



Neonatal Diabetes Mellitus Testing

Clinical Features of Neonatal Diabetes (NDM)

Neonatal diabetes mellitus (NDM) is a rare form of diabetes that is likely to have a monogenic cause, particularly when diagnosed before 6 months of age [1]. NDM is a relatively rare disorder that affects approximately 1:215,000 to 1:260,000 live births. Approximately half of the NDM cases are transient (TNDM) but can ultimately relapse [2, 3]. In contrast, permanent NDM (PNDM) cases need continual treatment from diagnosis. NDM cases diagnosed before 6 months of age are usually autoantibody negative and have human leukocyte antigen (HLA) types similar to the general population, rather than HLA types known to be associated with type 1 diabetes that are found in those diagnosed at older ages [4, 5]. Clinical manifestations at the time of diagnosis include intrauterine growth retardation (IUGR), hyperglycemia, glycosuria, osmotic polyuria, severe dehydration, and failure to thrive. Although the majority of cases of neonatal diabetes involve isolated diabetes, many of the known monogenic causes are characterized by a variety of syndromic features.

Molecular Genetics of Transient Neonatal Diabetes (TNDM)

- The most common cause of transient neonatal diabetes is overexpression of imprinted genes *PLAGL1* and *HYMAI* at 6q24, due to either paternal uniparental disomy of chromosome 6 (UPD6), duplication of 6q24 on the paternally inherited allele, or hypomethylation of the maternally inherited allele [6]. Recurrence risk for siblings and offspring of an affected proband depends on the cause of the imprinting defect, but may be up to 50% if caused by an inherited duplication of 6q24.
- Other rarer causes of TNDM include heterozygous mutations in *ABCC8* [7], *KCNJ11* [8], and biallelic mutations in *ZFP57* [9]. Very rare mutations in *INS* may also cause a phenotype that could be described as transient neonatal diabetes [10].

Molecular Genetics of Permanent Neonatal Diabetes (PNDM)

- Nearly half of all cases of PNDM are due to activating mutations in *KCNJ11* and *ABCC8* which encode the two components of the beta-cell plasma membrane ATP-dependent potassium channel [11, 12]; the majority of mutations in these genes associated with PNDM are *de novo* in origin. For patients with *ABCC8*- and *KCNJ11*-related PNDM their diabetes can be remarkably well controlled by oral sulfonylureas tablets instead of injected insulin [13, 14] leading to improved glucose regulation and quality of life, and lower costs of treatment. Activating *KCNJ11* mutations are associated wide range of clinical features ranging from isolated diabetes to more severe phenotypes such as DEND syndrome, which is associated with developmental delay, epilepsy and neonatal diabetes [15].
- Approximately 20% of PNDM is attributed to mutations of *INS*, the gene encoding insulin [16, 17]. Testing for the c.188-31G>A intronic mutation in the *INS* gene, which is thought to account for a significant proportion of patients with NDM, is included in our panel [18].
- Heterozygous mutations in *GATA6* are associated with pancreatic agenesis and permanent neonatal diabetes [19]. Extra-pancreatic findings such as congenital heart defects may also be observed in affected individuals.
- Biallelic *EIF2AK3* mutations cause Wolcott-Rallison syndrome (WRS; OMIM#226980) which is associated with permanent neonatal or early infancy insulin-dependent diabetes [20]. Other findings, including epiphyseal dysplasia, osteoporosis, and growth retardation, develop at a later age. Other frequent multisystem manifestations include hepatic and renal dysfunction, intellectual disability, and cardiovascular abnormalities.
- Mutations in the X-linked gene *FOXP3* cause IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked) syndrome, which is characterized by systemic autoimmunity, typically beginning in the first year of life. Presentation is most commonly the clinical triad of watery diarrhea, eczematous dermatitis, and endocrinopathy (most commonly insulin-dependent diabetes mellitus) [21].
- A heterozygous mutation in the *GATA4* gene has been identified in a patient with pancreatic agenesis (a rare cause of neonatal diabetes), white matter changes and multi-organ failure [22]. Heterozygous mutations in *GATA4* have also been identified in individuals with a variety of heart defects, including atrial septal defects, ventricular septal defects, and tetralogy of Fallot [23].

- In rare cases, PNDM is caused by inactivating mutations of *GCK*, the gene encoding glucokinase [24, 25]. Carrier parents have mild fasting hyperglycemia (previously known as monogenic diabetes of the young (MODY) type 2, or GCK-MODY).
- Homozygous missense mutations in *MNX1* have been identified in individuals with permanent neonatal diabetes mellitus from three different consanguineous families [26, 27]. All patients exhibited severe intrauterine growth retardation. One patient additionally exhibited sacral agenesis and imperforate anus, which are features of Currarino syndrome, a condition caused by heterozygous mutations in *MNX1* [OMIM#176450].
- Homozygous nonsense or frameshift mutations have been identified in *NXK2-2* in three individuals with severe defects in insulin secretion and presentation of diabetes at an early age without evidence of exocrine insufficiency. All three patients had intrauterine growth retardation and moderate-to-severe developmental delays [26].
- Rarely, PNDM is caused by inactivating mutations of *PDX1* [28]. Carrier parents have mild, adult-onset diabetes mellitus (*PDX1*-familial monogenic diabetes, previously known as MODY4) [28].
- Biallelic loss-of-function mutations in *LRBA* were identified in patients diagnosed with diabetes within the first 15 months of life and presenting with some degree of early onset autoimmunity with immune dysregulation [29].
- Heterozygous mutations in *STAT3* are associated with infantile-onset multisystem autoimmune disease-1, which can manifest as insulin-dependent diabetes mellitus and autoimmune enteropathy during childhood [30,31]. Other features include short statures and non-specific dermatitis. *STAT3* has been found to have a role in maintaining glucose-mediated insulin secretion and thus its dysregulation was hypothesized as a probable cause for neonatal diabetes [32].
- Maternally inherited diabetes and deafness (MIDD) is caused by specific mutations in the mitochondrial DNA. The most common mitochondrial mutation associated with MIDD is m.3243A>G [29]. The m.3243A>G mutation is also associated with MELAS (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes) in some individuals. Other findings in patients with the m.3243A>G mutation include macular retinal dystrophy, short stature, myopathy, and cardiac and renal disease [29]. The clinical phenotype is highly variable, even within the same family [30]. Other mitochondrial mutations identified in patients with MIDD are m.8296A>G [31] and m.14709T>C [32]. The age of diagnosis of diabetes in MIDD ranges from 11 to 68 years [33, 34].

Available testing options

Neonatal Diabetes Mutation Analysis (methylation-specific MLPA testing for 6q24 abnormalities, analysis for mitochondrial mutations m.3243A>G, m.8296A>G, and m.14709T>C, and sequence and deletion/duplication analysis of included genes)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
 Cost: \$3000
 CPT codes: See test page
 Turn-around time: 4 weeks

6q24 Methylation-Specific MLPA

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
 Cost: \$600
 CPT codes: See test page
 Turn-around time: 4 weeks

Neonatal Diabetes Mellitus (NDM) Panel (analysis for mitochondrial mutations m.3243A>G, m.8296A>G, m.14709T>C, and sequence and deletion/duplication analysis of included genes)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
 Cost: \$2500
 CPT codes: See test page
 Turn-around time: 4 weeks

UPD 6 testing (samples from both of patient's parents are required for analysis)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube

Cost:	\$540 (cost of patient's testing, no charge for parent's samples)
CPT codes:	See test page
Turn-around time:	4 weeks

Test methods:

- **Methylation-Specific MLPA:** Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is performed using a pre-designed kit that contains probes within the 6q24 region associated with transient neonatal diabetes. This test can detect copy number and methylation abnormalities in the 6q24 region associated with transient NDM.
- **Sequence analysis:** Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel will be performed. Targets of interests will be captured and amplified using Agilent SureSelect System. The constructed genomic DNA library will be sequenced using Illumina technology and reads will be aligned to the reference sequence. Variants will be identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All novel and/or potentially pathogenic variants will be confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20bp. For the mitochondrial mutations m.3243A>G, m.14709T>C, and m.8296A>G, heteroplasmy levels of lower than 25% may not be detected by this assay.
- **Deletion/duplication analysis:** Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.
- **UPD6 testing:** UPD testing is performed by microsatellite analysis, which compares microsatellite markers from both parents and the child or fetus. In order for UPD to be determined, a significant number of informative microsatellite markers must be present. Although testing is possible if only one parent is available, the chance of obtaining a sufficient number of informative markers is decreased.

Results:

Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. One report will be issued for the entire panel. All abnormal results are reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

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