RESEARCH PAPER

Clinical and Molecular Characterization of Children with Neonatal Diabetes Mellitus at a Tertiary Care Center in Northern India

VANDANA JAIN, AMIT SATAPATHY, JAIVINDER YADAV, RAJNI SHARMA, *VENKATESAN RADHA, *VISWANATHAN MOHAN, #ELISA DE FRANCO AND #SIAN ELLARD

*From Division of Pediatric Endocrinology, Department of Pediatrics, AIIMS, New Delhi, and *Madras Diabetes Research Foundation, Chennai, India; and #Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK.*

Correspondence to:
Prof. Vandana Jain,
Division of Pediatric Endocrinology,
Department of Pediatrics, AIIMS,
Delhi-110 029, India.
drvandanajain@gmail.com
Received: May 19, 2016;
Initial review: August 31, 2016;
Accepted: March 28, 2017.

Objective: To study the genetic mutations and clinical profile in children with neonatal diabetes mellitus **Methods:** Genetic evaluation, clinical management and follow-up of infants with neonatal diabetes **Results:** Eleven infants were studied of which eight had permanent neonatal diabetes. Median age at presentation was 8 weeks and mean (SD) birth weight was 2.4 (0.5) kg. Pathogenic genetic mutations were identified in 7 (63.6%) children; 3 infants with mutations in *KCNJ11* gene and 1 in *ABCC8* were switched to oral sulfonylureas; 2 infants had mutations in *INS* and 1 in *ZFP57*. **Conclusion:** Neonatal diabetes mellitus is a heterogeneous disorder. Identification of genetic cause guides clinical management.

Keywords: *Diabetic ketoacidosis, Genetic analysis, HbA1c, Sulfonylureas*.

Neonatal diabetes mellitus (NDM) is a constellation of rare monogenic disorders, with an estimated prevalence of 1 in 89,000 [1]. The condition usually manifests within the first 6 months of life, and occasionally in second half of the first year with polyuria, dehydration and diabetic ketoacidosis [1]. In the past three decades, monogenic mutations in more than 20 genes have been identified that lead to impaired development, reduced function or progressive destruction of the pancreatic beta cells [2,3].

There are two distinct subtypes of NDM: (*i*) permanent; and (*ii*) transient form characterized by resolution of diabetes within weeks to months [4]. Activating mutations in the genes *ABCC8* and *KCNJ11*, which encode for the Kir6.2 and SUR1 subunits, respectively, of the ATP-sensitive potassium (KATP) channel of the pancreatic beta cells are the commonest cause of permanent NDM [2]. Of the transient NDM cases, nearly 70% of are caused by loss of methylation at the differentially imprinted locus on chromosome 6q24 [4], while *KCNJ11* and *ABCC8* mutations account for majority of the remaining cases [5]. The present study was conducted with the objective of describing the genetic mutation profile and the clinical presentation, management and outcome of children with neonatal diabetes mellitus at our institute over the last seven years.

METHODS

We reviewed the case records of all infants with NDM presenting to All India Institute of Medical Sciences (AIIMS), New Delhi from January 2009 to December 2015. NDM was diagnosed when the child presented within the first 6 months of life with symptoms of polyuria and dehydration with random blood sugar more than 200 mg/dL, or when the presentation was later in the first year but proven to be of monogenic etiology [1,2].

All infants were hospitalized at presentation. Diabetic ketoacidosis (DKA) was managed as per protocol [6]. Subsequent management was with 0.3 to 0.7 U/kg/day of subcutaneous insulin in multiple divided doses. Majority of the infants needed 1-2 doses of Neutral Protamine Hagedorn (NPH) insulin and 3-4 doses of Regular insulin. Parents were instructed to dilute the insulin with normal saline and administer it using insulin syringe. They were given simple written instructions to monitor blood glucose at home, manage hypo- and hyperglycemia, and check urinary ketones when baby was sick [7]; close follow-up was maintained.

Genetic mutation analysis was done after obtaining informed consent from the parents. Genetic testing for six infants (cases 1, 6, 7, 8, 9 and 11 in ***Table*** I) was carried out at Department of Molecular Genetics, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK. It

TABLE I SUMMARY OF THE CLINICAL PROFILE AND MUTATIONS IN THE PATIENTS WITH NEONATAL DIABETES MELLITUS

Case No.	*City, State*	*Gender, Birth weight, Age at presentation*	*Family history*	*Mutation*		*Management*	*Current age and HbA1c*
				Index case	*Parents*		
1.	Jalandhar, Punjab	M, 2.90 Kg,4 weeks	Paternal grandfather and great grandmother had type 2 DM	Compound heterozygous missense mutations in *ABCC8* (p.Arg168Cys and p.Gly1256Ser)	Heterozygous missense mutation in *ABCC8* in both parents (p.Arg168Cys in mother and p.Gly1256 Ser in father)	Glibenclamide @ 0.4 mg/kg/day	7 y; 6.8%
2	Delhi	F,1.50 Kg,16 weeks	Adopted child	No mutation in *KCNJ11*, *ABCC8* or *INS*	Not tested	Insulin @0.6 u/kg/day	6 y;7.6%
3	Delhi	F,3.10 Kg44 weeks	Nil	Heterozygous missense mutation in *INS* (p. Arg89Cys)	No mutation	Insulin@0.3 u/kg/day	4 y; 7.1%
4	Indore, Madhya Pradesh	M,2.50 Kg3 weeks	Paternal grand mother had type 2 DM	Heterozygous mutation in *KCNJ11* (p. Arg50Gln)	Heterozygous mutation in *KCNJ11* (p.Arg50Gln) in mother, No mutation in father	Glibenclamide @2 mg/kg/day	3 y;6.2%
5	Manipur	M, 2.20 Kg,10 weeks	Nil	No mutation in *ABCC8*, *KCNJ11* or *INS*	Not done	Insulin@0.4 u/kg/day	2.5 y; 8.9%
6	Jaipur Rajasthan	M,3.20 Kg,12 weeks	Nil	Heterozygous missense mutation in *KCNJ11* (p. Phe333Leu)	No mutation	Glibenclamide @1 mg/kg/day	2 y;7.4%
7	Ranchi, Chattishgarh	F,2.05 Kg,4 weeks	Nil	Heterozygous missense mutation in *KCNJ11* (p.Arg201Cys)	No mutation	Glibenclamide @0.9 mg/kg/day	2 y;6.2%
8	Delhi	M, 2.30 Kg,1 week	Sibling died on day 21 due to delayed diagnosis of neonatal diabetes	Homozygous frameshift mutation in *ZFP57* (p.Gln469fs)	Not done	Off insulin after 4 months of age	1.5 y; 6.0%
9	Jhansi Uttar Pradesh	F,1.85 Kg8 weeks	Nil	No mutation in *KCNJ11*, *ABCC8* and *INS*	No mutation	Off insulin after 4 months of age	1 y;8.1%
10	Meerut Uttar Pradesh	M, 2.70 Kg10 weeks	Nil	Not done	Not done	Insulin@0.2 u/kg/day	Died at 14 weeks
11	Delhi	M,1.75 Kg,4 weeks	Nil	Heterozygous missense mutationin *INS* (p.Ser101Cys)	No mutation	Insulin@0.8u/kg/day	6 mo;7.1%

consisted of rapid Sanger sequencing of the *KCNJ11*, *ABCC8* and *INS* genes with additional *EIF2AK3* analysis in infants born to related parents. In patient 8, a custom designed targeted next generation sequencing of all known NDM genes [3] was also done; and methylation analysis for chromosome 6q24 was conducted at Wessex Regional Genetics Laboratory, Salisbury [8,9]. Mutation testing for cases 2, 3, 4 and 5 was done at Department of Molecular Genetics, Madras Diabetes Research foundation, Chennai. Sequencing was carried out on ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) using the Big Dye terminator V3.1, and the sequences were compared with the database. Published primers were used to amplify the DNA for *KCNJ11*, *ABCC8* and *INS* [10,11].

After receiving the genetic report, four infants with mutations in *KCNJ11* or *ABCC8* were switched to oral sulfonylureas as per the protocol [12]. All children were followed-up for growth, development and glycemic control.

RESULTS

Eleven infants (8 boys) were diagnosed with NDM. The clinical, demographic and genetic mutation profile of the cases is summarized in ***Table*** I. The median (range) age at initial presentation was 8 (1- 44) weeks. Mean (SD) birth weight was 2.4 (0.5) kg, with 7 (63.6%) infants being low birth weight. Six (54.5%) infants had presented with DKA; 4 (36.4%) with polyuria, irritability and increased demand for feeds, and one was diagnosed by surveillance of blood glucose in view of previous sibling's death with NDM.

Eight (72.7%) infants in our cohort had permanent NDM, 2 had transient NDM and the type was uncertain in one child who died at 3 months. Pathogenic genetic mutations were identified in seven (63.6%) infants (***Table* I**). The most commonly affected gene was *KCNJ11* in three cases, followed by *INS* gene in two cases. One patient each had mutation in *ABCC8* and *ZFP57* genes. None of the common mutations were identified in three infants. In one patient, initial sample was insufficient and the baby died before repeat sample could be obtained.

We were able to successfully transfer all four children with mutations in either *KCNJ11* or *ABCC8* genes from insulin to oral glibenclamide. The age at transfer ranged from 2 to 9 months. The initial dose of glibenclamide on which glycemic control was achieved ranged from 0.5 to 2 mg/kg/day, and was reduced on follow-up. Glycemic control was excellent in these children with no episode of DKA or symptomatic hypoglycemia. At last follow-up, the mean (SD) HbA1c level in these 4 children was 6.6 (0.5)%. Two infants having mutations in the *INS* gene were continued on subcutaneous regular and NPH insulin, and are under regular follow-up.

Case 8 was diagnosed within the 1st week of life. He was born out of consanguineous marriage, with the conspicuous history of death of a sibling with hyperglycemia at 3 weeks of age. A homozygous frameshift mutation in the *ZFP57* gene (c.1405del, p.Gln469fs) was identified. Complete loss of maternal methylation at the transient neonatal diabetes differentially methylated region on 6q24 was confirmed. Insulin could be stopped at 4 months of age. In the two other children in whom a mutation in the more common NDM genes was not identified (cases 2 and 5), insulin was continued, and glycemic control was satisfactory. We lost one baby in our series at 3 months of age to probable sepsis and DKA. The current median (range) age of our cohort is 27 (6-84) months, and the mean (SD) HbA1c is 7.1 (0.9)%. Growth and development of all the children is within normal limits.

DISCUSSION

We have presented the clinical and molecular characteristics for our series of 11 infants with NDM; pathogenic mutations were identified in seven (63.6%). Four cases of permanent NDM were caused by mutations in the K_{ATP} channel (*KCNJ11* and *ABCC8*) genes, and could be successfully switched over to oral glibenclamide. *INS* gene mutations were present in 2 children and transient NDM related to loss of 6q24 methylation due to a *ZFP57* homozygous mutation was identified in one child. Seven out of the 11 cases in our cohort had low birth weight. This is a relatively consistent feature of NDM, and reflects on the role of insulin as an important growth factor in fetal life [2].

Limitations of this study are: small sample size and incomplete genetic evaluation for some of the patients. Of the four cases without confirmed genetic mutation, two had undergone evaluation for only the three most common genes (*KCNJ11*, *ABCC8* and *INS*), one had transient NDM but could not be tested for loss of methylation and one baby died before genetic testing.

In a recent large study, it was reported that in offspring of non-consanguineous parents, mutations in *KCNJ11*, *ABCC8*, *INS* and 6q24 methylation abnormalities accounted for approximately 28%, 18%, 10% and 10%, respectively of the NDM cases; while among children of consanguineous parents, *EIF2AK3* mutations were the commonest [2]. In an earlier Indian series, Jahnavi, *et al*. [14] had reported a total of 12

WHAT THIS STUDY ADDS?

- The commonest cause of permanent neonatal diabetes mellitus in our series of north Indian infants was mutations in the K_{ATP} channel (*KCNJ11* and *ABCC8*) genes, and these children could be switched over to oral sulfonylureas.
- Mutations in *INS* gene were the next common and affected children require life-long insulin therapy.

mutations in 33 children with infancy-onset diabetes; *ABCC8* in 7, *KCNJ11* in 3 and *INS* in 2. In another recent series from Chennai [15], mutation profile of 10 children with NDM (six of whom were born to consanguineous parents) was presented. Nine of these had permanent NDM, attributable to mutations in *ABCC8* and *INS* in 2 each, and *KCNJ11*, *EIF2AK3*, *SLC19A2*, *NEUROD1* and *PDX1* in one each; while one had transient NDM due to loss of methylation in chromosome 6q24.

The identification of mutations in *KCNJ11* or *ABCC8* genes highlights the importance of establishing early genetic diagnosis, as these patients can be switched over to oral sulfonylureas, that are not only more convenient, but also associated with reduction in episodes of hypo- and hyper-glycemia [12,13]. Insulin gene (*INS*) mutations are associated with synthesis of misfolded preproinsulin or proinsulin that is retained in endoplasmic reticulum and results in apoptosis of the beta cells, leading to permanent insulin-dependence. The age at onset is variable with many patients presenting beyond 6 months or even beyond infancy. *INS* mutations are typically sporadic and heterozygous [10], as in both our cases. Abnormalities in 6q24 are typically associated with moderate intrauterine growth retardation and diabetes within first week of life. Hyperglycemia remits by few weeks to months, but may recur during illness [4], and nearly half relapse during adolescence or early adulthood [16].

To conclude, genetic testing is essential in children with neonatal diabetes mellitus. It not only confirms the diagnosis, but also defines the prognosis of the affected child, and predicts the risk in subsequent pregnancies. Since mutations in *KCNJ11* and *ABCC8* have major therapeutic implications, these genes should be screened first. Testing of 6q24 must be considered in infants with NDM who had intrauterine growth retardation, and go into spontaneous remission. The role of adequate counseling and education of the parents in appropriate management of the young infants with NDM cannot be over-emphasized. Early diagnosis, identification of the genetic defect and appropriate clinical management can be very rewarding.

Acknowledgements: Dr Deborah Mackay and Prof Karen I Temple for performing the methylation specific analysis, and to Mr Anil Kumar for extracting DNA and ensuring safe transport of the clinical samples from AIIMS to the laboratories in Chennai or Exeter.

Contributors: VJ: conceptualized the study design, critically revised the paper and will act as guarantor; VJ, AS: drafted the paper, VJ, AS, JY, RS: acquired the data; VR, VM, EDF, SE: performed and interpreted the mutation analysis. All authors contributed to manuscript writing and approved the final draft.

Competing interests: None stated.

Funding: SE is the recipient of a Wellcome Trust Senior Investigator award (grant number: WT098395/Z/12/Z). The genetic analysis carried out at Institute of Biomedical and Clinical Science, Exeter has been supported by this grant.

REFERENCES

1. Grulich-Henn J, Wagner V, Thon A, Schober E, Marg W, Kapellen TM, *et al*. Entities and frequency of neonatal diabetes: data from the diabetes documentation and quality management system (DPV). Diabet Med. 2010;27: 709-12.
2. De Franco E, Flanagan SE, Houghton JA, Lango AH, Mackay DJ, Temple IK, *et al*. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: An international cohort study. Lancet. 2015;386:957-63.
3. Ellard S, Lango AH, De Franco E, Flanagan SE, Hysenaj G, Colclough K, *et al*. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. Diabetologia. 2013;56:1958-63.
4. Temple IK, Gardner RJ, Mackay DJ, Barber JC, Robinson DO, Shield JP. Transient neonatal diabetes: Widening the understanding of the etiopathogenesis of diabetes. Diabetes. 2000;49:1359-66.
5. Ioannou YS, Ellard S, Hattersley A, Skordis N. KCNJ11 activating mutations cause both transient and neonatal diabetes mellitus in Cypriot patients. Pediatr Diabetes. 2011;12:133-7.
6. Wolfsdorf JI, Allgrove J, Craig ME, Edge J, Glaser N, Jain V, *et al*; International Society for Pediatric and Adoloscent Diabetes. ISPAD Clinical practice Consensus Guidelines 2014. Diabetes ketoacidosis and hyperglycemia hyperosmolar state. Pediatr Diabetes. 2014;15:154-79.
7. Jain V. Management of type 1 diabetes in children and adolescents. Indian J Pediatr. 2014;81:170-7.
8. Mackay DJ, Temple IK, Shield JP, Robinson DO. Bisulphite sequencing of the transient neonatal diabetes mellitus DMR facilitates a novel diagnostic test but reveals no methylation anomalies in patients of unknown aetiology. Hum Genet. 2005;116:255-61.
9. Mackay DJ, Callaway JL, Marks SM, White HE, Acerini CL, Boonen SE, *et al*. Hypomethylation of multiple

imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. Nat Genet. 2008;40:949-51.
10. Stoy J, Steiner DF, Park Y, Ye H, Philipson LH, Bell GI. Clinical and molecular genetics of neonatal diabetes due to mutations in the insulin gene. Rev Endocr Metab Disord. 2010;11:205-15.
11. Ellard S, Flanagan SE, Girard CA, Patch AM, Harries LW, Parrish A, *et al*. Permanent neonatal diabetes caused by dominant, recessive or compound heterozygous SUR1 mutations with opposite functional effects. Am J Hum Genet. 2007:81:375-82.
12. Pearson ER, Flechtner I, Njølstad PR, Malecki MT, Flanagan SE, Larkin B, *et al*. Neonatal Diabetes International Collaborative Group Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med. 2006;355:467 77.
13. Jain V, Kumar S, Flanagan SE, Ellard S. Permanent neonatal diabetes caused by a novel mutation. Indian Pediatr. 2012;49:486-8.
14. Jahnavi S, Poovazhagi V, Mohan V, Bodhini D, Raghupathy P, Amutha A, *et al.* Clinical and molecular characterization of neonatal diabetes and monogenic syndromic diabetes in Asian Indian children. Clin Genet. 2013;83:439-45.
15. Ganesh R, Suresh N, Vasanthi T, Ravikumar KG. Neonatal diabetes: A case series. Indian Pediatr. 2017;54:33-6.
16. Shield JP, Temple IK, Sabin M, Mackay D, Robinson DO, Betts PR, *et al.* An assessment of pancreatic endocrine function and insulin sensitivity in patients with transient neonatal diabetes in remission. Arch Dis Child Fetal Neonatal Ed. 2004;89:341-3.