroad and these labs catering to the needs of other developing countries, we now have excellent published data on genetic mutations available.[3] Nevertheless, both genetic and clinical heterogeneity still remain across all ethnicities. Also, practical management of these patients and outcomes with regard to growth and glycaemic control are poorly described as noted in Table 3. This study is an attempt to bridge that gap and highlights the management of NDM from a developing country perspective.

Table 3: Summary of Indian case-series with NDM

Study and year	Number of patients with NDM	Genetic mutation distribution	Follow-up period and outcome	Glycaemic control	Growth	Unique features of the study
Jahnavi *et al*., 2013, Multi-centre, India	22	22- PDM KCNJ11-3 ABCC8-4 INS - 1	Follow-up period – not described	Mean HbA1c- 6.8% among five on glibenclamide, Rest- not mentioned	Not reported	Good outcome with sulphonylureas mentioned.
Ganesh *et al*., 2016, Chennai, India	10 (Five boys)	9 – PDM, 1-TDM ABCC8-2 KCNJ11-1 INS-2 PDX-1 EIF2AK3-1 NEUROD1-1 SLC1A2-1	One died Nine followed-up Mean age at follow up – 4.3 years	Not mentioned	Reported to be normal	Predominant (33%) KATP channel mutation reported.
Jain *et al*., 2017, Delhi, India	11 (Eight boys)	8- PDM, 2- TDM, 1-uncertain KCNJ11-3 ABCC8-1 INS-2 ZFP57-1	Median age at follow up- 2.3 years	Mean HbA1c- 7.1%	Reported to be normal	63.6% pathogenic mutation rate, Follow-up age and HbA1c highlighted.
Dalvi *et al*., 2017, Mumbai, India	Six	5-PDM, 1-TDM ABCC8-3 INS-1 EIF2AK3-1		HbA1c - 5.9-7.1%, for three on glibenclamide - 9 and 10.3% for two on insulin	Not mentioned	Late manifestation at 9 years for one with homozygous ABCC8 mutation described. Low birth weight not a striking feature.
Nayak *et al*., 2021, Lucknow, India	12	7-PDM, 4-TDM GCK-2 TRMA-2 EIF2AK3-2 FOXp3-1	Nine alive and on follow-up (1.5-10) 3-expired- 1 post-transplant, 1 with neg mutation and 1 with WRS and pneumonia	HbA1c at follow-up for all not mentioned.	Not mentioned	Novel disease causing mutations in EIF2AK3, GCK, ABCC8 described Homozygous ABCC8 presenting as TDM described Excellent summary of genetic data of all previous Indian patients with NDM
Lakshmanan *et al*., 2021, Cochin, India	15	9-PDM, 6- NDM ABCC8-3 INS-3 KCNJ11-2		Not mentioned for all	Not mentioned	All syndromic forms included have age on onset 1 year and above (13 with Wolfram, 1 with TRMA, 1 mitochondrial) Association of DEND with ABCC8 mutation described
Current study, 2021, India	12	All PDM EIF2AK3-2 INS-1 PDX-1 FOXp3-1 IL2RA-1	Eight alive and on follow-up (1.8-12.1 years)[#]. Median follow-up age 5 years. DAMA 2 died- 1 post-transplant, 1 unclear cause Lost to follow-up	Mean HbA1c – 8.2% (1-year average)	Weight for age from 2nd to 13th percentile Height for age from 6.5 to 20th percentile	The outcome of NDM in terms of both growth and glycaemic status included -Rare forms of immune dysfunction with NDM described -No K channel mutation in a cohort

TDM=transient diabetes mellitus. *Distribution of genetic mutations among PDM in each group alone included in the table. [#]Age range at follow-up

Limitations

This was a retrospective study cohort with only a 54% genetic mutation-positive rate detected.

Conclusion

Our study demonstrates good growth and glycaemic outcome in NDM patients without immune dysfunction when treated with SC insulin therapy. The use of diluted SC insulin is often necessary for the management of NDM infants. When manufacturer-provided diluents are not available, dilution with normal saline can be used for rapid-acting analogues, regular insulin as well as glargine. The molecular diagnosis was attained in a smaller proportion (54%) of patients with a conspicuous absence of mutations in *KCNJ11* and *ABCC8,* which are in contrast to other Indian or international studies. The major utility of molecular diagnosis to identify sulphonylurea-responsive NDM patients is well known. In this study, we emphasize the role of molecular diagnosis in identifying the need for therapies other than insulin such as HSCT and/or immunosuppressive therapy in NDM patients.

Author contribution

SK- Concept, data collection, analysis, clinical management, manuscript writing, editing
LR- genetic analysis, editing
PGP- clinical management, editing
J- Genetic analysis
AC- Genetic analysis, data collection, editing
SS- clinical management, editing
AS- clinical management, editing
SM- clinical management, editing.

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Conflicts of interest

There are no conflicts of interest.

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Appendix 1: Thirty gene next-generation *genetic* sequencing (NGS) panel for neonatal diabetes includes *HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, KCNJ11, ABCC8, AKT2, CISD2, CP, EIF2AK3, GATA6, GLUD1, HADH, IER3IP1, INSR' NEUROG3, PTF1A, RFX6, SLC2A2, WFS1, ZFP57, GLIS3, FOXP3* genes.