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Genetics of Neonatal Diabetes: An Update

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Abstract

Neonatal diabetes mellitus (NDM) is defined as persistent hyperglycemia in infants within the first six months of life or rarely within one year of life, along with absent or insufficient circulating insulin. It can be either transient or permanent diabetes, depending on the duration of insulin requirement. Transient NDM is frequently associated with aberrations of 6q24 locus resulting in overexpression of imprinted genes. Other pathogenic variations which cause transient NDM are activating mutations of K_{ATP} channel-related genes (*ABCC8* and *KCNJ11*), and insulin (*INS*) and *ZFP57* gene mutations. Permanent NDM is caused by a wide range of pathogenic variants causing beta cell functional defects, endocrine pancreas development abnormality, and beta cell destruction which is either immune mediated or secondary to endoplasmic reticulum stress. Most common pathogenic variants associated with permanent NDM are *KCNJ11*, *ABCC8*, *INS*, *GCK* and *PDX1* mutations. Several syndromic variants with extra-pancreatic features are also described to cause neonatal diabetes. Molecular genetic testing is important for prognostication, to decide on the appropriate therapeutic option (insulin or sulfonylureas) and for genetic counselling.

Keywords: Genetic basis, neonatal diabetes mellitus

Introduction

Hyperglycemia is one of the common metabolic disturbances seen in neonates, especially in the preterm, low-birthweight or very sick infants. To define as neonatal diabetes mellitus (NDM), there must be persistent hyperglycemia (plasma glucose >150 mg/dL) within the first six months or rarely within one year of life along with absent or insufficient circulating insulin (Beltrand et al., 2020). The global incidence varies from 1 in 90,000 to 160,000 live births (Lemelman et al., 2019). More than 50% of the cases are transient NDM (TNDM), in which hyperglycaemia usually resolves within 18 months (Polak et al., 2007). The remaining are permanent NDM which requires lifelong insulin therapy. The basic mechanism is either an abnormal development of pancreatic beta cells or a functional abnormality in insulin secretion. Consequently, neonatal diabetes may also be associated with pancreatic and extra-pancreatic malformations. It is a predominantly monogenic disease with 80% having an identified genetic mutation (Polak et al., 2007). Apart from conventional insulin replacement therapy, specific genetic mutations also respond to oral sulfonylureas. Hence, the role of genetic testing in NDM is crucial.

Molecular Pathology and Genetic Basis

Neonatal DM is broadly classified into **transient** or **permanent,** based on the duration of insulin requirement. **Syndromic NDM** can be either transient or permanent, associated with extra-pancreatic features that are part of a known inherited syndrome.

A) Transient Neonatal DM The mutations contributing to TNDM predominantly fall in two major categories- aberrations in 6q24 locus and activating mutations of K_{ATP} channel-related genes (*ABCC8* and *KCNJ11*).

Normally, 6q24 locus is subject to imprinting (by methylation of CpG islands) when maternally inherited. A disorder of imprinting resulting in overexpression of *PLAGL1/ZAC* (pleiomorphic adenoma gene-like 1) and *HYMAI* (Hydatidiform mole-associated and imprinted transcript) genes can cause a functional beta cell defect with variable expression based on age. The mechanisms described are paternal uniparental disomy of

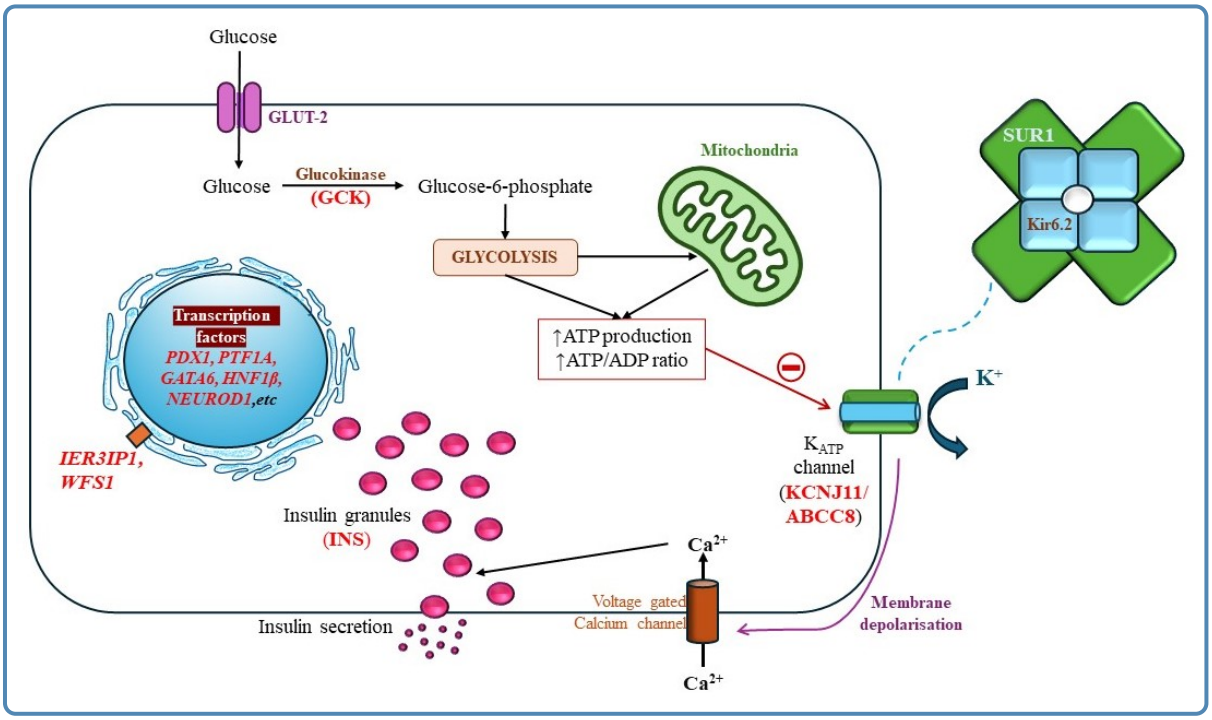

Figure 1 Pictorial representation of glucose response and insulin secretion pathway in the pancreatic beta cells and the genes associated with the pathway.

chromosome 6 (UPD6), paternal duplication of 6q24 and hypomethylation of maternal 6q24.

ZFP57 pathogenic variants contribute to TNDM as part of multi-locus imprinting disturbance with recessive inheritance.

B) Permanent Neonatal DM The mechanism of insulin secretion by beta cells of pancreas is depicted in Figure-1. Any defect in the genes and molecules involved will hamper insulin synthesis or secretion, resulting in neonatal diabetes. These mutations are classified as follows:

1. **Genetic causes of beta cell functional defect**- K_{ATP} channel mutations (*ABCC8/ KCNJ11*), insulin (*INS*) gene mutations with an altered protein expression and glucokinase (*GCK*) mutations

2. **Beta cell destruction by early immune destruction or endoplasmic Reticulum stress**- Accumulation of abnormal proteins because of *INS, EIF2AK3* (enzyme), *IER3IP1* (endoplasmic Reticulum protein) and *WFS1* (Wolframin- ER protein) mutations can cause beta cell apoptosis due to ER stress. Alternatively, immunodysregulation in the IPEX (Immunodysregulation, polyendocrinopathy, and enteropathy, X-linked) syndrome causes immune mediated beta cell destruction.

3. **Endocrine pancreas developmental abnormality**- Transcription factor mutations which are associated with congenital malformations include pathogenic variants of *PDX1, PTF1A, GATA6, GATA4, GLIS3, HNF1β, NEUROD1, NEUROG3,* etc.

Most common mutations are *KCNJ11* (30%), *ABCC8* (19%), *INS* (20%), *GCK* (4%) and *PDX1* (<1%) (De León et al., 2024). Salient clinical features of these pathogenic variants are described in **Table 1**.

K_{ATP} channels consist of the ion transporting Kir6.2 subunits (coded by the *KCNJ11* gene) and regulator subunit SUR1 (sulfonylurea receptor coded by the *ABCC8* gene). Activating heterozygous variants cause membrane hyperpolarisation, and prevent calcium influx and thereby insulin secretion. Since these channels are also present in the brain, neurological symptoms may be seen. Oral sulfonylureas can bind to the SUR1 subunit and induce closure of potassium channels, thereby reestablishing the normal physiology.

Heterozygous variants in the insulin (*INS*) gene affect the structure of preproinsulin. Abnormal protein accumulates, causing severe endoplasmic reticulum stress and β cell death. This variant

Table 1 Salient clinical features of common pathogenic variants of NDM

Gene/ Chromosome	Locus	Clinical features
Chr 6	6q24	• Transient NDM • Onset within 6 weeks • Hyperglycemia, dehydration, and failure to thrive at presentation • Ketoacidosis is rare • Macroglossia or umbilical hernia may be associated. • Remission within 18 months • Recurs in adolescence or adulthood- resembles type 2 diabetes.
ABCC8/ KCNJ11	11p15.1	• Transient/ permanent NDM • Onset before 6 months • IUGR in antenatal period and SGA at birth • Neurological symptoms- attention deficit hyperactivity disorder, sleep disruptions, seizures, and developmental delay
INS	11p15.5	• Transient/ permanent NDM • Presents any time before 1 year • Ketoacidosis at presentation
GCK	7p15.3-p15.1	• Permanent NDM • Presents from even neonatal period • Hyperglycemia of both parents • Heterozygotes present with MODY type 2
PDX1	13q12.1	• Permanent NDM • Agenesis/ hypoplasia of pancreas

NDM- Neonatal diabetes mellitus; IUGR- Intrauterine growth retardation; SGA- Small for gestational age; MODY- Maturity-onset diabetes of the young

usually causes permanent NDM. However, certain rare, recessive mutations are also reported, which alter the protein expression and can cause transient or permanent DM. These respond well to insulin therapy.

The first step in glucose metabolism inside β cell is catalysed by the glucokinase enzyme (coded by the *GCK* gene). It is a "sensor" of blood glucose and controls insulin secretion. Nonsense mutations cause neonatal diabetes when homozygous as glucokinase is completely deficient. Similar heterozygous mutations can

cause glucose intolerance (MODY 2) and hence parents of those with homozygous mutations can have fasting hyperglycemia.

Homozygous mutations of *PDX1* gene can present with pancreatic agenesis or hypoplasia. **Table 2** lists the other significant pathogenic variants.

Clinical features

Insulin being an anabolic hormone plays a critical role in fetal and extrauterine growth. In this context of insulin deficiency, many patients present with intrauterine growth retardation and low birth weight. Postnatal faltering of growth manifests when untreated. Clinical differentiation of transient from permanent NDM is difficult, except for the rapid fall in insulin requirement over 12-14 weeks. However, findings such as macroglossia and umbilical hernia have been described in 6q24-associated phenotypes. Fifty to sixty percent of TNDM cases can present with relapse of diabetes around puberty and in adulthood, which resembles early onset type 2 diabetes mellitus (Temple and Shield, 2002). This is proposed to be due to insulin resistance and could be prevented with lifestyle modifications and avoiding the potential risk factors (fast-food, smoking, lack of exercise). Neurological features like developmental delay and epilepsy (DEND syndrome), attention-deficit hyperactivity disorder (ADHD) or sleep disruptions are suggestive of K_{ATP} channel mutations. Ketoacidosis is rare at presentation and almost unlikely in transient NDM. Around 30% of individuals with *INS* mutations present with diabetic ketoacidosis (DKA) (Letourneau et al., 2017). The specific gene-related clinical features are listed in **Tables 1 and 2**.

Diagnosis of NDM

Hyperglycemia in the neonatal period may be caused by prematurity, extremely low birthweight, sepsis, necrotising enterocolitis, parenteral nutrition, use of drugs (such as glucocorticoids, catecholamine, caffeine, etc.) and any forms of stress such as mechanical ventilation or surgery. Neonatal DM is a rare cause, but there should be a strong suspicion when persistent (>150-200 mg/dL and insulin dependent more than seven days) or acute extreme hyperglycaemia (>1000mg/dL) is noted. Low or undetectable plasma insulin and C-peptide levels relative to hyperglycemia can confirm the diagnosis of NDM. Hyperketonaemia and ketonuria are not usually seen in initial presentation. An abdominal ultrasonogram must be done to look for presence or absence of pancreas. Further delineation of pancreatic morphology is done with computed tomography (CT) or magnetic resonance imaging (MRI) of the pancreas, whenever indicated. Stool fat examination and fecal elastase is tested in those with pancreatic agenesis/ hypoplasia to rule out exocrine deficiency.

Molecular genetic testing is recommended for all diabetes mellitus detected in less than 6 months of age. Additionally, those presenting between 6 months and 1 year should be tested if any extra-pancreatic features, negative autoantibodies, unusual family history, associated congenital defects or multiple autoimmune disorders are noted (ISPAD Clinical Practice Consensus Guidelines, 2022). Different testing strategies are used (**Figure 2**), such as serial single gene testing, multigene panel including the most common genes like *ABCC8, KCNJ11, INS, GCK* and *PDX1*, or a comprehensive genomic testing with whole exome or whole genome sequencing. Syndrome specific clinical phenotypes should be tested for the corresponding gene as per **Table 2**. Serial testing of single genes is a time consuming and expensive procedure with a high chance to miss rare genetic variants, except when clinical phenotypes specific to certain genes are identified. Whole exome sequencing is a cost-effective approach in a country like India, enabling detection of both common and uncommon pathogenic variants.

Management of NDM

Initial management consists of emergency stabilisation by rehydration and intravenous insulin infusion to control hyperglycemia. Once the child is stable and tolerates oral feeds, an appropriate regimen of subcutaneous insulin must be started. Infants requires very minimal doses of insulin only and hypoglycemia is more dangerous to the growing brain. Any inappropriate dose of rapid and short acting insulin can cause severe hypoglycemia and should be avoided. Longer acting insulin analogues such as Glargine or Detemir are preferred, as it maintains a basal insulin level without significant hypoglycemia. Intermediate acting insulins are not as effective but may be used in low-income settings.

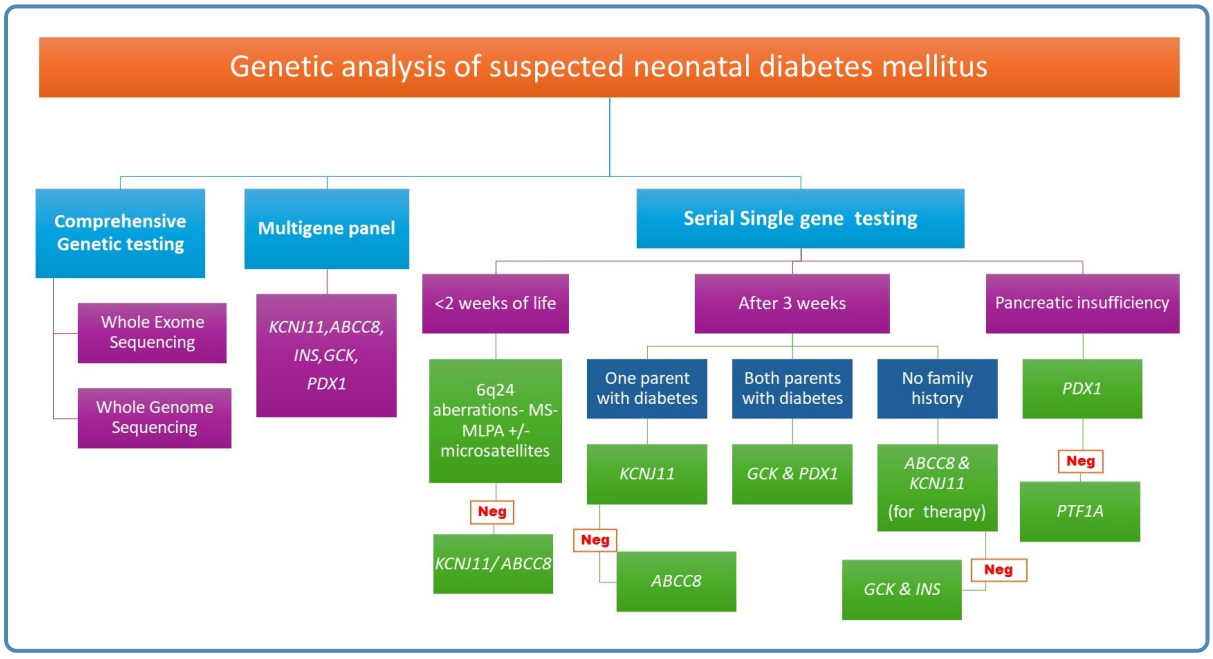

Figure 2 Algorithm for genetic testing of suspected neontal diabetes

Continuous subcutaneous insulin infusion (CSII) can deliver accurate insulin doses corresponding to blood glucose levels. This is more physiologic, safer, and reduces HbA1c better when compared to other regimens.

Sulfonylurea therapy is beneficial in *KCNJ11* and *ABCC8* mutations and some pathogenic variants of *GCK*. Around 90-95% of these patients achieve glycemic control with oral sulfonylureas when weaned off insulin therapy. It also improves the neurological symptoms. Various transfer protocols are available online for insulin to sulfonylurea transition. High doses (0.4-1.0 mg/kg/day of Glibenclamide) may be required. Crushed tablets are poorly soluble in water. Recently, a sulfonylurea suspension (Amglidia^{R}) has been approved by the European Medicines Agency (EMA). Common side effects are transitory diarrhoea and nausea.

Diet modification is better than diet restriction in children. A high calorie diet is recommended to maintain adequate weight gain. Pancreatic enzyme replacement must be provided for those with exocrine insufficiency.

Relapse of transient neonatal DM can respond to diet alone or needs addition of oral hypoglycemic agents with occasional insulin requirement.

Genetic Counselling

Confirmation of pathogenic variants can help in several aspects such as provision of targeted gene specific therapeutic modalities, early prediction of other associated system involvement, and for counselling and testing other family members. In transient NDM due to 6q24 mutations, paternal duplications are autosomal dominant, and hence carry a 50% transmission risk if inherited from the father (Temple and Shield, 2002). UPD6 is usually sporadic with a low recurrence risk. Maternal hypomethylation can be theoretically transmitted to 50% offspring of affected female individuals, but only de-novo and non-recurrent cases have been detected till now. Pathogenic variants of *KCNJ11* have autosomal dominant inheritance when familial, but 90% are de novo heterozygous mutations (ISPAD CPCG 2022). Phenotypes related to *ABCC8* and *INS* variants are either autosomal dominant or recessive and *GCK* and *PDX1*- related NDM follows an autosomal recessive pattern of inheritance.

Genetic counselling begins with identification of the variant(s) in the proband. There are several methods available to determine the pathogenicity of the variant such as database searches, in silico modelling with web-based applications, in-vitro functional studies of the protein product and

Table 2 Molecular genetics of syndromic forms of neonatal diabetes

Clinical phenotype	Genes affected	OMIM Phenotype
Pancreatic exocrine insufficiency or agenesis and cardiac abnormalities	*GATA6*	Neonatal and childhood Onset diabetes/ Pancreatic agenesis and congenital heart defects (OMIM # 600001)
Enteropathy and dermatitis	*FOXP3*	Immunodysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX syndrome) (OMIM # 304790)
Cerebellar involvement	*PTF1A*	Pancreatic agenesis 2/ Pancreatic and cerebellar agenesis (OMIM # 609069)
Congenital hypothyroidism, hepatic fibrosis, cystic renal dysplasia, congenital glaucoma	*GLIS3*	Diabetes mellitus, neonatal, with congenital hypothyroidism (OMIM # 610199)
Cerebellar hypoplasia, sensorineural deafness, and visual impairment	*NEUROD1*	Maturity-Onset Diabetes of the Young 6 (MODY 6) (OMIM # 606394)
Pancreatic hypoplasia, intestinal atresia, and gall bladder hypoplasia	*RFX6*	Mitchell-Riley syndrome (OMIM # 615710)
Congenital malabsorptive diarrhoea	*NEUROG3*	Diarrhea 4, malabsorptive, congenital (OMIM # 610370)
Skeletal abnormalities (epiphyseal dysplasia) and liver dysfunction	*EIF2AK3*	Wolcott-Rallison syndrome (OMIM # 226980)
Megaloblastic anemia and deafness	*SLC19A2*	Thiamine-responsive megaloblastic anemia (TRMA) syndrome (OMIM # 249270)
Renal and genital abnormalities	*HNF1B*	Renal cysts and diabetes syndrome (OMIM # 137920)
Optic atrophy, diabetes insipidus and deafness	*WFS1*	Wolfram syndrome 1 (OMIM # 222300)

clinical studies such as familial co-segregation studies. The mode of inheritance is communicated, and recurrence risk of parents and siblings are predicted. Pathogenic variants of *GCK, INS, PDX1, RFX6*, etc. can present with neonatal diabetes when homozygous and milder forms of diabetes such as Maturity Onset Diabetes of Young (MODY) when heterozygous. In these cases, heterozygous parents and siblings should undergo a screening blood glucose test even if asymptomatic. Prenatal counselling is advised for those with a known pathogenic variant in a family member. Option of prenatal/ preimplantation genetic testing can be suggested for the identified variant in the proband.

Follow–up and surveillance

Periodic daily blood glucose monitoring with conventional glucometers or continuous glucose monitoring systems (CGMS) is done to assess therapeutic adequacy. Target HbA1c should be

less than 7.5. ISPAD suggests yearly HbA1c monitoring for transient NDM patients after remission for early identification of relapse. Long term follow-up should include metabolic work-up and socio-education. Periodic developmental assessment is needed, especially in *KCNJ11* and *ABCC8* pathogenic variants. Yearly screening for microalbuminuria and retinopathy should start from 10 years of age.

Conclusion

Neonatal diabetes mellitus is a rare cause of hyperglycemia in neonates, with a predominant monogenic origin. After confirming the diagnosis, genetic testing is recommended for prognostication and management. Pancreatic malformations and extra-pancreatic features should be specifically looked for. Initial therapy is with insulin replacement, but a transition to oral sulfonylureas must be attempted as soon as a favourable pathogenic variant (*KCNJ11* and *ABCC8*) is identified. Lifestyle modifications and periodic follow-up are the key to disease control.

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