Neonatal diabetes caused by disrupted pancreatic and beta cell development

Short running title: Neonatal diabetes and beta cell development

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Abstract

Neonatal diabetes is diagnosed before the age of 6 months and is usually caused by single-gene mutations. Over 30 genetic causes of neonatal diabetes have been described to date, resulting in severely reduced beta cell number or function. Seven of these genes are known to cause neonatal diabetes through disrupted development of the whole pancreas, resulting in diabetes and exocrine pancreatic insufficiency. Pathogenic variants in 5 transcription factors essential for beta cell development cause neonatal diabetes without other pancreatic phenotypes. However, additional extra-pancreatic features are common.

This review will focus on the genes causing neonatal diabetes through disrupted beta cell development, discussing what is currently known about the genetic and phenotypic features of these genetic conditions, and what discoveries may come in the future.

Introduction

Neonatal diabetes is a rare monogenic condition, with an estimated incidence of 1/100,000 live births. It is characterised by diabetes diagnosed before the age of 6 months. Previous studies have shown how individuals diagnosed with diabetes before this time point are very unlikely to have type 1 diabetes, and most likely to have a single genetic variant causing their disease [1].

Neonatal diabetes is clinically divided into three broad subgroups depending on disease progression. Roughly half of the cases have transient neonatal diabetes, which is typically diagnosed in the first few weeks of life. The treatment requirements progressively decrease in these individuals and the diabetes remits after a few months (typically before the age of 18 months) [2]. However, for most cases with transient neonatal diabetes, diabetes relapses later in life, typically in late adolescence/early adulthood. Permanent neonatal diabetes is the other common subtype, characterised by treatment requirement throughout these people' lives. A smaller proportion of individuals develop diabetes in the neonatal period as the presenting feature of a more complex syndrome (syndromic neonatal diabetes) [3]. There is a strong correlation between genotype and phenotype, with some genetic causes only causing one specific subtype of neonatal diabetes (for example 6q24 methylation defects cause transient neonatal diabetes [4]). Some genes however can cause all three types of neonatal diabetes, for example pathogenic activating variants in *KCNJ11* and *ABCC8*, can cause either transient, isolated neonatal, or syndromic neonatal diabetes (DEND syndrome) [5-9]. In this case, the severity of the clinical phenotype depends on the severity of the DNA change [9].

1. Genetic causes of neonatal diabetes

Neonatal diabetes is a genetically heterogeneous conditions, with over 30 genetic causes identified so far accounting for >85% of the cases [3]. Pathogenic variants causing neonatal diabetes result in disrupted beta cell development or function through 5 mechanisms, 1) abnormal methylation at the imprinted 6q24 region (known to cause transient neonatal diabetes), 2) disrupted glucose sensing/insulin secretion, 3) increased beta cell death resulting from deregulated endoplasmic reticulum stress, 4) increased beta cell death resulting from deregulated autoimmunity, and 5) disrupted beta cell/pancreas development.

2. Neonatal diabetes caused by disrupted beta cell development

Pathogenic variants in genes involved in beta cell development and maturation can result in neonatal diabetes through severely reduced number of mature beta cells (Table 1). These genes are all developmental factors involved in regulation of transcription and are essential either to the development of the whole pancreas or to formation and maturation of the beta cell specifically. In the first case, pathogenic variants often result in exocrine pancreatic insufficiency in addition to diabetes,

with or without severe reduction in pancreatic size (a condition called pancreatic agenesis/hypoplasia). In the second case, the exocrine pancreatic function is not affected. However, additional extra-pancreatic features are often associated with mutations in both categories of genes, highlighting the shared role of developmental factors in regulating formation of multiple organs.

3. Neonatal diabetes caused by failure of pancreatic development

Mutations in 7 genes which are essential at different stages of pancreatic development have been reported to cause pancreatic agenesis/hypoplasia, characterized by neonatal diabetes and exocrine pancreatic insufficiency [10]. Individuals with pancreatic agenesis/hypolasia have intrauterine growth retardation, reflecting severe insulin secretion deficiency *in utero* [11]. Neonatal diabetes usually presents within the first month of life and requires insulin treatment. Diagnosis of exocrine pancreatic insufficiency is challenging as symptoms are non-specific (malabsorption, failure to thrive) and pancreatic imaging is very difficult in young babies [10].

An early genetic diagnosis is essential to prompt investigation of exocrine pancreatic function, as oral replacement therapy of pancreatic enzymes (lipase, protease, and amylase) is often needed to prevent growth delay.

3.1. Neonatal diabetes caused by autosomal recessive *PDX1* mutations

PDX1 is a member of the homeodomain family of transcription factors and is essential for both development of the pancreas where it is essential for the specification of a pancreatic progenitor cell population, and maintenance of beta cell function since it is one of the main regulators of the (insulin) *INS* gene [12].

Autosomal recessive mutations in the *PDX1* (also known as *IPF1*) gene were the first reported to cause isolated pancreatic agenesis. Stoffers et al analysed the *PDX1* gene, the knock-out of which was known to result in pancreatic agenesis in mouse, in a female infant with neonatal diabetes and pancreatic exocrine insufficiency [13]. Subsequent abdominal ultrasound failed to show a pancreas. This patient was found to be homozygous for a frameshift mutation, resulting in a premature stop codon within the second and last exon of the *PDX1* gene. The same mutation was later identified in another patient diagnosed with permanent neonatal diabetes due to pancreatic agenesis at age two weeks, needing enzyme supplementation [14] Compound heterozygous missense *PDX1* variants were identified in a child with permanent neonatal diabetes due to pancreatic agenesis reported by Schwitzgebel et al.[15]. Both mutations affected residues within the DNA-binding domain (the homeodomain) and were shown to significantly decrease the protein half-life.

Later reports have highlighted the variability of the phenotypic spectrum of diabetes associated with biallelic *PDX1* missense mutations. Nicolino et al. reported two cousins born to consanguineous parents who were homozygous for a missense mutation within the PDX1 homeodomain [16]. Both cases had permanent neonatal diabetes diagnosed in the first days of life but no clinical signs of pancreatic insufficiency. Biochemical studies conducted on the two probands and their unaffected parents showed sub-clinical exocrine insufficiency in the two patients and marginally impaired glucose responses in their parents. De Franco *et al* later reported 4 cases from 3 families with biallelic *PDX1* mutations [17]. All had permanent neonatal diabetes but no clinical symptoms of exocrine pancreatic insufficiency. Normal exocrine function was confirmed by fecal elastase measurement in two cases.

A recent case report has raised the possibility that, in addition to a variable pancreatic phenotype, *PDX1* mutations may also cause extra-pancreatic features. Kulkarni et al reported the identification of a novel homozygous missense variant in a proband with neonatal diabetes, exocrine pancreatic insufficiency duodenal atresia, annular pancreas and gall bladder hypoplasia [18]. The presence of a gut phenotype in this individual is consistent with evidence from the *Pdx1* knock-out mouse suggesting that Pdx1 is required for rostral duodenum development [19]. Identification and characterisation of further cases with *PDX1* mutations and 'atypical' phenotype is required to have a clearer picture of the clinical features caused by recessive mutations in this gene.

3.2 Neonatal diabetes caused by autosomal recessive *PTF1A* mutations

PTF1A is the DNA-binding subunit of the trimeric complex PTF1 and belongs to the basic helix-loop-helix family of proteins. Mouse studies highlighted important dosage-dependent roles of Ptf1a during pancreas development, including determination of pancreas size, development of the exocrine pancreas, and regulation of islets morphogenesis and endocrine cell function in the neonatal period [20].

In 2004 Sellick *et al* reported two consanguineous families where all affected members showed an identical phenotype, consisting of neonatal diabetes due to pancreatic agenesis, severe intrauterine growth retardation, microcephaly, and cerebellar hypoplasia/agenesis [21]. All affected members died within a few weeks after birth. These features closely resemble that of the *Ptf1a* knock-out mice phenotype. Using a combination of homozygosity mapping and candidate gene analysis, Sellick *et al* identified homozygous loss of function mutations in the *PTF1A* gene in both families. Both mutations were predicted to result in the translation of a truncated PTF1A protein lacking the DNA binding domain. Two further pancreatic agenesis and cerebellar hypoplasia cases have subsequently been reported, both of them harboring homozygous *PTF1A* truncating mutations [22, 23].

In 2014, Weedon *et al* used a combination of genome sequencing and epigenetic mapping in pancreatic progenitors to identify biallelic mutations in a distal *PTF1A* enhancer in 10 families with pancreatic agenesis but no additional neurological features [24]. Functional studies showed that the enhancer was pancreatic-specific and active during differentiation of pancreatic progenitors. This report suggested that *PTF1A* enhancer mutations are the most common cause of isolated pancreatic agenesis. A recent report of 30 patients with biallelic *PTF1A* enhancer mutations further explored the phenotype caused by these pathogenic variants. None of the patients had severe neurological features, however additional features such as growth retardation, anemia, and cholestasis were common [25].

Isolated pancreatic agenesis has also been reported in two families who were homozygous for the *PTF1A* missense mutation, p.(Pro191Thr). *In vitro* studies suggested that this mutation is likely to result in partial loss of function, thus potentially explaining the absence of the severe neurological features caused by coding loss of function mutations [26].

Overall biallelic mutations in *PTF1A* account for ~2% of cases of neonatal diabetes, which rises to >8% in individuals born to consanguineous parents [3] (Table 1).

3.3 Neonatal diabetes caused by autosomal dominant *HNF1B* mutations

HNF1B (also known as TCF2 or vHNF1) is a homeodomain-containing transcription factor that plays an essential role in nephrons and liver development, Mullerian duct formation and development of the embryonic pancreas.

Heterozygous loss of function mutations and deletions resulting in haploinsufficiency of the *HNF1B* gene cause a multi-developmental disorder known as RCAD (Renal cysts and diabetes syndrome). Patients with RCAD syndrome usually develop diabetes outside the neonatal period, after presentation of renal disease [27]. Pancreatic atrophy and defects of pancreatic exocrine function are common in individuals with *HNF1B* mutations and deletions, pointing toward a defect in pancreatic development [28].

Heterozygous *HNF1B* mutations can sometimes cause neonatal diabetes, with two cases reported in the literature [29, 30] and an estimated frequency of 0.002% in a large neonatal diabetes cohort [3] (Table 1). In both previously reported cases, after an initial diagnosis in the first month of life, the children's insulin requirement decreased, with insulin treatment becoming intermittent in the first case and interrupted in the second. In both cases however the diabetes relapsed in infancy. The individual reported by Edghill *et al* [30] had marked pancreatic atrophy and further investigations confirmed exocrine as well as endocrine dysfunction, although this did not result in clinical symptoms. *HNF1B* mutations are therefore a rare cause of transient neonatal diabetes, and they are more often associated with early-onset diabetes and various degrees of renal dysfunction.

3.4 Neonatal diabetes caused by autosomal recessive *RFX6* mutations (Mitchell-Riley syndrome)

RFX6 is a winged-helix transcription factor belonging to the family of Regulator Factors X (Rfx), a group of 7 highly conserved proteins. Two members of the Rfx family, RFX3 and RFX6 are highly expressed in pancreas and observations in knock-out mouse models showed that they are both involved in pancreatic development [31, 32].

Biallelic loss of function mutations in *RFX6* (including missense, frameshift and mutation predicted to affect splicing) were first identified in 5 individuals with suspected Mitchell-Riley syndrome, a condition characterised by neonatal diabetes, bowel atresia, and gallbladder agenesis/hypoplasia [32]. Eleven further cases have been reported in the literature since [33], including 4 cases from two families with diabetes onset in infancy rather than in the neonatal period. In both families, the disease was caused by compound heterozygous mutations, with one allele being predicted to preserve some degree of activity [34, 35]. The condition was reported to be lethal before the first year of life in at least 7 cases suggesting that prognosis is poor in ~40% of cases.

3.5 Neonatal diabetes caused by autosomal dominant mutations in *GATA6*

GATA6 is a transcription factor containing two tandem Zinc-Finger domains which mediate DNA binding and protein-protein interactions. GATA6 is involved in early embryonic development is essential for the correct development of all endoderm-derived organs, including pancreas, liver, and thyroid [36].

Heterozygous loss of function mutations in the *GATA6* gene cause a wide spectrum of developmental defects. *GATA6* mutations were first reported to cause congenital heart defects [37]. In 2012 Allen *et al* reported the identification of *de novo GATA6* mutations as the most common cause of pancreatic agenesis in humans, having identified loss of function variants (including missense, frameshifts and splicing variants) in 15 individuals with neonatal diabetes and exocrine pancreatic insufficiency [10]. These individuals also presented additional extra-pancreatic features, including congenital heart defects, hepatobiliary malformations (particularly gallbladder agenesis), and gut abnormalities. The broad spectrum of phenotypes caused by *GATA6* haploinsufficiency reflects this gene's essential role during endodermal development. The pancreatic phenotype caused by *GATA6* mutations is very variable, with pathogenic variants in *GATA6* resulting in a wide spectrum of diabetes phenotypes, including transient neonatal diabetes and adult-onset diabetes without exocrine pancreatic insufficiency [36].

It is still unclear what are the factors influencing the phenotypic presentation of *GATA6* haploinsufficiency. Systematic assessment of *GATA6* mutations have shown that *de novo* loss of function mutations and mutations within the second Zinc-Finger domain are more likely to result in a pancreatic phenotype [38]. However, the phenotypic variability observed among individuals with the

same mutation highlights the presence of other factors influencing the phenotype in addition to the *GATA6* pathogenic variants. A recent report suggested a potential role of a common polymorphism (rs12953985) located ~8kb downstream of *GATA6* in affecting the pancreatic phenotype in individuals with *GATA6* mutations [39]. More studies, including *in vitro* characterisation of stem cell models of pancreatic differentiation and genetic studies in individuals with *GATA6* mutations and different phenotypes, are needed to clarify whether the phenotypic spectrum observed in individuals with *GATA6* haploinsufficiency is due to genetic, environmental, or stochastic factors, or a combination of all three. This information would be extremely valuable for families of individuals with *GATA6* mutations, allowing more accurate information on recurrence risk.

3.6 Neonatal diabetes caused by autosomal dominant mutations in *GATA4*

GATA4 belongs to the same protein family as GATA6 and has a similar protein structure characterised by the presence of two tandem Zinc-Finger domains. Similarly to GATA6, GATA4 is involved in early embryonic development and studies in mice have suggested redundant roles of these two factors during rodent pancreas development [40, 41].

Haploinsufficiency of *GATA4* is a known cause of congenital heart defects with over 120 cases reported in the literature [42]. Two studies have reported 3 cases with heterozygous mutations in *GATA4* and pancreatic agenesis: two cases also had congenital heart defects [42, 43]. The third one, who was heterozygous for a novel missense variant predicted to severely affect GATA4's ability to activate target genes, died soon after birth. Shaw-Smith *et al* [42] reported two individuals harboring heterozygous *GATA4* deletions, who were diagnosed with diabetes in infancy rather than in the neonatal period. These two children had congenital heart defects but normal pancreatic exocrine function. This suggests that mutations in *GATA4* can cause pancreatic agenesis and neonatal/~~early~~ infancy onset diabetes in addition to congenital heart defects.

Given the suggested redundancy of Gata4 and Gata6 in mice, it is interesting that in humans, heterozygous loss of either gene can result in diabetes through defective pancreatic development. Whilst mutations in these genes result in a spectrum of phenotypes, haploinsufficiency of *GATA4* does not appear to commonly result in multi-system involvement as in the case of *GATA6*. The occurrence of a pancreatic phenotype in individuals with *GATA4* deletions/mutations also appears to be much rarer than in individuals with *GATA6* mutations, suggesting a different role of these two genes during human pancreas development compared to rodents.

3.7 Neonatal diabetes caused by a specific heterozygous *CNOT1* mutation

The protein encoded by the *CNOT1* gene acts both as a repressor of transcription during development and as the scaffold protein of the CCR4-NOT complex, which is one of the main regulators of RNA

metabolism [44]. In its role as a transcriptional repressor, CNOT1 has been suggested to be essential for maintaining stem cell pluripotency [45].

Four individuals with the same heterozygous *CNOT1* missense mutation, p.(Arg535Cys), and pancreatic agenesis have been reported by two studies [46, 47]. The mutation had arisen *de novo* in all cases where both parental samples were available for testing. Three individuals also had holoprosencephaly and the fourth had facial features potentially consistent with holoprosencephaly but a brain scan was not available. A fifth case with holoprosencephaly but without neonatal diabetes was also reported.

The initial identification of an identical *de novo CNOT1* mutation in 3 individuals with pancreatic agenesis had suggested a potential specific effect of the p.(Arg535Cys) variant. De Franco *et al*, based on the results in a mouse model harbouring this variant in homozygosity (the heterozygous mice showed no phenotype, as often observed in mouse models of these early stages of pancreatic development), suggested that the mutation may be specifically impairing the ability of stem cells to exit their pluripotent state and affect de-activation of the SHH pathway (which is known to be essential for pancreatic development; *SHH* mutations cause holoprosencephaly) [46]. The hypothesised specific role of the p.(Arg535Cys) variant in causing pancreatic agenesis and holoprosencephaly has been further supported by the recent report of 39 individuals with heterozygous loss of function *CNOT1* mutations resulting in a neurodevelopmental disorder. None of them had holoprosencephaly or pancreatic agenesis [48].

Whilst a very rare cause of pancreatic agenesis, the identification of this *CNOT1* mutation was important to further highlight the mechanisms regulating pancreatic development, as CNOT1 had never before been suspected to be important for pancreatic development.

4. Neonatal diabetes caused by failure of beta cell development

Mutations resulting in disruption of at least 5 genes involved in the transcriptional pathways regulating beta cell development, from differentiation of endocrine progenitors to generation of mature insulin-producing beta cells, have been reported to cause neonatal diabetes. Extra-pancreatic features are associated with mutations in each of these genes which therefore cause syndromic neonatal diabetes.

4.1 Neonatal diabetes caused by autosomal recessive *GLIS3* mutations (NDH syndrome)

The Kruppel-like zinc finger protein GLIS3 is a transcription factor with both activation and repression activity. It is mostly expressed in the kidney, pituitary, pancreas, uterus, and thyroid gland. Mouse studies have showed that *Glis3* is expressed during beta cell differentiation and mouse knock-out for *Glis3* present neonatal diabetes, polycystic kidneys and congenital hypothyrodisim [49].

Biallelic loss of function mutations in *GLIS3* were first found to cause NDH (neonatal diabetes and hypothyroidism) syndrome in 3 consanguineous families with 6 affected individuals [50]. Using linkage analysis followed by analysis of candidate genes in the linkage region, Senee *et al* identified homozygous multi-exon deletions in two families and a frameshift variant predicted to result in a truncated GLIS3 protein in the third [50]. Over 15 patients with biallelic *GLIS3* mutations have been reported so far, all with a very consistent phenotype of intrauterine growth retardation, diabetes diagnosed in the first month of life and congenital hypothyroidism. Polycystic kidneys, hepatomegaly, cholestasis, and characteristic facial features have also been [51, 52]. The majority of cases described in the literature harbor deletions involving one or more of the 11 *GLIS3* exons, however protein truncating and missense mutations affecting the DNA-binding domain have also been reported.

Only one case with compound heterozygous mutations (a deletion and a missense mutation) has been reported in the literature so far [51]. Interestingly, this is the only individual reported to have neonatal diabetes but normal thyroid function, suggesting some potential phenotypic variability depending on genotype. The identification and characterisation of additional individuals with this neonatal diabetes subtype is needed to further assess this possibility.

4.2 Neonatal diabetes caused by autosomal recessive *NEUROD1* mutations

NEUROD1 belongs to the basic helix-loop-helix family of transcription factors. Its function is essential for endocrine pancreas and brain development. *Neurod1* knock-out in mouse results in diabetes onset soon after birth [53] in addition to neurological abnormalities, such as inner ear defects caused by severe reduction of sensory neurons [54]. Consistent with its role in pancreas and brain development, mouse studies have shown that Neurod1 transcription is regulated by two development factors which are known to cause neonatal diabetes and have a role in development of both organs, Neurog3 and Nkx2-2.[55, 56],

Homozygous mutations in *NEUROD1* have been reported in 3 families [57, 58]. The first two cases were reported by Rubio-Cabezas *et al* who identified homozygous frameshift pathogenic variants in two unrelated individuals with permanent neonatal diabetes diagnosed in the first 10 weeks of life, intrauterine growth retardation, and multiple neurological features (including developmental delay, sensorineural deafness, myopia and diffuse retinal dystrophy). The mutations were predicted to result in a truncated protein lacking the transactivation domain [58]. The third case had clinical features very similar to the previously reported cases and is homozygous for a novel missense variant affecting the DNA binding domain. Given the striking similarity between the human disease and the phenotype in the knock-out *Neurod1* mouse, it is likely that all three variants cause neonatal diabetes and additional neurological features as a result of NEUROD1 loss of function [59].

4.3 Neonatal diabetes caused by autosomal recessive *NEUROG3* mutations

NEUROG3 is a member of the basic helix-loop-helix family of transcription factors and is considered the master regulator of pancreatic endocrine cells differentiation. Loss of *Neurog3* in mouse has been reported to result in complete loss of islets, highlighting the essential role of this gene during beta cell development [56].

Homozygous *NEUROG3* mutations were first reported as a cause of Congenital malabsorptive diarrhea in three individuals. Two of them were diagnosed with diabetes in infancy whilst the third case died during childhood [60]. In 2011 the first case with a homozygous *NEUROG3* frameshift mutation and neonatal diabetes was reported [61], followed by 3 additional cases with biallelic *NEUROG3* missense and frameshift mutations. All these individuals were diagnosed with permanent neonatal diabetes in the first month of life, had intrauterine growth retardation, in addition to enteric anendocrinosis and malabsorptive diarrhea [62, 63].

The variable age at onset of diabetes (neonatal vs infancy-inset) in individuals with biallelic *NEUROG3* mutations was initially proposed to be genotype-dependent, with complete loss of function mutations causing neonatal diabetes whilst missense pathogenic variants resulting in a partial loss of function would cause later onset diabetes. However, two individuals with complete loss of function pathogenic variants in *NEUROG3* who were diagnosed with diabetes in infancy (aged 3 and 7) have recently been reported [64]. This new evidence shows that the variability of diabetes onset in individuals with biallelic *NEUROG3* mutations is not completely explained by the genotype, but other factors may influence the pancreatic phenotype.

4.4 Neonatal diabetes caused by autosomal recessive *NKX2-2* mutations

The *NKX2-2* gene encodes a homeobox transcription factor involved in development of the nervous system and of the pancreatic beta cells, as firstly recognised by mouse knock-out studies showing neonatal diabetes and severe neurological defects in the absence of *Nkx2-2* [65, 66].

Biallelic loss of function *NKX2-2* mutations were identified by Flanagan *et al* in three children from two families using a candidate gene approach in patients born to consanguineous families [67]. A recent report [68] has brought the total number to 4 cases, suggesting that this is likely to be a rare cause of neonatal diabetes. The pathogenic variants identified so far were all protein truncating, likely resulting in loss of function. The 4 individuals reported so far had severe intrauterine growth retardation and were diagnosed with diabetes soon after birth. They also all had moderate or severe developmental delay. Hypoplasia of the corpus callosum was reported in one case. These individuals also appear to

develop severe infantile-onset obesity. Identification of further cases will be crucial to define the spectrum of disease associated with these rare pathogenic variants.

4.5 Neonatal diabetes caused by autosomal recessive *MNX1* mutations

MNX1 is a homeobox transcription factor essential for development of the pancreas and nervous system. *Mnx1* knock-out mouse presents a severe phenotype characterised by aberrant motorneuron differentiation, dorsal pancreas aplasia with reduced number of beta cells, and pulmonary paralysis resulting in death soon after birth [69-71].

Autosomal dominant pathogenic variants in *MNX1* have been reported in individuals with Currarino syndrome, a congenital disorder involving multiple organs. Neonatal diabetes is not a feature of Currarino syndrome. However, homozygous *MNX1* missense mutations predicted to affect the DNA binding domain of the MNX1 protein have been identified in two unrelated individuals with permanent neonatal diabetes [67, 72]. One case, who died at 10 months of age, also had additional clinical features similar to those identified in individuals diagnosed with Currarino syndrome, including sacral agenesis and developmental delay. The second individual developed diabetes in the first day of life and was not reported to have additional extra-pancreatic features. Since only two cases have been reported so far, it is unclear whether *MNX1* mutations cause isolated permanent neonatal diabetes, syndromic neonatal diabetes or both. Identification of other cases will be essential to define the clinical phenotype of this rare neonatal diabetes subtype.

5. Candidate genes

The identification of neonatal diabetes-causing mutations in genes such as *PDX1*, *PTF1A*, *NKX2-2*, *MNX1*, *NEUROD1*, and *NEUROG3*, which were known to be important for pancreatic and beta cell development based on mouse studies, confirmed that these genes are essential for pancreatic development in humans as well [67]. However, there are other genes, which have been shown by knock-out studies to regulate pancreatic development and/or function, where the association with neonatal diabetes is still unclear.

5.1 *PAX6*

Autosomal dominant pathogenic variants in *PAX6* cause aniridia and other eye disorders (MIM:106210). Autosomal recessive loss of function mutations in *PAX6* have been reported in two individuals so far. One died at the age of 1 week and had complex brain abnormalities, but no endocrine phenotype was reported [73]. A second individual with compound heterozygous *PAX6* variants had a complex brain and multi-system phenotype including neonatal diabetes [74]. However, this child also had Trisomy 21 which has been recently reported to be a cause of neonatal/early onset diabetes [75]. The identification

of additional unrelated cases with neonatal diabetes and autosomal recessive *PAX6* pathogenic variants is needed to confirm whether mutations in these gene are a cause of neonatal diabetes.

5.2 *SOX9*

Mouse studies have shown that *Sox9* is essential for development of the endocrine pancreas. In humans, heterozygous loss of function mutation in *SOX9* cause the congenital multisystem disorder Campomelic dysplasia, but diabetes is not reported to be a feature of this condition (MIM:114290). No recessive mutations in the gene have been reported so far. It is possible that in humans *SOX9* biallelic mutations are not compatible with life. Alternatively, a disease mechanism similar to what is observed for *MNX1* may be possible, with heterozygous mutations causing a severe multi-system disease and only specific, less severe missense variants resulting in a diabetes phenotype. Investigation of *SOX9* in individuals with diabetes is needed to confirm or refute these hypotheses.

5.3 *NKX6-1* and *NXK6-2*

The homeobox factor NKX6-1 is essential for beta cell development and function. *Nkx6.1* knock-out in mice displays 85% reduction in beta cells, whereas double knock-out of both *Nkx6.1* and the closely related factor *Nkx6.2* have a 92% reduction [76], suggesting an important role for Nkx6.1 in the generation of beta cells, with some compensatory ability of Nkx6.2. Neither of these genes has so far been conclusively shown to cause diabetes (or any other disorders) in humans.

5.4 *MAFA* and *MAFB*

Mouse studies suggested a role for the transcription factor Mafb during beta cell development and an important function of Mafa for insulin secretion in beta cells. Whilst neither has been found to cause neonatal diabetes, mutations in both genes have been recently report to cause human diseases. A heterozygous activating missense variant in *MAFA* has been found to cause insulinomatosis and adult-onset diabetes, confirming the important role of MAFA in insulin secretion [77]. Autosomal dominant missense mutations in *MAFB* have been reported to cause a skeletal disorder (MIM:166300), whilst loss of function mutations cause a subtype of Duane syndrome (MIM:617041). Diabetes is not a feature of either condition.

6. Future developments

The identification of genetic mutations in known pancreatic and beta cell genes is essential for the patients' diagnosis, their family counselling and confirming these genes' role in human disease.

The identification of many genes essential for pancreatic development through mouse studies has been instrumental to the discovery of pathogenic variants in some of these genes as causing disease in humans [67]. However, differences in the phenotype observed with heterozygous mutations in genes

causing neonatal diabetes in humans such as *GATA6*, *GATA4*, and *CNOT1* highlight the possibility of species-specific mechanisms regulating pancreatic development in humans compared to mice. Furthermore, the identification of a mutation in *CNOT1* as causing pancreatic agenesis has highlighted two important limits of exclusively using gene candidacy to identify novel genetic causes of disrupted pancreatic development: 1) CNOT1 was not a candidate gene for pancreatic development, and 2) it is likely that *Cnot1* knock-out in mice would not have highlighted a pancreatic phenotype since the disease is caused by a specific mutation. The increased availability of transcriptome and epigenome data in the human developing pancreas [78-80] represents an exciting novel resource to identify novel genes essential for regulation of pancreas development in humans, but not necessarily in other species. This knowledge can in turn be leveraged to aide in the identification of novel genetic causes of monogenic diabetes.

The use of genome sequencing, the most comprehensive genetic analysis approach we currently have, to identify novel genetic causes of neonatal diabetes has allowed the identification of >10 novel causative genes in the last 10 years. Whilst genome sequencing is a powerful tool for genetic variants detection, to identify the causal mutation careful selection of candidate variants depending on presumed inheritance and population frequency is necessary. Furthermore, access to genetic data from individuals with a similar phenotype is essential to replicate the findings and confirm causality.

Overall, the advent of genome sequencing and the development of large genomic databases are valuable resources which are likely to result in a step-change in our ability to understand and genetically characterise subtypes of neonatal diabetes caused by disrupted pancreatic or beta cell development.

Table 1. Summary of the mode of inheritance, diabetes phenotype, additional features, and approximate prevalence among neonatal diabetes cases (based on [3]) of the genes reported to cause neonatal diabetes >2 unrelated individuals.

Gene	OMIM ID	Mode of inheritance of neonatal diabetes	Diabetes phenotype	Additional features	Approximate prevalence in neonatal diabetes
PDX1	260370	Autosomal recessive	NDM	Exocrine pancreatic insufficiency (in some patients)	0.59%
PTF1A	609069, 615935	Autosomal recessive	NDM	Exocrine pancreatic insufficiency, cerebellar agenesis (for loss of function mutations)	2.16%
HNF1B	137920	Autosomal dominant	NDM (rare), MODY	Exocrine pancreatic insufficiency, renal cysts	0.02%
RFX6	615710	Autosomal recessive	NDM, infancy-onset diabetes	Annualar pancreas, bowel atresia, intestinal malrotation, gallbladder agenesis/hypoplasia	0.01%
GATA6	600001	Autosomal dominant	NDM, infancy-onset diabetes, MODY (rare)	Congenital heart defects, umbilical hernia, diaphragmatic hernia, gallbladder agenesis/hypoplasia, hypothryodisim	2.84%
GATA4	na	Autosomal dominant	NDM, infancy-onset diabetes	Congenital heart defects	0.39%
CNOT1	618500	Autosomal dominant mutation-specific	NDM	Holoprosencephaly	0.29%
GLIS3	610199	Autosomal recessive	NDM	Congenital hypothyroidism, polycystic kidneys, liver dysfunction, facial features	0.88%
NEUROD1	na	Autosomal recessive	NDM	Developmental delay, sensorineural deafness, myopia and diffuse retinal dystrophy	0.29%
NEUROG3	610370	Autosomal recessive	NDM, infancy-onset diabetes	Malabsorptive diarrhea	0.20%
NKX2-2	na	Autosomal recessive	NDM	Developmental delay, severe infantile-onset obesity, corpus callosum agenesis (one case so far)	0.20%
MNX1	na	Autosomal recessive	NDM	Sacral agenesis (one case so far)	0.01%

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