

NEONATAL DIABETES – FROM GENE DISCOVERY TO CLINICAL PRACTICE CHANGES

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Abstract

Diabetes mellitus is one of the most common chronic diseases but also one of the most heterogeneous. Apart the common phenotypes of type 1 and type 2 diabetes, around 1-2% of all cases arise from a single gene mutation and are known as monogenic diabetes. Diabetes diagnosed within the first 6 months of life is known as neonatal diabetes and has been extensively studied during the last two decades. Unraveling the genetic cause and molecular mechanism of this rare diabetes phenotype led to a dramatic change in the treatment of these children who often can be switched from insulin to sulphonylurea treatment. The aim of this paper is to review the known genetic causes of neonatal diabetes and to highlight the most recent aspects of the disease caused by mutations in the K_{ATP} and insulin genes, with a special focus on the individualized treatment of these cases.

key words: neonatal diabetes, *KCJN11*, *ABCC8*, insulin gene, sulphonylurea

Introduction

Diabetes mellitus is one of the most common chronic diseases in human populations across the globe. Thus, the 2012 update to the Fifth Edition of the International Diabetes Federation (IDF) Atlas published in 2011 reports that currently 371 million people have diabetes [1]. Moreover, the prevalence of diabetes continues to rise in both the Western world and in the developing countries as changing lifestyles lead to reduced physical activity, and increased obesity. The same 2011 IDF Atlas [1] predicts that the prevalence of diabetes will reach number will reach almost 10% by 2030, meaning ~552

million subjects. Unfortunately, the epidemic of diabetes affects also children, with significant increases in incidence for both type 1 (T1DM) and type 2 (T2DM) diabetes.

During the last decades, major progresses have been made in unraveling the genetics of diabetes for both the two major diabetes phenotypes, the polygenic forms of T1DM and T2DM. These genetic studies have led also to the precise description of the rare forms of diabetes with exclusively genetic pathogenesis known as monogenic diabetes, including *neonatal diabetes mellitus* (NDM) and *maturity onset of diabetes in young* (MODY). These were included in the last classification of diabetes [2]

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in group 3 - *Other Specific Types of Diabetes*, subgroup 1 - *Genetic defects of the β -cell*. As we shall further discuss below, monogenic diabetes provided a unique opportunity for using genetics to improve the care and treatment of patients.

Neonatal diabetes – Definition and clinical forms

Diabetes in neonates and infants has been first described more than two centuries ago [3] but before the discovery of insulin by Nicolae Paulescu in 1921 these infants did not survive. After insulin discovery, the number of NDM cases surviving till adulthood increased, so that in the 1950s series of tens of cases followed up for more than 10 years were reported [4]. In parallel with the deciphering of the autoimmune nature of T1DM and the advent of beta cell auto-antibodies testing, clinicians have begun to suspect a distinct underlying etiology for NDM [5].

Currently, diabetes clinically diagnosed within the first 6 months of life is defined as neonatal diabetes mellitus [6-8]. Depending on whether diabetes resolves later in life or is permanent throughout life, two phenotypes were described: Permanent neonatal diabetes (PNDM) or transient neonatal diabetes (TNDM).

TNDM accounts for approximately for 50% cases of NDM, with an incidence of ~1/100,000 live births [9]. Its main clinical feature is that it may either spontaneously remit or be so mild as not to require treatment. However, usually diabetes will often relapse, most often during adolescence [10]. The majority (about 80%) of cases of TNDM are caused by abnormalities of an imprinted locus on chromosome 6q24 that results in the over-expression of a paternally expressed gene [11]. PNDM accounts for the other 50% of NDM cases. Although the majority of cases of PNDM involve isolated diabetes, some of the known monogenic causes are characterized by a variety of syndromic features.

Main genetic defects leading to NDM or congenital syndromes including diabetes

There are five main classes of β -cell dysfunction that encompass most cases of monogenic diabetes [6]: 1) Defective glucose sensing (Glucokinase - *GCK* gene); 2) Abnormal potassium ATP-sensitive (K_{ATP}) channels (*KCNJ11* - Potassium channel (subfamily J) member 11 and *ABCC8* - Sulfonylurea Receptor genes); 3) Mutated transcription factors (Hepatocyte Nuclear Factors *HNF-4 α* , *HNF-1 α* , *HNF-1 β* , *IPF-PDX1*, *NEUROD1*, etc); 4) Defective mitochondria (A3243G mutation in mitochondrial DNA) and 5) Endoplasmic reticulum stress (*EIF2AK3* - Pancreatic EIF2 alpha kinase, *INS* – insulin gene, *WFS1* – Wolfram gene). The identification of the etiological genes helped the recognition of novel clinical subgroups, including neonatal diabetes or genetic syndromes that include neonatal diabetes. A brief description of these syndromes is given in [Table 1](#).

It should be stated that a form of PNDM can be induced also by homozygous mutation in all the MODY genes. Homozygous mutations in *IPF-PDX1* and *NEUROD1* lead to pancreatic agenesis with permanent diabetes accompanied by signs of exocrine pancreatic insufficiency.

Transient Neonatal Diabetes Mellitus

TNDM is characterized by severe intrauterine growth retardation (due to deficient insulin secretion in utero) and diagnosis of diabetes within days from birth [12]. It was the first form of NDM for which the genetic basis was unraveled. Most often, TNDM is induced by the overexpression of some paternally imprinted genes located on chromosome 6q24 [13]. Most often involved is over-expression of the genes *PLAGL1/ZAC* (pleiomorphic adenoma gene-like 1) and *HYMAI* (hydatidiform mole - associated and imprinted transcript), whose exact function is still not fully elucidated [14]. The

expression of these genes is normally restricted to the paternal allele as a result of maternal DNA methylation. More recently it was shown that TNDM is not associated with mutations of the PLAGL1 or HYMAI genes, but rather with their overexpression via uniparental disomy, chromosome duplication, or relaxation of imprinting [15]. The main cause seems to be a defect in maternal methylation, most often due to recessive mutations in the ZPF57 (Zinc Finger Protein) gene [13,15,16]. The treatment of TNDM cases classically relied on insulin. More

recently, some attempts of sulfonylurea treatment have been made [17]. Data on the largest case-series of 6q24 TNDM, including 163 patients from Europe, the Americas, Asia and Australia, were recently published [12].

Less frequent causes of TNDM are represented by mildly activating mutations in the genes encoding the KATP channel KCJN11 and ABCC8 but in these cases the clinical picture is dominated by repeated episodes of hyperglycemia that require intermittent treatment during childhood [18].

Table 1. Neonatal diabetes and other rare monogenic syndromes including diabetes (adapted after [6]).

Disease	Gene	Locus	Detailed name	Clinical features
TNDM	<i>PLAGL1</i> (ZAC)	6q24	Pleomorphic adenoma gene-like 1	Intrauterine growth retardation, acute onset diabetes, insulin treatment, remission between 3-6 months, usually relapses later in life
	<i>KCNJ11</i>	11p15.1	Potassium channel (subfamily J, member 11)	
	<i>ABCC8</i> (SUR1)	11p15.1	Sulfonylurea Receptor	
PNDM	<i>KCNJ11</i>	11p15.1	Potassium channel (subfamily J, member 11)	Intrauterine growth retardation (IUGR), acute onset diabetes, insulin treatment, KCNJ11 and ABCC8 types can be treated successfully with high dose sulphonylurea therapy, usually with better results than insulin
	<i>ABCC8</i> (SUR1)	11p15.1	Sulfonylurea Receptor	
	<i>INS</i>	11p15.5	Insulin gene	
	<i>GCK</i> (homozygous)	7p15-p13	Glucokinase	
Mitochondrial diabetes	<i>mtDNA</i>	mt3243A>G	Mitochondrial DNA	Maternally inherited, usually diagnosed later in life, almost all carriers develop diabetes, 75% deafness, increased risk for stroke, epilepsy, renal and cardiac disease
Wolfram Syndrome	<i>WFS1</i>	4p16.1	Wolframin	Childhood onset; associates optic atrophy, deafness, diabetes insipidus, gonadal atrophy, neurological and psychiatric disease. Median age at death is 30 years.
Wolcott-Ralisson Syndrome	<i>EIF2AK3</i> (PERK)	2p12	Pancreatic EIF2 alpha kinase	Childhood onset, associates epiphyseal dysplasia, renal and hepatic dysfunction and mental retardation. Most cases do not survive beyond 15 years.

Permanent Neonatal Diabetes Mellitus

PNDM may be either isolated or form part of a syndrome associating diabetes and other extra-pancreatic manifestations, as briefly described in [Table 1](#). The most common causes

of isolated PNDM are represented by mutation in the genes that encode the K_{ATP} channel components Kir6.2 (*KCNJ11* gene) and Sulphonylurea Receptor – SUR (*ABCC8* gene) on chromosome 11p15.1 and insulin (*INS* gene) on chromosome 11p15.5 [9].

PNDM due to KCJN11 mutations

The pancreatic beta cell K_{ATP} channel is an octamer complex formed by four Kir6.2 (*Potassium Inward Rectifier 6.2*) subunits and four regulatory SUR1 (*Sulphonylurea Receptor-1*) subunits [19]. Kir6.2 is an inwardly rectifying K-channel that forms the potassium-selective pore and possesses an inhibitory site for ATP. SUR1 is a member of the ATP binding cassette (ABC) superfamily and plays multiple regulatory roles [9,20]. At low glycemic levels, the K_{ATP} channels from the beta cell membrane are open and a continuous efflux of potassium through the channel keeps a negative potential of the beta cell membrane, with the consequent closure of the voltage dependent calcium channels [21]. When blood glucose levels rise, it is transported inside the beta cell through specific glucose transporters and subsequently metabolized, a process that increases the ATP-to-ADP ratio. This leads to closure of the K_{ATP} channel which stops the K^+ efflux with subsequent membrane depolarization which leads to the opening of the voltage dependent calcium channels and Ca^{2+} influx in the beta cell. Finally, increased calcium levels inside the beta cell induce exocytosis of the insulin granules.

Due to the key role of the K_{ATP} channel for insulin secretion, it was hypothesized that mutations in the genes encoding its two components might lead to disease states characterized either by hypo or hyperglycemia [22]. In fact, it was already known that loss of function mutations in both *KCJN11* and *ABCC8* genes can cause over-secretion of insulin and lead to the congenital hyperinsulinism of infancy (CHI) [23]. Actually more than 100 mutations of the K_{ATP} channel associated with CHI have been described. Most these mutations result in non-functional channels associated with continuous depolarization of the beta cell membrane and subsequent excessive and un-regulated insulin secretion [24]. Based on these facts, it was

hypothesized that the opposite state – overactive K_{ATP} channels induced by gain of function mutations in the *KCJN11* and *ABCC8* genes – might be associated with diabetes mellitus [25]. Such mutations will block the K_{ATP} channels in an opened state maintaining the beta cell membrane hyperpolarized, thus preventing the Ca^{2+} influx and impairing insulin secretion.

The hypothesis was first confirmed in 2004 by Anna Gloyn and colleagues who reported PNDM caused by activating dominantly-inherited mutations in the *KCJN11* gene [26]. In this article it was shown that 10 out of 29 patients with PNDM had heterozygous mutations in this gene. The number of mutations increased continuously so that currently more than 30 are described as being associated with PNDM [27]. All *KCJN11* gene mutations associated with PNDM are heterozygous. Depending on their location, they affect either ATP mediate closure of the channel alone (with lower K_{ATP} electric currents) or combined with a defect in channel gating and channel conformation which keeps it opened (usually associated with higher K_{ATP} electric currents) [20,28]. In addition, depending on the severity of the mutations, function of the K_{ATP} channels in other tissues (mainly neuronal) can be altered.

Patients carrying *KCJN11* mutations can be classified in four distinct clinic phenotypes, usually dependent on the severity of the mutations. The most frequent are isolated NDM, usually PNDM but, more rarely, TNDM also [9,20]. The most severe disease phenotype is known as the *Developmental Delay, Epilepsy and neonatal Diabetes Syndrome* (DEND). These patients exhibit also severe neurological impairment, including motor development delay, muscle weakness, epilepsy and dismorphic features [28,29]. Finally, some patients have an intermediate phenotype between isolated PNDM and DEND. These are considered to have the intermediate DEND known as iDEND and are

characterized by some degree of motor development delay (usually by 1-2 years), muscle weakness and late development of speech [30]. Specific *KCNJ11* mutations associated with iDEND have been described [31].

PNDM due to *ABCC8* mutations

Mutations of the *ABCC8* gene (encoding for the SUR1 component of the K_{ATP} channel) that cause PNDM were first identified in 2006, almost simultaneously, by the groups of Proks et al. [32] and Babenko et al. [33]. Most subjects carrying *ABCC8* mutations have isolated PNDM or, more rarely, TNDM. Up to 30% of patients have additional neurological features, including developmental delay, muscle weakness, epilepsy, learning difficulties, etc. [18,34], up to the most severe form of DEND [9]. To date, only two *ABCC8* mutations (F132L and I49F) were identified to be associated with the DEND syndrome [9] while one mutation (L213R) with the iDEND [33].

Currently over 60 mutations of the *ABCC8* gene have been identified. All are missense mutations, can be either heterozygous (dominant) or homozygous (recessive). They account for more than 10% of PNDM cases and sometimes for TNDM [35]. Generally no correlation was found between the location of the *ABCC8* mutations and the clinical phenotype.

PNDM due to *INS* mutations

Heterozygous autosomal dominantly inherited mutations in the insulin gene (*INS*) are the second most common cause of PNDM (after mutations in *KCNJ11*) [10]. Thus, in the large Prof. Hattersely Exeter cohort of PNDM subjects, ~14% of patients were found to carry *INS* mutations [36]. Sometimes the diagnosis of diabetes is made after 6 months of age which is the limit of diagnosis of PNDM. *INS* mutations

associated with PNDM were identified independently by the groups of Støy et al. in 2007 [37] and Colombo et al. in 2008 [38].

Most often *INS* mutations associated with PNDM are heterozygous mutations that determine the synthesis of a preproinsulin or proinsulin molecule with structural abnormalities [39]. The probable mechanism of diabetogenesis involves β -cell endoplasmic reticulum stress secondary to the misfolding of the proinsulin encoded by the mutated alleles. This is explained by the altered formation of the disulfide-bonds between the A-chain and B-chain of the proinsulin molecule. The misfolded protein does not progress normally through the endoplasmic reticulum, finally impairing the normal beta cell function and leading to beta cell death [39]. This mechanism has been repeatedly demonstrated both in vitro and in animal models [40,41].

Less frequently, recessive homozygous mutations in *INS* can affect proinsulin biosynthesis per (via different mechanisms including deletion of exons, decreased transcription or translation) and finally lead to NDM [42]. Finally, it should be mentioned that *INS* mutations have been reported to be associated also with infancy-onset diabetes (between 6 and 12 months of age, non-autoimmune T1DM, MODY and early onset T2DM [39]).

Congenital syndromes including infancy/childhood onset diabetes

The extensive investigation of the monogenic forms of diabetes during the last two decades has led to the identification of an expanding list of causal genes inducing congenital diabetes associated with other non-diabetic phenotypic features. The list includes syndromes for which diabetes is diagnosed beyond the neonatal period but frequently in early infancy and this is the reason for including them in our review. Despite the fact they are

very rare, considering these clinical entities in the differential diagnosis of diabetes in early infancy is important. This is why, a complete list

of these syndromes, including the genetic defect and main clinical features are given in [Table 2](#).

Table 2. Monogenic forms of diabetes occurring during early infancy accompanied by syndromic features.

(Adapted after [10]).

Gene	Locus	Detailed name/function	Clinical features
<i>RFX6</i>	6q22.2	DNA-binding protein (RFX6)/ winged-helix transcription factor	Diabetes diagnosed within the first days of life, pancreatic hypoplasia, intestinal atresia, gall bladder agenesis/hypoplasia, and congenital diarrhea
<i>IER3IP1</i>	18q21.1	Immediate early response 3 interacting protein 1	Neonatal diabetes with simplified gyral pattern microcephaly and severe infantile-onset epileptic encephalopathy.
<i>NEUROG3</i>	10q21.3	NeuroG3 or Neurogenin 3/ bHLH transcription factor	Diabetes and chronic intractable malabsorptive diarrhea starting soon after birth
<i>NEUROD1</i>	2q32	(Neurogenic Differentiation 1) /bHLH transcription factor	NDM, small for gestational age, cerebellar hypoplasia, developmental delay, sensorineural deafness, and visual impairment.
<i>PTF1A</i>	10p12.3	Pancreas transcription factor 1, subunit α / bHLH transcription factor	Congenital diabetes, paucity of subcutaneous fat, optic nerve hypoplasia, complete agenesis of the cerebellum and complete absence of the pancreas,
<i>GLIS3</i>	9p24.3	Glioma-associated oncogene-similar family zinc finger 3 (GLIS3)/ transcription factor	NDM within the first few days of life, low birth weight, mild facial dysmorphism, and congenital primary hypothyroidism
<i>PDX1</i>	13q12.1	Pancreas/duodenum homeobox protein 1 /transcription factor	PNDM and exocrine dysfunction following pancreatic agenesis
<i>HNF1B</i>	17q12	Hepatocyte nuclear factor 1 β /transcription factor	NDM, dysplastic kidneys (without cysts or evidence of renal failure), pancreatic hypoplasia
<i>PAX6</i>	11p13	Paired box gene / transcription factor	NDM, brain malformations, microcephaly, and microphthalmia
<i>WFS1</i>	4p16.1	Wolframin	Childhood onset; associates optic atrophy, deafness, diabetes insipidus, gonadal atrophy, neurological and psychiatric disease. Median age at death is 30 years.
<i>SLC19A2</i>	1q23.3	Thiamine transporter 1/ transports thiamine across the plasma membrane	Thiamine-responsive megaloblastic anemia (Rogers syndrome), infancy diabetes, sensorineural deafness
<i>SLC2A2/ GLUT2</i>	3q26.1	GLUT2/facilitative glucose transporter	Hepatic and renal glycogen accumulation; renal proximal tubular dysfunction, glycosuria, phosphate wasting, rickets, delay of puberty and short stature; hypergalactosemia; mild fasting hypoglycemia but postprandial hyperglycemia and diabetes or impaired glucose tolerance
<i>FOXP3</i>	Xp11.23	Forkhead box protein P3/ transcription factor	Neonatal monogenic autoimmune diabetes, enteropathy, severe diarrhea and malnutrition, severe eczema, autoimmune thyroid disease.
<i>EIF2AK3 (PERK)</i>	2p12	Pancreatic EIF2 alpha kinase	Childhood onset, associates epiphyseal dysplasia, renal and hepatic dysfunction and mental retardation. Most cases do not survive beyond 15 years.

From gene discovery to paradigm shift in the treatment of PNDM

PNDM has been treated invariably with insulin before the era of modern genetics. In fact, until then it was not distinguishable from the

common form of autoimmune T1DM, patients being assumed to suffer from this disease. Recognition of the fact that approximately 2/3 of PNDM patients carry mutations in the K_{ATP} channel subunits [35,36] led to the hypothesis that these subjects might be treated with

sulphonylureas (SUs) instead of insulin. The hypothesis was based on the fact that SUs bind to both Kir6.2 and SUR1 subunits of the K_{ATP} channel, though with very different affinities: high for SUR1 and low for Kir6.2 [9]. Subsequently, studies of the mutant proteins *in vitro* confirmed this hypothesis [3], followed quickly by isolated case reports [43] and small clinical series [44,45] proving the efficacy of SU treatment in cases of PNDM caused by *KCJN11* gene mutations. The precise molecular mechanism of the SU block of mutant K_{ATP} channels was reviewed recently [46].

The largest series of PNDM treated successfully with SUs was published in 2006 [47] and included 49 subjects. In this study, 90% of the patients successfully discontinued insulin after switch to SUs, while HbA1cd improved in all patients, (from 8.1 percent before treatment to 6.4 percent after 12 weeks of treatment, $P < 0.001$). Subsequently, studies showed the success of SU treatment in most cases of PNDM secondary to SUR1 mutations [48]. The most commonly used drug for the treatment of PNDM is glibenclamide (glyburide). The usual dose used is 0.4-1 mg/kg/day which is higher than the current dose used for the treatment of T2DM [43,47,48]. However, the improvement in the quality of life of the patients and families is heartbreaking. Interestingly, patients continue to maintain near normal HbA1c for years after switching to SU treatment [49]. Even more, the incidence of hypoglycemia in these patients seems to be very low, with no cases of severe hypo's reported even in patients receiving very high doses (> 2 mg/kg) of glibenclamide [3]. However, long-term monitoring of the PNDM cases treated with SUs will be required in order to assess the safety of these drugs on the long term.

It should be mentioned though that there is a group of patients with PNDM due to K_{ATP} channel mutations that do not respond to SU treatment. It includes the patients who are older at the time of insulin/SU switch attempt [3] and those with DEND syndrome [50]. For the former category, the probable explanation is the progressive decline in functional beta cell mass over time, so that SU "rescue" of beta cell function on a decreased beta cell mass is not enough to restore efficacious insulin response [47].

Although it is not clear to what extent SUs can cross the blood-brain barrier, several case report studies reported improvements in neurological disabilities of the patients with DEND syndrome following SU treatment [51,52]. Usually these are cases of iDNED, require higher doses of glibenclamide (up to 2.3 mg/kg/day) and none exhibit complete resolution of the neurologic disorders. However, the improvements in both glucose control and neurodevelopmental outcome warrants at least attempting SU treatment in these cases [3].

Conclusions

Major progresses have been made in deciphering the genetics of monogenic diabetes. Unraveling the molecular mechanism leading to PNDM led to a paradigm shift in the treatment of cases associated with mutations in the K_{ATP} channel. SU treatment in these cases is not only more efficacious but also safer and associated with greatly improved quality of life for the children and their families. Deciphering the exact genetic mechanisms will be hopefully useful in establishing the most appropriate treatment of the rare recessive monogenic forms of diabetes associated with other syndromic features. Finally, the study of monogenic diabetes provides a unique opportunity to elucidate the beta cell function.

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