

Neonatal diabetes mellitus: A model for personalized medicine

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Neonatal diabetes mellitus occurs in approximately 1 out of every 100,000 live births. It can be either permanent or transient, and recent studies indicate that is likely to have an underlying genetic cause, particularly when diagnosed before 6 months of age. Permanent neonatal diabetes is most commonly due to activating mutations in either of the genes encoding the two subunits of the ATP-sensitive potassium channel. In most of these patients, switching from insulin to oral sulfonylurea therapy leads to improved metabolic control, as well as possible amelioration of occasional associated neurodevelopmental disabilities. It remains to be determined what is the most appropriate treatment of other causes. The diagnosis and treatment of neonatal diabetes, therefore, represents a model for personalized medicine.

Monogenic diabetes: an opportunity to elucidate beta-cell function

Diabetes mellitus is a heterogeneous group of disorders that can present from birth to old age (Box 1). The most common forms, type 1 and type 2 diabetes, are polygenic in origin, whereas neonatal diabetes mellitus (NDM) and maturity-onset diabetes of the young (MODY) are likely to have a monogenic cause (Table 1). The monogenic forms of diabetes, once thought to be rare, could represent as much as 1–2% of all cases of diabetes [1] and are primary genetic disorders of the insulin-secreting pancreatic β -cell [2]. These monogenic forms offer a unique opportunity for using genetics to improve the care and treatment of patients with diabetes [3].

Clinical and molecular heterogeneity in neonatal diabetes

Diabetes in neonates and infants has been recognized since at least 1789 [4]. In 1955, Keidan reviewed 22 NDM cases reported subsequent to 1947, including five cases of transient NDM diagnosed before 6 weeks of age [5]. Prior to the advent of insulin therapy, these neonates did not survive. Even after increased understanding of the autoimmune nature of the most common form of childhood diabetes (type 1 diabetes) and the advent of autoantibody testing, treatment other than insulin was very rarely used, even if clinicians might have astutely suspected a distinct underlying etiology [6,7]. Indeed, 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 [8,9].

Glossary

6q24: chromosome 6q24. Includes paternally expressed genes *PLAGL1* (ZAC) and *HYMAI* within an imprinted region. Overexpression of these genes represents the most common cause (70%) of TNDM, via paternal uniparental disomy (UPD6), paternal duplication or loss of maternal methylation. It remains unclear how these genes contribute to causing diabetes.

DEND: developmental delay, epilepsy and neonatal diabetes. Syndrome found in some PNDM-causing mutations in *KCNJ11* and rarely in *ABCC8*.

iDEND: intermediate DEND. Syndrome with less severe features than DEND, usually without seizures. DEND and iDEND can be seen in approximately 20% of NDM caused by mutations in *KCNJ11*.

INS: insulin gene. Heterozygous dominant-acting mutations in *INS* are the second most common monogenic cause of PNDM diagnosed less than 6 months of age and can also occur at later ages. Rare cause of autoantibody-negative MODY or autosomal recessive PNDM/TNDM.

KATP channel: ATP-sensitive potassium (K) channel. A heterooctameric complex composed of two subunits (Kir6.2 and SUR1).

Kir6.2: (encoded by the *KCNJ11* gene) potassium inward rectifier. Smaller protein subunit of the KATP channel that helps to maintain β -cells, neurons and other tissues in a hyperpolarized state.

SUR1: (encoded by *ABCC8* gene) sulfonylurea receptor. Much larger protein subunit of KATP channel with three transmembrane domains (TMD0–2) and two nucleotide binding domains (NBD1–2). Sulfonylureas bind to this subunit to induce closure of KATP channel.

MODY: maturity-onset diabetes of the young. A phenotype characterized by autoantibody-negative autosomal dominantly inherited diabetes occurring at a younger age, usually with detectable preservation of β -cell function. Such patients can carry mutations in any of at least seven genes (OMIM 606391), although an uncertain percentage could result from an unknown cause. Whenever possible, the specific gene names causing the disease should be used; however, the term MODY is useful for facilitating recognition of patients suitable for genetic screening by clinicians familiar with this phenotypic description.

NDM: neonatal diabetes mellitus. Variably defined in the literature, but recent consensus is based on evidence that most diabetes before 6 months of age is likely to be monogenic, although various monogenic causes can be diagnosed at later ages. As many patients are not diagnosed in the first month of life (usual definition of the neonatal period), other terms have been suggested, such as monogenic diabetes of infancy (MDI); however, we note that not all patients with early onset diabetes have a known monogenic cause and not all are diagnosed in infancy. We suggest using specific gene names, or even mutations, when a monogenic cause is uncovered. As others have suggested, a more appropriate term for the phenotype of diabetes occurring soon after birth would be *congenital diabetes*; however, to maintain consistency with the literature until a consensus is reached, we maintain the accepted terminology *neonatal diabetes*.

PNDM: permanent neonatal diabetes. Requires continuous treatment from the time of diagnosis.

Sulfonylurea (sulphonylurea): class of hypoglycemic agents used in the treatment of type 2 diabetes for over 50 years to stimulate insulin secretion by inducing closure of KATP channels by binding to the SUR1 subunit. Most common agent used in NDM is glyburide (same as glibenclamide).

TNDM: transient neonatal diabetes. Characterized by spontaneous remission within the first few months to a year of life, and relapse later in life in most cases.

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Box 1. Distinguishing between diabetes types not secondary to other conditions**Type 1 diabetes**

Typical onset in childhood/adolescence, rarely in infancy; autoimmune destruction of insulin-producing islets characterized by positive autoantibodies; polygenic disorder in which specific HLA haplotypes ascribe the highest risk.

Type 2 diabetes

Onset at older age, although occurring with increasing frequency in adolescent youth owing to increasing obesity; characterized by relative insulin deficiency resulting from a combination of excessive obesity related insulin resistance and a defective ability of β -cells to compensate; a complex polygenic disorder.

Monogenic diabetes

Two main clinical phenotypes are suggestive of monogenic diabetes: (i) onset in neonates/infants and (ii) onset of autoantibody-negative (e.g. anti-GAD65, anti-IA2, anti-insulin) diabetes in adolescence/early adulthood with diabetes often present in multiple generations. More rarely, recessive causes could also occur and often have other features as part of a syndrome (Box 2).

A genetic etiology for NDM was first discovered in transient NDM (TNDM), most cases of which were found to result from chromosomal abnormalities of chromosome 6q24, although the role of the two candidate genes implicated in this disorder (*PLAGL1/ZAC*, pleiomorphic adenoma gene-like 1/zinc finger protein that regulates apoptosis and cell cycle arrest; and *HYMAI*, hydatidiform mole-associated and imprinted transcript) remains poorly understood [10,11].

It was subsequently found that permanent NDM (PNDM) can result from recessively inherited mutations in the genes encoding the pancreatic transcription factor insulin promoter factor (*IPF1*, also with exocrine deficiency from pancreatic agenesis; OMIM 260370) [12–15], or the glycolytic enzyme glucokinase (*GCK*, causing diabetes in isolation), a key enzyme linking glucose metabolism to insulin secretion [16]. Other causes that are associated with pancreatic atrophy and/or extrapancreatic features have been described, including mutations in *EIF2AK3* [17,18] (termed Wolcott-Rallison syndrome; OMIM 226980), which is the most common autosomal recessive cause of PNDM, usually involving epiphyseal dysplasia and other features; *PTF1A* [19] characterized by autosomal recessive PNDM with cerebellar hypoplasia (OMIM 609069); *HNF1B* [20,21], a rare dominant cause of TNDM with renal anomalies (the same gene causes renal cysts and diabetes syndrome, OMIM 137920); *FOXP3* [22,23], which causes the X-linked severe autoimmune condition IPEX (Immunodysregulation, Polyendocrinopathy, Enteropathy, X-Linked; OMIM 304790) syndrome; and *GLIS3*, which involves congenital hypothyroidism and other features (OMIM 610199) [24].

In 2004, Gloyn *et al.* first reported NDM to be caused by activating dominantly inherited mutations in *KCNJ11*, which encodes the Kir6.2 subunit of the ATP-sensitive potassium (KATP) channel [25]. Activating mutations in *ABCC8*, encoding the SUR1 subunit of the KATP channel, were also reported to cause both PNDM and TNDM, similar to *KCNJ11* [26–31]. Studies of the mutant proteins *in vitro* suggested that it would be possible to treat NDM

due to mutations in these two genes with oral sulfonylureas rather than insulin, owing to their ability to block KATP channels. This concept was subsequently confirmed in case reports and small clinical series [32–43], culminating in the groundbreaking report in 2006 that definitively showed the benefit of such treatment [44].

Recent studies have shown that misfolding mutations in proinsulin can also cause PNDM [45]. Approximately 40% of NDM diagnosed before 6 months of age continues to have no currently identifiable cause, although some patients with a rare syndrome of pancreatic hypoplasia and intestinal atresias were recently found to carry recessive mutations in the pancreatic transcription factor *RFX6* [46]. Genetic testing now allows for a molecular diagnosis in a majority of cases of NDM with the hope for the discovery of new diabetes genes in the near future.

Sulfonylurea-responsive NDM: revolutionized care through molecular genetics

PNDM had been treated indistinguishably from type 1 diabetes until elucidation of the genetic basis of NDM and identification of patients with activating mutations in *KCNJ11* or *ABCC8*. This discovery led to the radically different and successful approach of oral sulfonylureas in lieu of insulin. Case reports [47–55] and series [56–59] have confirmed the efficacy of oral sulfonylureas, with the largest series of 49 subjects in whom sulfonylureas strikingly improved metabolic control in 90% of patients [44]. Not only is treatment with oral agents significantly easier for families, but years later patients continue to maintain normal or near normal hemoglobin A1c levels [54,60,61]. Although some patients continue to limit carbohydrate intake, it appears that many with no specific dietary plan report only transient glucose elevations that normalize without further intervention [53,59,60]. To the best of our knowledge, there has been only a single episode of severe hypoglycemia [62] (altered mental status and/or inability to assist in care [63]) in any patient, even in those taking very high doses (>2 mg/kg/day of glyburide). However, long-term monitoring of all cases will be critical for surveillance of possible rare side effects previously associated with sulfonylurea treatment, such as liver dysfunction, dermatologic reactions, pancytopenia, hyponatremia or increased cardiovascular mortality. Although any medication could bear the risk of very rare unpredictable effects, it is worth noting that the above adverse associations were seen in the treatment of adult type 2 diabetic patients who are likely to have other medical problems and take other medications. Still, NDM patients will initiate life-long treatment at a young age and at higher doses. Before sulfonylurea initiation, clinicians should thus have a thorough discussion of benefit and risk, including unknown possible effects, such as the recent observation of tooth discoloration [64].

The minority of patients who do not readily respond to sulfonylureas tend to be either older at the time of transition or exhibit the most severe neurodevelopmental disability, termed DEND (developmental delay, epilepsy, neonatal diabetes) syndrome [44,49,53–55,59]. In a 2006 report, two out of the five patients who failed to transition completely off insulin (out of 49 in total) were adult parents

Table 1. Genetic basis of neonatal diabetes and diabetes with syndromic features

Gene/syndrome name; gene symbol	Protein name	Protein function	Inheritance	Approximate no. of cases	Other features	Refs
<i>Transient neonatal diabetes mellitus (TNDM)</i>						
Owing to overexpression of paternally expressed genes within the imprinted region of chromosome 6q24; including <i>PLAGL1</i> and <i>HYMAI</i> (non-protein coding)	Implicated: zinc finger protein <i>PLAGL1</i> (ZAC tumor suppressor)	Causal genes/proteins remain uncertain, although glucose-stimulated insulin secretion appears impaired	UPD6 (40%; <i>de novo</i> , non-recurrent), paternal duplication (40%, could be inherited) or maternal methylation defect (20%; autosomal recessive)	>200	Low birth weight; macroglossia (25%); umbilical hernia	[10,11,82–84,88,89]
Potassium channel, inwardly rectifying, subfamily J, member 11; <i>KCNJ11</i>	Inward rectifier K(+) channel Kir6.2	ATP-sensitive potassium channel; inwardly rectifying potassium channel subunit	Spontaneous (80%) and autosomal dominant	~50	Low birth weight; possible developmental delay; usually responsive to sulfonylurea therapy	[29,36]; others
ATP-binding cassette, subfamily C, member 8; <i>ABCC8</i>	Sulfonylurea receptor 1 SUR1	ATP-sensitive potassium channel; sulfonylurea receptor 1 subunit	Spontaneous (80%) and autosomal dominant	>50	Low birth weight; usually responsive to sulfonylurea therapy	[26,29–31]; others
Insulin; <i>INS</i>	Insulin	Hormone	Autosomal recessive	5	Low birth weight	[101]
HNF1 homeobox B; <i>HNF1B</i>	HNF-1 β	Transcription factor	Spontaneous and autosomal dominant	2	Renal and genitourinary abnormalities; atrophy of the pancreas	[20,21]
<i>Permanent neonatal diabetes mellitus (PNDM)</i>						
Potassium channel, inwardly rectifying, subfamily J, member 11; <i>KCNJ11</i>	Inward rectifier K(+) channel Kir6.2	ATP-sensitive potassium channel; Inwardly rectifying potassium subunit channel	Spontaneous (80%) and autosomal dominant	>200	Low birth weight; developmental delay (20%); epilepsy (6%); usually responsive to high doses of sulfonylureas	[25,32,33]; others
ATP-binding cassette, subfamily C, member 8; <i>ABCC8</i>	Sulfonylurea receptor 1 SUR1	ATP-sensitive potassium channel; sulfonylurea receptor 1 subunit	Spontaneous (80%) and autosomal dominant	~50	Low birth weight; rare developmental delay; usually responsive to high doses of sulfonylureas	[26,27]; others
Insulin; <i>INS</i>	Insulin	Hormone	Spontaneous (80%), autosomal dominant, or recessive	>50	Low birth weight	[45,94–100]
Glucokinase (hexokinase 4); <i>GCK</i>	Glucokinase	Glycolytic enzyme	Autosomal recessive	<10	Parents have impaired fasting glucose – GCK MODY	[16]

Table 1 (Continued)

Gene/syndrome name; gene symbol	Protein name	Protein function	Inheritance	Approximate no. of cases	Other features	Refs
<i>Syndromes that include diabetes mellitus</i>						
Immunodysregulation polyendocrinopathy, enteropathy, X-linked; <i>IPEX</i> ; Forkhead box P3; <i>FOXP3</i>	Forkhead box protein P3; FoxP3	Transcription factor	X-linked recessive	~20	Only males affected; severe immune dysregulation; chronic diarrhea with villus atrophy (95%); pancreatic and thyroid autoantibodies (75%); thyroiditis (20%); eczema (50%); anemia (30%); often die before 1 year	[22,23]
Wolcott–Rallison syndrome; epiphyseal dysplasia with early onset diabetes mellitus; eukaryotic translation initiation factor 2-alpha kinase 3; <i>EIF2AK3</i>	EIF2AK3	Kinase involved in regulation of translation	Autosomal recessive	30	Epiphyseal dysplasia (90%); osteopenia (50%); acute liver failure (75%); developmental delay (80%); hypothyroidism (25%); exocrine pancreatic dysfunction (25%)	[17,18]
Diabetes mellitus, permanent neonatal, with pancreatic agenesis, congenital; pancreatic and duodenal homeobox 1; <i>PDX1</i> (Insulin promoter factor 1; <i>IPF1</i>)	Pancreas/duodenum homeobox protein 1 PDX1 (IPF1)	Transcription factor	Autosomal recessive	5	Pancreatic agenesis; parents have <i>IPF1</i> MODY	[12–15]
Diabetes mellitus, permanent neonatal, with cerebellar agenesis; pancreas specific transcription factor, 1a; <i>PTF1A</i>	Pancreas transcription factor 1, subunit alpha PTF1A	Transcription factor	Autosomal recessive	2	Pancreatic and cerebellar agenesis	[19]
Diabetes mellitus, permanent neonatal, with pancreatic hypoplasia, intestinal atresia and gallbladder aplasia; regulatory factor X, 6; <i>RFX6</i>	DNA-binding protein RFX6	Transcription factor	Autosomal recessive	5	Pancreatic hypoplasia, intestinal atresias, gall bladder hypoplasia or aplasia and diarrhea	[46]
Diabetes mellitus, neonatal, with congenital hypothyroidism; GLIS [GLI (glioma-associated oncogene)-similar] family zinc finger 3; <i>GLIS3</i>	Zinc finger protein GLIS3	Transcription factor	Autosomal recessive	4	Congenital hypothyroidism, glaucoma, liver fibrosis and cystic kidney disease	[24]
<i>Syndromic diabetes with onset typically beyond the neonatal period</i>						
Wolfram syndrome 1; <i>WFS1</i>	Wolframin	Membrane glycoprotein	Autosomal recessive	>50	Diabetes insipidus and mellitus with optic atrophy and deafness	OMIM # 222300
Woodhouse–Sakati syndrome; DDB1 and CUL4 associated factor 17; <i>DCAF17</i> (chromosome 2 open reading frame 37; <i>C2ORF37</i>)	DDB1- and CUL4-associated factor 17 DCAF17	Ubiquitous low nucleolar expression of alpha and beta isoforms	Autosomal recessive	<20	Hypogonadism, alopecia, diabetes mellitus, mental retardation, and extrapyramidal symptoms	OMIM # 241080
Thiamine-responsive megaloblastic anemia (TRMA) syndrome; solute carrier family 19 (thiamine transporter), member 2; <i>SLC19A2</i>	Thiamine transporter 1	Transports thiamine across the plasma membrane	Autosomal recessive	~20	Thiamine-responsive megaloblastic anemia with diabetes mellitus and sensorineural deafness	OMIM # 249270
Maternally inherited diabetes and deafness; MIDD (most often m.3243A>G)	Mitochondrial leucine tRNA	Protein synthesis	Mitochondrial mutation – maternal inheritance	>200	Various features in addition to deafness and diabetes depend on heteroplasmy	OMIM # 520000

carrying the same mutations as their children who did successfully transition to exclusive sulfonylurea therapy [44]. Mouse models support the hypothesis that a decline in functional β -cell mass occurs in the absence of sulfonylurea “rescue” of healthy β -cell function [65,66]. Patients with a severe DEND phenotype, in general, seem less likely to respond to sulfonylureas [67–69]. In contrast, those with the less severe intermediate DEND phenotype, such as from the V59M mutation, exhibit good responsiveness to sulfonylureas [32,44,52,59,70–73]. The unresponsiveness seen in severe DEND cases could reflect the degree of neuronal inhibition as a result of impaired closure of KATP channels, which would serve to make neurons expressing open KATP channels less excitable.

In our experience, most patients whether older or with neurological features show at least partial responsiveness to sulfonylureas as indicated by improved glucose levels on reduced insulin doses, although some require a high sulfonylurea dose or additional medications. Even if complete insulin independence cannot be achieved, potential improvement in metabolic control and neurocognitive function argue strongly for consideration of sulfonylurea treatment. There are many possible modifiable and/or familial factors, such as obesity and diet, that could contribute to sulfonylurea resistance. Anecdotal reports suggest benefit from additional oral agents such as dipeptidyl peptidase 4 inhibitors but larger trials are required to confirm their efficacy. Registries will facilitate such studies.

As sulfonylureas would be expected to maintain closure of KATP channels, it is remarkable how highly regulated insulin secretion appears to be in relation to meals in treated patients. Indeed, sulfonylurea-treated cases exhibit robust responsiveness to oral glucose or mixed meal challenge, but a lesser degree of responsiveness to intravenous glucose [44]. Thus, sulfonylurea treatment seems to allow for the effect of various possible insulin secretion modulators that probably include incretin hormones; however, other glucose-responsive β -cell elements or a direct effect through “rescued” KATP channels cannot be excluded.

Impact of sulfonylurea treatment on neurodevelopmental disabilities

KATP channels are widely expressed, not only in β -cells but also in brain, muscle and other tissues, where they are also metabolically responsive; however, their function in these tissues remains incompletely understood [74–76]. Although the majority of KATP-related NDM is characterized by isolated NDM, given the expression of Kir6.2 and SUR1 in the brain [77,78], it is perhaps not surprising that relatively common intermediate DEND cases have variable motor, speech or cognitive delay, whereas very rare severe DEND cases exhibit dysmorphic features with significant neurological impairment and seizures [40,79]. The severity of features appears to be on a phenotypic spectrum that is at least partially correlated to genotype, although several as yet incompletely characterized factors lead to a high degree of variability even among patients with the same mutation. Among these variables could be the age at diagnosis and level of metabolic control of the diabetes, as

hyperglycemic and hypoglycemic episodes can have independent effects on neurological function.

Several case reports have demonstrated measurable improvement in the neurodevelopmental outcome in intermediate DEND subjects, including those with *KCNJ11* mutations V59M, G53D and H46L [48,51,52,59,70,72,80]. Patients of various ages with severe motor, speech and/or cognitive delay exhibited notable but variable improvement within months of treatment; however, none had complete resolution of their disabilities. Furthermore, one case of severe DEND including intractable seizures exhibited significant neurological improvement at particularly high doses (2.3 mg/kg/day of glyburide/glibenclamide compared with typical doses of <1 mg/kg/day in other NDM cases, and a maximal dose of approximately 0.3 mg/kg/day in type 2 diabetes) [81].

Sulfonylurea treatment should be considered in all cases of KATP-related NDM and could arguably be continued in cases with neurodevelopmental impairment even if little reduction in insulin dose is achieved. Furthermore, as no data are available on the efficiency with which glyburide crosses the blood–brain barrier, the dose in such cases should perhaps be maximized unless significant hypoglycemia occurs. It is important to note that many factors (such as time, age at diabetes diagnosis, level of diabetes control, age at sulfonylurea initiation) may influence neurodevelopmental outcome, and negative consequences of sulfonylurea therapy are possible. Thus, such treatment should be done under careful monitoring, preferably in the context of long-term outcome studies. In this regard, treatment of patients with DEND-associated mutations soon after birth will shed light on the relative importance of very early glyburide treatment in ameliorating or even preventing neurological sequelae. Improved outcomes could provide a rationale for empiric sulfonylurea therapy, particularly if there is any possibility of delay in obtaining the necessary genetic diagnosis; however, this should be considered with caution as NDM caused by *INS* mutations might be worsened by sulfonylurea therapy.

Although much attention has appropriately been focused on those patients with more obvious DEND-like impairment, it is becoming apparent that even patients with common “mild” mutations (such as R201H) can exhibit subtle problems, such as learning disorders [53]. Whether these difficulties are related to brain expression of mutated channels will become clear only through careful assessment of all patients with KATP-related NDM, to determine whether a significant percentage of patients are affected and whether improvement is observed following sulfonylurea treatment.

TNDM: a spectrum of defects cause transient neonatal, relapsing or late-onset diabetes

TNDM is most commonly (70%) due to overexpression of paternally expressed imprinted genes, including *PLAGL1* and *HYMAI*, as a consequence of paternal uniparental disomy of chromosome 6q24 (UPD6), paternal duplication of 6q24 or loss of maternal methylation [11,82]. Poor *in utero* growth, presumably resulting from insulin deficiency, leads to intrauterine growth retardation and

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admission to neonatology intensive care units, which is probably related to their very early diagnosis (first few days of life) compared with other causes of NDM. Despite their dramatic presentation, 6q24-related TNDM cases tend to need lower replacement doses of insulin, and the condition remits spontaneously within the first year or so of life, usually within the first few months [83,84]. Subsequently, an uncertain but estimated two-thirds of patients experience relapse of diabetes around the time of adolescence, although the reasons for the recurrence and why only in some individuals are poorly understood with very few patients followed longitudinally. Numerous lines of evidence suggest that β -cell mass normally expands in the postnatal period [85,86]. Although β -cell function is probably impaired in 6q24-related TNDM, it could be that neonatal β -cell expansion accounts for the early remission. Such a mechanism appears to be supported by a mouse model of the disorder in which euglycemia occurs after β -cell numbers increase; however, β -cell development, not just function, also seems to be impaired [87]. In the few patients who have been studied, β -cell function as assessed by intravenous glucose tolerance testing appears to be on the lower end of normal during the remission phase [88]. Subsequent relapse might be a consequence of physiologic or acquired decrease in insulin sensitivity to which the β -cells in these patients cannot appropriately respond [89]. A single case after relapse exhibited a ready response to lower-dose monotherapy with sulfonylureas [90]. However, most cases have been treated with insulin, presumably either because of misdiagnosis as type 1 diabetes, or because other non-insulin therapies are not approved for use in children. As such, the most appropriate treatment has yet to be defined.

Approximately 30% of TNDM cases have mutations in *ABCC8*, or less often *KCNJ11*, with these mutations having less effect on KATP channel function than those causing PNDM. Moreover, patients within families carrying the same mutation can exhibit an impressive heterogeneity of phenotype, including TNDM, relapsing diabetes or onset of mild diabetes much later in life without any apparent neonatal phase [26,30,31,36,55,59,91–93]. In our experience, patients with KATP-TNDM during the remission phase maintain a subclinical degree of metabolic impairment that is unmasked in times of stress or illness, consistent with underlying mild β -cell dysfunction. Cases with onset of diabetes only later in life (a MODY-like phenotype) have invariably only been recognized because of another family member with TNDM, although they might have experienced a subclinical degree of neonatal hyperglycemia that went unrecognized.

Neonatal and infancy-onset diabetes caused by mutations in the insulin gene

Genetic diagnosis has the potential to optimize treatment in NDM (Box 2). Increased awareness of the potential of genetic testing has resulted in frequent inquiries from parents (more often than physicians) about genetic testing and enrollment in registries (particularly in the US) [59]. After screening for *KCNJ11* and *ABCC8* mutations, however, a large fraction of patients remained with early diagnosis of diabetes but no underlying genetic cause.

Box 2. When should testing for monogenic diabetes be considered?

- In any patient diagnosed with diabetes before 6 months of age:
If isolated diabetes $\pm$ neurodevelopmental disabilities: *KCNJ11*, *INS*, *ABCC8*. *GCK* can also be considered for rare compound heterozygous cases, especially if there is any family history of diabetes. Include testing of 6q24 if TNDM or still in neonatal phase before possible remission, especially if very low birth weight.
If any syndromic characteristics or suspected consanguinity: *GCK*, *IPF1*, *INS*, *PTF1A*, *EI2AK3*, *FOXP3* and *GLIS3* or if later onset *WFS1* (OMIM 606201), *DCAF17* (*C2orf37*, OMIM 241080), *TRMA* (OMIM 249270) and mitochondrial 3243A>G (OMIM 520000). Clinical features pointing to a diagnosis in the neonatal period could include symptoms of exocrine insufficiency such as diarrhea/malabsorption (*FOXP3* or *IPF1*, fecal fat or elastase could be helpful and ultrasound could reveal pancreatic hypoplasia seen with *IPF1* or unknown causes), neurological dysfunction (*PTF1A*), liver or kidney dysfunction or epiphyseal dysplasia (*EI2AK3*), or hypothyroidism (*GLIS3*, *EI2AK3* or *FOXP3*). Other as yet unknown causes could involve intestinal atresia or cardiac defects.
- If between 6 and 12 months of age at diagnosis or older but antibody-negative or autosomal dominant family history: *INS* or other causes could be considered, preferably in the context of research studies investigating less likely but still possible monogenic causes.
- In cases with a MODY phenotype: significant family history of diabetes mellitus at younger ages or if atypical for type 1 or type 2 diabetes, even in isolated cases (e.g. “type 1 diabetes” that is autoantibody-negative or requires a low dose of insulin even after more than a year of treatment or “type 2 diabetes” without expected features suggestive of insulin resistance or in those who are normal weight): *HNF1A*, *HNF4A*, *GCK*, *HNF1B* (especially if renal cysts/pathology; analysis for deletions missed by standard sequencing should also be considered), *INS* and rarely *IPF1*, *NEUROD1* or *CEL*. As family members of NDM probands can exhibit diabetes later in life without a neonatal phase, *ABCC8*, *KCNJ11* or even and arguably 6q24 could be considered, although usually at least one family member might be expected to have a history of TNDM.
- In patients exhibiting consistent mild elevation of glucose levels (that might have been discovered incidentally and could be relatively unresponsive to treatment): *GCK*.

Identification of mutations in the insulin gene (*INS*) as a common cause of PNDM further decreased the fraction of cases with an unknown etiology [45]. The initial report also showed that *INS* mutations could be found in patients diagnosed with NDM after the age of 6 months, thus extending the age under which screening for monogenic causes of diabetes should be considered. Further studies extended the phenotype to include rare cases of MODY or type 1B (autoantibody negative) diabetes, with variable early childhood onset as late as 2–6 years of age [94–100]. Recently, rare recessively inherited mutations reducing insulin biosynthesis were discovered to cause PNDM and TNDM [101].

The *INS* mutations causing PNDM tend to affect either directly or indirectly cysteine residues critical for disulfide bridge formation, suggesting that proinsulin misfolding disrupts protein processing and secretion and leads to activation of endoplasmic reticulum (ER) stress [45,99,102,103]. In fact, the C96Y mutation in human patients is also found in the Akita non-obese mouse model of diabetes [104]. Both the Akita and Munich mouse models of insulin-related diabetes show β -cell activation of the

Box 3. Internet resources for information on neonatal and monogenic diabetes

- For Registries and testing on a research basis:

US Neonatal Diabetes Mellitus Registry at the University of Chicago: <http://NeonatalDiabetes.org>

UK registry in Exeter: <http://DiabetesGenes.org>

Many other well-established national registries can be found in countries around the world, including Austria, France, Israel, Italy, Japan, Norway, Slovakia, Spain, Poland and others.

- JDRF: <http://MonogenicDiabetes.org>
- NIH: <http://diabetes.niddk.nih.gov/dm/pubs/mody/>
- For more information on research-based and CLIA-certified commercial testing: <http://GeneTests.org>
- Other resources: <http://www.DiabetesTrialNet.org>; <http://ClinicalTrials.gov>

unfolded protein response and ER stress [104,105]. A better understanding of the effects of misfolded proinsulin proteins on β -cell function could lead to future improved therapy [106]. Although genetic diagnosis does not currently alter treatment in these patients, they still benefit from correct diagnosis for the purpose of genetic counseling.

Concluding remarks and future considerations

Neonatal diabetes is a paradigm for the application of genetics for the diagnosis and treatment of other metabolic diseases, eventually including common forms of diabetes such as type 1 and type 2 diabetes. Genetic studies have greatly improved our understanding of the causes and pathophysiology of NDM, leading to improved treatment in many cases (Box 3). Further studies (Box 4) are needed to document the incidence, prevalence and natural history of the various forms of NDM, the risk for specific complications, the long-term effectiveness of sulfonylurea treatment in responsive cases and possible treatment alternatives in non-KATP-related NDM. Patient registries will not only facilitate these studies but they will also allow for patient and clinician support, genetic counseling and screening of family members, as well as novel opportunities for multidisciplinary efforts, including ethical and policy considerations regarding genetic testing and use of novel therapies in vulnerable populations.

Box 4. Outstanding Questions

- Will earlier recognition and diagnosis of KATP-NDM lead to improved metabolic and neurodevelopmental outcome, and to what extent will early sulfonylurea treatment prevent neurodevelopmental disability?
- What is the best therapy for other forms of NDM?
- Should all newly diagnosed NDM patients be given an empiric trial on glyburide while awaiting results of necessary genetic testing?
- How many other genetic causes of NDM remain to be discovered?
- Will common variants in the genes responsible for NDM affect risk of type 1 or type 2 diabetes?
- What will be the cost utility of screening for monogenic diabetes, either in those with early onset diabetes or possibly in the population?

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