The lessons of early-onset monogenic diabetes for the understanding of diabetes pathogenesis

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Monogenic diabetes consists of different subtypes of single gene disorders comprising a large spectrum of phenotypes, namely neonatal diabetes mellitus or monogenic diabetes of infancy, dominantly inherited familial forms of early-onset diabetes (called Maturity-Onset Diabetes of the Young) and rarer diabetes-associated syndromic diseases. All these forms diagnosed at a very-young age are unrelated to auto-immunity. Their genetic dissection has revealed major genes in developmental and/or functional processes of the pancreatic \(\beta\)-cell physiology, and various molecular mechanisms underlying the primary pancreatic defects. Most of these discoveries have had remarkable consequences on the patients care and patient’s long-term condition with outstanding examples of successful genomic medicine, which are highlighted in this chapter. Increasing evidence also shows that frequent polymorphisms in or near monogenic diabetes genes may contribute to adult polygenic type 2 diabetes. In this regard, unelucidated forms of monogenic diabetes represent invaluable models for identifying new targets of \(\beta\)-cell dysfunction.

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Lessons from neonatal diabetes and non auto-immune diabetes of infancy

*Ætiopathogenic characteristics of early-infancy diabetes*

Neonatal diabetes mellitus (NDM) is defined by diabetes, either isolated or with syndromic features, diagnosed within the first 6 months of life.\(^1,2\) It is a relatively rare entity including many clinically and genetically heterogeneous disorders that affect \(\sim 1:100,000–260,000\) live births.\(^3–^5\) NDM can be either permanent (PNDM) requiring lifelong treatment or transient (TNDM) with insulin dependence in the first months only and a spontaneous remission of diabetes usually by 18 months of age.\(^5–^7\) The severe hyperglycaemia and minimal ketosis appearing in the first days of life may have dramatic complications in the neonate, such as failure to thrive, acidosis, dehydration and neurological alterations.\(^6,7\) Very early-onset diabetes mellitus is mostly unrelated to auto-immunity, and appears to be conferred by single gene disorders related to developmental and/or functional defects of the endocrine pancreas, as demonstrated by us and many other groups in the last 15 years.\(^5,8\) Since many patients can be diagnosed after the neonatal period (i.e. after 6 months of life, as used in the literature), we will also refer here to monogenic diabetes of infancy (MDI).\(^5,8\)

In most of cases presenting with early-infancy diabetes, the disease is not present in the parents, and *de novo* mutations or chromosomal abnormalities were demonstrated to explain the child disorder.\(^5,9\) However, in some families, diabetes is also diagnosed in one parent and/or relatives (either at young ages or in adulthood), highlighting a vertical dominant transmission of the disease and also marked phenotype variabilities.\(^2,10\) The most frequent causes of NDM/MDI are mutations in the pancreatic \(\beta\)-cell expressed ATP-sensitive potassium (\(K_{\text{ATP}}\)) channel genes and in the preproinsulin (INS) gene, accounting for \(>50\%\) of cases with PNDM diagnosed before 6 months of age.\(^3,5,9–^11\)

In some NDM cases born from consanguineous parents, a recessive transmission of a homozygous or compound heterozygous mutation was demonstrated in families mostly of Middle-East origin.\(^12,13\) In rare conditions, diabetes may also associate with extra-pancreatic symptoms (including neurological, cardiac, renal, intestinal or skeletal abnormalities, developmental delay and also dysmorphic features). At least 14 genes have been identified to date in these uncommon syndromic disorders (*Table 1*)\(^2,5,8;\) as well as homozygous or compound heterozygous mutations in *PDX1*, encoding the pancreatic duodenal homeobox-1 (PDX1) transcription factor, which cause pancreas agenesis or hypoplasia (with endocrine and exocrine failure)\(^14,15\) or in *GCK*, encoding the glycolytic enzyme glucokinase, which lead to a complete insensitivity of the pancreatic \(\beta\)-cell to glucose and severe hyperglycemia.\(^16\)

*Genetic anomalies of TNDM as an imprinting disorder*

TNDM patients present with intra-uterine growth retardation (IUGR) and very low birth weights (usually below the 2nd percentile for gestational age in \(\sim 80\%\) of cases), resulting from *in utero* insulin deficiency, and macroglossia.\(^1,6\) Diabetes develops in the first weeks of life, then remits spontaneously within 1–18 months with a possible relapse to a permanent diabetes state at the time of adolescence, or later in early adulthood, in \(\sim 2/3\) of patients.\(^6\) A major factor in the onset of recurrent diabetes is likely a metabolic stress with significant insulin resistance, such as in puberty or pregnancy, whereas the primary \(\beta\)-cell defect may have a variable phenotypic expression.

This condition is due to genetic or epigenetic alterations at an imprinted locus on chromosome 6q24 and can be sporadic or inherited.\(^1,7\) At least three molecular mechanisms have been shown to result in overexpression of a paternally expressed allele within a critical 400-kb region of 6q24: i) paternal uniparental disomy of the region, ii) paternal inherited duplication of chromosomal 6q24, or iii) loss of methylation in a CpG island of the maternally inherited chromosome 6q24 (i.e. loss of imprinting and altered expression of the maternal allele).\(^17,18\) Two paternally expressed genes are located in the 6q24 region: *HYMA1* whose function is unknown, and *PLAGL1* (or *ZAC*), which encodes a zinc-finger transcription factor known to regulate cell cycle and apoptosis and also to be a transcriptional regulator of the pituitary-adenylate-cyclase-activating-polypeptide receptor-1 (PACAP1, being a potent insulin secretagogue) (*Table 1*). *PLAGL1/ZAC* is located 85 kb from the imprinted CpG island on 6q24 and is imprinted in foetal tissues.\(^17\) Thus, overexpression of these genes, which is normally restricted to the paternal allele, is the more likely cause of the disease.\(^5,18\) In some patients, diabetes may be the initial
Table 1
Subtypes of monogenic diabetes: Neonatal diabetes (NDM/MDI), with or without syndromic features, and MODY.

<table>
<thead>
<tr>
<th>Gene name and locus</th>
<th>Protein/Function</th>
<th>Inheritance</th>
<th>Phenotypes/syndromes</th>
<th>OMIM</th>
<th>Impact in adult T2D (ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NDM/MDI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>PLAGL1</em> 6q24.2</td>
<td>Pleiomorphic adenoma gene-like 1, zinc-finger protein or ZAC tumour suppressor</td>
<td>UPD6 (de novo), paternal duplication (may be inherited), maternal methylation defect</td>
<td>Autosomal recessive</td>
<td>TNDM; Chromosome 6q structural anomalies with imprinting mechanisms (methylation defects)</td>
<td>601410 + 603044</td>
</tr>
<tr>
<td><em>ZFP57</em> 6p22.1</td>
<td>Zinc-finger protein 57, role in maintenance of imprinted DNA methylation</td>
<td>Autosomal recessive</td>
<td>TNDM with macroglossia, developmental delay, umbilical defect, visual impairment, CHD</td>
<td>601410</td>
<td></td>
</tr>
<tr>
<td><em>KCNJ11</em> 11p15.1</td>
<td>Kir6.2, inward rectifier K+ channel pore-forming subunit</td>
<td>Spontaneous (80%) or autosomal dominant</td>
<td>PNDM (more often), TNDM (less often), DEND (rarely)</td>
<td>606176 (97-99, 102)</td>
<td></td>
</tr>
<tr>
<td><em>ABCC8</em> 11p15.1</td>
<td>Sulfonylurea receptor-1 (SUR1) subunit of KATP channel</td>
<td>Spontaneous (80%) or autosomal dominant</td>
<td>TNDM (more often), PNDM (less often), DEND (very rare)</td>
<td>606176 + (35,82, 99,101)</td>
<td></td>
</tr>
<tr>
<td><em>INS</em> 11p15.5</td>
<td>Proinsulin, insulin, hypoglycaemic hormone, effect on anabolism</td>
<td>Spontaneous (80%), autosomal dominant or recessive (rarely)</td>
<td>PNDM and early-infancy diabetes; Heterozygous mutations in INS coding regions, homozygous mutations in INS regulatory regions</td>
<td>606176</td>
<td></td>
</tr>
<tr>
<td><em>GCK</em> 7p13</td>
<td>Glucokinase, glycolytic enzyme</td>
<td>Autosomal recessive</td>
<td>PNDM (homozygous or compound heterozygous mutations)</td>
<td>606176 + (93,105, 106)</td>
<td></td>
</tr>
<tr>
<td><strong>NDM/MDI associated with developmental anomalies and/or extra-pancreatic features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>PDX1</em> 13q12</td>
<td>Pancreatic duodenal homeobox protein 1 (PDX1) or IPF1 transcription factor</td>
<td>Autosomal recessive</td>
<td>PNDM with pancreas agenesis/hypoplasia for homozygous mutations</td>
<td>260370</td>
<td>600733</td>
</tr>
<tr>
<td><em>EIF2AK3</em> 2p12</td>
<td>Eukaryotic translation initiation factor 2-α kinase 3, or pancreatic eIF2-α kinase (PERK)</td>
<td>Autosomal recessive</td>
<td>Wolcott Rallison syndrome (diabetes associated with epiphyseal dysplasia)</td>
<td>226980</td>
<td>604032</td>
</tr>
<tr>
<td><em>PTF1A</em> 10p12</td>
<td>Pancreas transcription factor 1, Subunit α</td>
<td>Autosomal recessive</td>
<td>PNDM with pancreatic and cerebellar hypoplasia</td>
<td>609069</td>
<td>607194</td>
</tr>
<tr>
<td><em>GLIS3</em> 9p24.2</td>
<td>GLI-similar family zinc-finger 3 transcription factor</td>
<td>Autosomal recessive</td>
<td>PNDM with congenital Hypothyroidism, glaucoma (NDH syndrome)</td>
<td>610199</td>
<td>610192</td>
</tr>
<tr>
<td><em>FOXP3</em> Xp11.23</td>
<td>Forkhead box protein P3 (FoxP3) or Scurfen transcription factor</td>
<td>X-linked recessive</td>
<td>X-linked IPEX syndrome (Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) with diffuse auto-immunity</td>
<td>304790</td>
<td>300292</td>
</tr>
<tr>
<td><strong>NEUROD1</strong> 2q31.3</td>
<td>NeuroD1 or Beta2 transcription factor</td>
<td>Autosomal recessive</td>
<td>PNDM with cerebellar hypoplasia, sensorineural deafness, visual impairment and developmental delay</td>
<td>601724</td>
<td></td>
</tr>
<tr>
<td><strong>NEUROG3</strong> 10q22.1</td>
<td>NeuroG3 or NGN3 transcription factor</td>
<td>Autosomal recessive</td>
<td>PNDM with severe diarrhoea, absence of intestinal enteroendocrine cells</td>
<td>610370</td>
<td>604882</td>
</tr>
</tbody>
</table>

(continued on next page)
presentation of a more complex imprinting disorder due to recessive mutations in the gene ZFP57, encoding a zinc-finger transcription factor expressed in early development, which associate with developmental anomalies (mainly developmental delay and cardiac defects).19

Early diagnosis of 6q24-related TNDM is performed by segregation analysis of polymorphic markers on chromosome 6q24 and by analyzing the methylation status at this locus.5–7 As recurrent diabetes is frequent in TNDM, a prolonged follow-up is strongly recommended.

<table>
<thead>
<tr>
<th>Gene name and locus</th>
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<th>Inheritance</th>
<th>Phenotypes/syndromes</th>
<th>OMIM</th>
<th>Impact in adult T2D (ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX6</td>
<td>Paired box 6 containing Transcription factor</td>
<td>Autosomal recessive</td>
<td>PNDM with brain malformations, microcephaly, microphthalmia, anhydrotic ebulius</td>
<td>165550</td>
<td>607108</td>
</tr>
<tr>
<td>HNF1B</td>
<td>HNF-1β Transcription factor</td>
<td>Spontaneous or autosomal dominant</td>
<td>TNDM/PNDM with pancreatic atrophy and/or renal abnormalities (GCKD: renal cysts) or renal dysplasia</td>
<td>189907</td>
<td>(91,93,95)</td>
</tr>
<tr>
<td>RFX6</td>
<td>Regulatory factor X, 6 Transcription factor</td>
<td>Autosomal recessive</td>
<td>PNDM with pancreatic hypoplasia, intestinal atresia, gall bladder hypoplasia or aplasia, diarrhoea</td>
<td>601346</td>
<td>612659</td>
</tr>
<tr>
<td>WFS1</td>
<td>Wolframin Membrane glycoprotein</td>
<td>Autosomal recessive</td>
<td>Wolfram syndrome (DIDMOAD)</td>
<td>222300</td>
<td>+</td>
</tr>
<tr>
<td>IER3IP1</td>
<td>Immediate early response 3 interacting protein 1</td>
<td>Autosomal recessive</td>
<td>PNDM with microcephaly, severe infantile epileptic encephalopathy</td>
<td>614231</td>
<td></td>
</tr>
<tr>
<td>SLC19A2</td>
<td>Thiamine transporter 1 Solute carrier family 19 member</td>
<td>Autosomal recessive</td>
<td>TRMA syndrome (meagaloblastic anaemia)</td>
<td>249270</td>
<td>603941</td>
</tr>
<tr>
<td>SLC2A2</td>
<td>GLUT2 Facilitated glucose transporter</td>
<td>Autosomal recessive</td>
<td>Fanconi-Bickel syndrome, NDM or IGT or diabetes in infancy/childhood</td>
<td>227810</td>
<td>138160</td>
</tr>
<tr>
<td>MODY</td>
<td>HNF4A</td>
<td>HNF-4α Transcription factor</td>
<td>MODY1, in adolescence or early adulthood (and neonatal hyperinsulinism)</td>
<td>125850</td>
<td>600281 (51,93)</td>
</tr>
<tr>
<td>GCK</td>
<td>Glucokinase Glycolytic enzyme</td>
<td>Autosomal dominant</td>
<td>MODY2, mild hyperglycaemia (onset in early-childhood, and lifelong) [frequent]</td>
<td>138079</td>
<td>125851 (93,105,106)</td>
</tr>
<tr>
<td>HNF1A</td>
<td>HNF-1α Transcription factor</td>
<td>Autosomal dominant</td>
<td>MODY3, in adolescence or early adulthood [frequent]</td>
<td>604096</td>
<td>+</td>
</tr>
<tr>
<td>PDX1</td>
<td>IPF1</td>
<td>Autosomal dominant</td>
<td>MODY4, in early adulthood (similar to HNF1A but rare)</td>
<td>142410</td>
<td>(91–94)</td>
</tr>
<tr>
<td>HNF1B</td>
<td>HNF-1β Transcription factor</td>
<td>Autosomal dominant</td>
<td>MODY5, in early adulthood, renal cysts and diabetes (RCAD)</td>
<td>137920</td>
<td>+</td>
</tr>
<tr>
<td>NEUROD1</td>
<td>NeuroD1 or Beta2 Transcription factor</td>
<td>Autosomal dominant</td>
<td>MODY6, in early adulthood (similar to HNF1A but rare)</td>
<td>189907</td>
<td>(91,93,95)</td>
</tr>
<tr>
<td>INS</td>
<td>Preproinsulin, insulin</td>
<td>Autosomal dominant</td>
<td>MODY7, in childhood and early adulthood</td>
<td>606394</td>
<td>601724</td>
</tr>
</tbody>
</table>

The OMIM (Online Mendelian Inheritance in Man) numbers depict phenotype and/or gene MIM numbers. CHD, congenital heart defect; DED, developmental delay, epilepsy, neonatal diabetes; DIDMOAD, diabetes insipidus, diabetes mellitus, optic atrophy, deafness; GCKD, glomerulocystic kidney disease; IGT, impaired glucose tolerance; KATP, ATP-sensitive potassium channel; MDI, monogenic diabetes of infancy; NDH, neonatal diabetes and congenital hypothyroidism; NDM, neonatal diabetes mellitus; PNDM, permanent neonatal diabetes mellitus; RCAD, renal cysts and diabetes; TNDM, transient neonatal diabetes mellitus; TRMA, thiamine-responsive megaloblastic anaemia; T2D, type 2 diabetes; UPD6, uniparental disomy of chromosome 6.
Neonatal diabetes due to ATP-sensitive potassium channel mutations and therapeutic consequences

Activating mutations of the $K_{\text{ATP}}$ channel genes (either $ABCC8$ or $KCNJ11$) are the most common cause of NDM, with $KCNJ11$ mutations mostly responsible for PNDM (arising as neomutations more often)\(^{11,20}\) and $ABCC8$ mutations mostly associated to TNDM.\(^{2,10,21}\) The $K_{\text{ATP}}$ channels couple cellular glucose metabolism to membrane excitability by regulating potassium flux in many cell types.\(^{22}\) The pancreatic $\beta$-cell and neurone-expressed channels are hetero-octameric complexes comprising four regulatory sulphonylurea receptor-1 (SUR1, encoded by $ABCC8$) subunits surrounding four pore-forming inwardly rectifying potassium channel subunits (Kir6.2, encoded by $KCNJ11$).\(^{23}\) $K_{\text{ATP}}$ channels possess an exquisite sensitivity to small changes in cellular metabolism, via a dual regulation by adenine nucleotides and ATP/ADP ratio within the cell.\(^{2,22}\) They are closed by binding of ATP to Kir6.2 and they are opened by interaction of ADP in presence of Mg\(^{2+}\) ions with SUR1, which antagonizes the ATP inhibitory effect on the channel. The $K_{\text{ATP}}$ channel activity is therefore determined by the balance between these inhibitory and stimulatory effects.\(^{22,23}\) The NDM-related mutations cause an increase in the $K_{\text{ATP}}$ channel open probability, by altering channel gating or reducing ATP-binding affinity on Kir6.2, and consequently lead to persistent channel overactivity, which enhances the whole-cell $K_{\text{ATP}}$ current and prevents $\beta$-cell membrane depolarization with severe impairment of insulin secretion\(^{2,22,23}\) (Fig. 1). $ABCC8$ mutations generally decrease ATP-sensitivity less than $KCNJ11$ mutations and may also act directly by enhancing Mg\(^{2+}\)-ATP activation of the $K_{\text{ATP}}$ channel.\(^{10,24}\) Patients with $K_{\text{ATP}}$ channel mutations generally have lower birth weights than average, due to in utero marked insulin secretory insufficiency, in keeping with the crucial role of insulin in foetal growth, especially during the last trimester of pregnancy.\(^{20,21,25}\)

The activating $K_{\text{ATP}}$ channel mutations result in isolated diabetes or, for the more deleterious ones, diabetes in association with a range of neurodevelopmental and neuromotor disabilities.\(^{2,10,11,25}\) A more detailed figure is shown in Fig. 1.
severe phenotype, termed DEND for “developmental delay, epilepsy and neonatal diabetes syndrome” is linked to some PNDM-\textit{KCNJ11} mutations (very rarely to \textit{ABCC8} mutations).\textsuperscript{26–28} An intermediate DEND syndrome (iDEND) with less severe symptoms, usually without seizures, has been described in several cases with \textit{KCNJ11} mutations.\textsuperscript{28} Other discrete neurological abnormalities, as minor dystonia and dyspraxic features, were also reported in NDM patients with various \textit{ABCC8} mutations.\textsuperscript{21,28} The clinical variability potentially reflects the magnitude of the changes in \textit{K_{ATP}} current (the larger the \textit{K_{ATP}} current, the more severe the disease phenotype) and also the differential expression of \textit{ABCC8}/\textit{SUR1} in endocrine cells and several populations of neurons and \textit{KCNJ11}/\textit{Kir6.2} in both neuroendocrine and skeletal muscle cells.\textsuperscript{2,20,25} For example, the most prevalent p.R201H/\textit{Kir6.2} mutation produces diabetes only, whereas the more severe p.V59M/\textit{Kir6.2} mutation causes DEND.\textsuperscript{2,25} In the latter case, the reduced motor function originates in the central nervous system rather than in muscle or peripheral nerves.\textsuperscript{29}

The most striking clinical implication of molecular diagnosis of a \textit{K_{ATP}} channel mutation is the radical change from insulin injections to an oral sulfonylurea (SU) drug to treat diabetes.\textsuperscript{10,11,30} SU drugs (e.g. glibenclamide, glipizide or gliclazide) specifically bind the β-cell expressed high-affinity \textit{SUR1} receptor and close the \textit{K_{ATP}} channels, and directly stimulate insulin secretion.\textsuperscript{2,22} Most of the patients carrying a \textit{Kir6.2} or \textit{SUR1} mutation retain sensitivity to SU,\textsuperscript{10,11,30} but the effectiveness of oral SU treatment is determined by the nature of the activating \textit{K_{ATP}} channel mutations.\textsuperscript{24} The largest European study of switching from insulin to oral SU reported in 2006 a number of 49 patients from 40 families (ages: 3 months–36 years), diagnosed with \textit{KCNJ11}-PNDM, of whom 44 (90%) were able to stop insulin injections.\textsuperscript{30} The median initial dose of glibenclamide was 0.45 mg/kg/day (range: 0.05–1.5 mg/kg/day), which is much higher than the doses commonly used for type 2 diabetes (T2D) treatment (i.e. 0.25 mg/kg/day). Glycemic control improved in 38 tested patients with a mean glycated haemoglobin falling from 8.1% before SU treatment to 6.4% after 12 weeks cessation of insulin (with no increase of hypoglycaemia events).\textsuperscript{30} From the French NDM study group, in 20 PNDM patients who entered a therapeutic change trial, the successful switching rate is >95% with a follow-up time of >5 years (Pr M. Polak, personal communication). A significant improvement in the neurological dysfunctions of patients with iDEND (including \textit{KCNJ11} mutations p.V59M, p.G53D and p.H46L) following SU therapy was reported.\textsuperscript{28,31} Interestingly, glibenclamide therapy in 11 PNDM patients with \textit{KCNJ11} mutations (including five iDEND/DEND cases with the p.V59M mutation) retained its efficacy after a median of 68 months (mean HbA1c: 6 ± 0.39%) with a progressive reduction of SU dose in all cases.\textsuperscript{32}

Furthermore, NDM patients treated with insulin for ≥15 years before initiating SU therapy showed an optimum metabolic control, meaning that no β-cell desensitisation nor β-cell mass deterioration has occurred.\textsuperscript{11,32,33} Thus, patients for whom a \textit{K_{ATP}} channel mutation is identified in childhood or young adulthood may also benefit from a therapeutic switch.\textsuperscript{11,33–35}

Overall, these findings represent a remarkable example of successful genomic medicine, in that knowledge of the mutation can definitively improve both patient care and the patient’s long-term condition.\textsuperscript{28} The issue of cost-effectiveness of a genetic testing policy for a disease like PNDM where there is an undoubted improved quality of life for patients having a \textit{K_{ATP}} channel mutation was recently evaluated by Greeley et al.\textsuperscript{36} This paradigm case study of the potential societal and economic benefits illustrates the cost-savings of applying the concept of personalized medicine in this and likely other diseases in the future, as far as the mutation prevalence would remained >3% for any gene defect tested.\textsuperscript{36}

\textit{Impaired insulin biosynthesis as a cause of permanent early-infancy diabetes}

Heterozygous missense mutations in the preproinsulin (\textit{INS}) gene are responsible for permanent NDM/MDI diagnosed before or after the age of 6 months.\textsuperscript{37–39} Further studies extended the phenotype spectrum with rare cases of MODY (“Maturity-onset diabetes of the young”) and autoantibody-negative type 1B diabetes, and showed a marked variability in age at diagnosis (early-childhood onset as late as 2–6 years of age or even later), in clinical presentation (ketoacidosis or not) and treatment (insulin dependence or not) depending on the mutations.\textsuperscript{37,38,40–42}

Most of the \textit{INS} mutations affect amino acid residues that are crucial for preproinsulin processing or insulin precursor folding, such as p.R55C/p.R89C mutations involving sites of proteolytic processing or p.C96Y that also causes diabetes in the Akita mouse model.\textsuperscript{39} Three mutations reported in humans (p.C95Y[or A6], p.C96Y[or A7], p.C43G[or B19])\textsuperscript{39} affect the formation of disulfide bonds linking the
B-chain and A-chain as well as the intra-A-chain disulfide bond, which lead to a misfolded proinsulin molecule that is retained in the endoplasmic reticulum (ER), thus resulting in abnormal unfolded-protein responses (UPR) and β-cell death.\textsuperscript{43,44} Electron microscopy studies of β-cells from diabetic Akita mice reveal severe organelle dysfunction with enlarged ER, swollen mitochondria, prominent lysosomes and a reduced number of secretory granules.\textsuperscript{45} These defects were also observed by overexpressing human INS mutants in β-cell lines, which is consistent with a block in the progression of mutant proinsulin (and possibly some normal proinsulin) from the ER to the Golgi apparatus accompanied by ER stress [Meur2010,Rajan2010]. The degree of ER engorgement and nature of the UPR, subsequently causing defective insulin trafficking to secretory granules and β-cell apoptosis, can widely differ between mutants, which likely explains the variability in β-cell dysfunction and in clinical presentation.\textsuperscript{41,44} This complex proteotoxic mechanism may also explain the time required for diabetes development, its severity and evolution, which can widely differ even among carriers of a same mutation.\textsuperscript{39,41}

Beside, and more rarely, recessively inherited INS mutations were identified in NDM patients from consanguineous families and explain diabetes through several mechanisms impairing insulin gene expression (decreased insulin biosynthesis via gene deletion, lack of translational initiation, altered mRNA stability and abnormal INS transcription).\textsuperscript{13,46} Homozygous deletions of the C1/E1 cis-regulatory elements of the INS promoter and single base-pair substitutions in a conserved CC box highlight the essential role of these sequence elements in the regulation of insulin biosynthesis. We demonstrated that the c.-331C>G mutation located in the proximal INS promoter affects a novel binding site for Krüppel-Like Factors (KLF) encoded by a family of genes known to be involved in obesity and T2D.\textsuperscript{47,48}

Among the 16 known KLF proteins, KLF11 is the actual activator of this site and it fails to bind to the mutated site, consequently impairing INS transcription in a p300-dependent manner.\textsuperscript{46} Importantly, mice deleted for Klf11 recapitulate the disruption in insulin production and the phenotype observed in the patients.\textsuperscript{46} Although rare, these mutations reveal a distinct pathogenic mechanism underlying NDM compared to the dominant missense mutations. Considering the different types of INS mutations, until now, the therapeutic implications are minimal apart from familial genetic counselling.

\textit{Perspectives for the discovery of new genes and for genetic testing in NDM}

In many cases with NDM/MDI, particularly when diabetes is associated with pancreatic developmental anomalies or with other extra-pancreatic defects, the cause of pancreatic dysfunction is still unknown. As the list of NDM/MDI genes is increasingly expanding thanks to the latest advances in genomic research, we and other groups are currently developing genome-wide next-generation sequencing and bioinformatics approaches to further improve an efficient and cost-saving molecular diagnosis.\textsuperscript{49} In this regard, whole-exome sequencing (WES) based on wide-genome exons capture (as proposed by the Agilent or Illumina protocols) and new targeted resequencing methods (such as the RainDance platform) combined with next-generation parallel sequencing allow to sequence hundreds or thousands of genes of interest for each patient tested in a same experiment. These new protocols are applicable to isolated cases with well-documented phenotypic features (like NDM) or family samples sharing a particular phenotype. In the meantime, appropriate and accurate analysis pipelines for ascribing the causality of any novel rare mutations to the disease have to be implemented.\textsuperscript{49} These new genetic technologies will also impact on other ethical concerns, and demand new evolving policies pertinent to genetic research and data integration into clinical care.

\textit{Lessons from the MODY subtypes of diabetes}

\textit{Clinical presentation and genetic heterogeneity of MODY}

A mild form of diabetes with a dominant mode of inheritance was first reported in three families by Tattersall in 1974, then a further clinical description was made and the term “MODY” for Maturity-Onset Diabetes of the Young was first used in 1975.\textsuperscript{50} MODY is defined as a dominantly inherited young-onset non-autoimmune diabetes that occurs in childhood, adolescence or young adulthood due to a primary defect in pancreatic β-cell function.\textsuperscript{50-52} However, a residual insulin secretion may be still maintained for some years after diagnosis and exogeneous insulin is generally not required at the time of diagnosis.
Depending on the genetic aetiology and other non genetic factors, age at diagnosis may widely differ either within the first two decades of life or later in young adulthood.\textsuperscript{51,52} An anticipation or progressive reduction in age at diagnosis in succeeding generations was observed in many reported MODY pedigrees (probably because of enhanced awareness of diabetes leading to earlier testing). The MODY phenotype is rarely associated with obesity which is not required for its development. MODY may account for 1–2% of all T2D cases, and a minimum prevalence of MODY has been estimated in the UK population to \( \sim 100 \) cases per million.\textsuperscript{53}

Heterozygous mutations or partial/whole gene deletions in seven susceptibility genes so far explain the clinical heterogeneity of the MODY subtypes (Table 1). The MODY genes encode the enzyme glucokinase (GCK),\textsuperscript{34} the transcription factors HNF-1\( \alpha \) (HNF1A), -1\( \beta \) (HNF1B), -4\( \alpha \) (HNF4A), PDX1 and NEUROD1,\textsuperscript{51,52} or the preproinsulin (INS),\textsuperscript{41} each having a crucial role in the development and/or function of the pancreatic \( \beta \)-cells (Fig. 1). A sub-classification of the MODY subtypes according to the gene involved was then proposed,\textsuperscript{50,52} as described in Table 1. Mutations in GCK, HNF1A and HNF4A are the most common causes of MODY although the mutation prevalence may vary across populations.

Since the first reports of GCK-related MODY subtype,\textsuperscript{50,54} more than 600 GCK mutations distributed throughout the gene have been reported worldwide in more than 1400 families.\textsuperscript{55} A growing number of heterozygous activating GCK mutations causing hypoglycaemia have also been reported.\textsuperscript{55} In rare circumstances when both parents carry a GCK mutation (more likely in consanguineous couples), there is a 25% chance for a child having a homozygous GCK mutation causing PNDM.\textsuperscript{16} Heterozygous loss-of-function GCK mutations result in alterations of both glucose-stimulated insulin secretion and hepatic glycogen synthesis, which cause mild lasting hyperglycaemia (5.5–8.0 mmol/l) with little deterioration with age (<50% of affected individuals having overt diabetes).\textsuperscript{56–58} The kinetic properties and relative enzymatic activity of the mutant GCK proteins are impaired, entailing a decreased glycolytic flux in pancreatic \( \beta \)-cells. These defects translate in vivo as a glucose-sensing defect leading to an increase in the blood glucose threshold that triggers insulin secretion.\textsuperscript{56,57} Decreased storage of hepatic glycogen and increased hepatic gluconeogenesis following standard meals contribute to the post-prandial hyperglycaemia in GCK-deficient subjects.\textsuperscript{58} Another effect of MODY-GCK mutations is a reduced birth weight, by affecting insulin-mediated foetal growth, whereas maternal GCK mutations indirectly increase the birth weight by increasing foetal insulin secretion, as a consequence of maternal hyperglycaemia during foetal life.\textsuperscript{59}

More than 200 different HNF1A mutations located in the promoter and coding regions have been described in \( \sim 370 \) families of various ethnic origins, whose the most common, p.P291fsinsC, was reported in 65 families.\textsuperscript{60} HNF4A mutations are rarer with at least 31 mutations reported in 40 families.\textsuperscript{60} HNF1A/HNF4A mutations are associated with diabetes onset in early adulthood and cause a progressive and severe deterioration in glucose tolerance requiring hypoglycemic drugs or even insulin therapy at a young age. This shared phenotype is consistent with the interdependence between HNF-1\( \alpha \) and HNF-4\( \alpha \) forming part of a regulatory network in the pancreatic \( \beta \)-cell.\textsuperscript{61} A key, unanticipated role of HNF-4\( \alpha \) was shown in determining foetal birth weight and hyperinsulinemia in utero and at birth evolving to decreased insulin secretion and diabetes later in life.\textsuperscript{62} In a study of 15 pedigrees with 12 different HNF4A mutations, a significant increase in median birth weight (790 g) was observed with a 56% prevalence of macrosomia in mutation carriers versus 13% in non carriers. Transient neonatal hypoglycaemia was reported in 8 of 54 neonates, and three of them had hyperinsulinemia.\textsuperscript{62} Thus, HNF4A mutations can be viewed as a cause of neonatal hypoglycaemia. Macrosomia and neonatal hypoglycaemia have not been associated with HNF1A mutations,\textsuperscript{62} raising the question as to which genes are regulated by HNF-4\( \alpha \) but not by HNF-1\( \alpha \) in early stages of \( \beta \)-cell development.

Mutations in HNF1B/TCF2 were at first associated with MODY in a few families.\textsuperscript{51} In addition to have an important role in early pancreas development, HNF-1\( \beta \) function is also crucial in kidney development and nephron differentiation, and HNF1B mutations were shown to be a more common cause of renal cystic diseases and multiple renal malformations including hypoplastic glomerulocystic kidney disease (GCKD), oligomeganephronia, or atypical familial juvenile hyperuricemic nephropathy.\textsuperscript{63,64} This has been recognized as a discrete clinical syndrome, called RCAD for renal cysts and diabetes syndrome.\textsuperscript{63} Other extra-renal phenotypes include uro-genital tract malformations (affecting the Mullerian tract), abnormal liver and biliary function, gout and hyperuricemia.\textsuperscript{63,65} More than 65 different mutations have been identified in 143 families, including point mutations, small deletions/insertions and large genomic rearrangements (most of them are private, arising as de novo in 50% of cases).\textsuperscript{65,66} Although HNF1B was
initially described as a MODY gene, patients usually present with renal disease or RCAD rather than with MODY.65 Pancreatic atrophy and exocrine dysfunction were also associated with a heterozygous HNF1B mutation in two NDM patients, who also presented a kidney disease (renal cysts or dysplasia), according to the crucial role of the transcription factor HNF-1b in early pancreas and renal development.67,68 Two other transcription factor genes, PDX1 and NEUROD1, have an important role in the development of the endocrine pancreas, although representing a rare cause of MODY. PDX1 is co-expressed with insulin in the developing β-cell and is required for maintaining the β-cell phenotype, yet not essential for pancreatic determination of the endoderm. A frame-shift mutation in the coding sequence of the PDX1 gene was found to co-segregate with MODY in a five-generation family presenting a consanguineous link.69 In heterozygous mutation carriers, the phenotypes range from impaired glucose tolerance to overt non insulin-dependent diabetes. One child homozygous for the mutation was born with pancreatic agenesis and suffers from diabetes as well as exocrine insufficiency.69 The basic helix-loop-helix (bHLH) transcription factor NEUROD1 (or BETA2) with other factors (like NEUROG3) specifies the pancreatic endocrine lineage. Heterozygous loss-of-function mutations in NEUROD1 were reported in a few MODY families,51 and very rare homozygous mutations associate with PNDM, cerebellar hypoplasia, learning difficulties, visual and hearing impairment.70 This syndrome highlights the critical role of NEUROD1 in the development of both endocrine pancreas and central nervous system.

Translation into clinical practice and pharmacogenetics

A molecular genetic diagnosis in patients suspected of MODY is important because it confirms a diagnosis of MODY, classifies the subtype, predicts the likely clinical course, defines risk for relatives and may change the patient’s treatment. Best practice guidelines for both diagnostic and predictive genetic tests have been established to guide testing and reporting of results52,71 (online practical information on genetic testing for monogenic diabetes can be found at http://www.diabetesgene.org, from the Peninsula Molecular Genetics Laboratory Exeter, UK).

The many genetic, clinical and metabolic studies carried out in patients with the more common MODY subtypes have shed light on their specific clinical features. The hyperglycemia associated with GCK mutations is often mild, despite the multiple defects in the pancreas and the liver, and is usually responsive to diet (without hypoglycaemic agents).72 However, the development of insulin resistance with age may impact on the long-term deterioration of glucose tolerance in the GCK-MODY patients.73 In these patients, there is a lower prevalence of microvascular complications (retinopathy and proteinuria) compared to other MODY subtypes and late-onset T2D.50,51,72

In contrast, the diabetic phenotypes of HNF1A- and HNF4A-MODY patients are more severe from diagnosis with a possible deterioration throughout life (or with superimposed environmental factors).31,72 Interestingly, patients with HNF1A mutations show a particular sensitivity to the hypoglycaemic effects of sulfonylureas, which directly bind the SUR1 regulatory subunit of the pancreatic KATP channel.74 According to animal and cellular models of HNF-1α deficiency, the pancreatic β-cell defect is indeed upstream of the KATP channel. The sensitivity to sulfonylureas means that patients who have been misdiagnosed as type 1 diabetic and treated with long-term insulin from diagnosis can successfully switch to low-dose oral sulfonylureas with an improved glycemic control in most cases.75

A drug discovery process aimed at increasing the activity of GCK has led to the production of several compounds that activate the enzyme, so-called GK-activators (GKAs), with a net effect by decreasing fasting plasma glucose and improving glucose tolerance.76 Since the discovery of the first orally active GKA (R00281675), several research groups have reported the identification of novel potent GKAs.76 This is an excellent example of translational research from a complex system such as the regulation of GCK towards the discovery of a new class of therapeutic agents which will be useful in the treatment of common T2D.

Usefulness of predictive biomarkers for MODY

As an accurate molecular diagnosis of diabetes subtypes confers many clinical benefits, serum biomarkers could greatly help to prioritise patients for genetic investigation. In this regard, the transcription factors HNF-1α and HNF-4α regulate the expression of genes encoding serum proteins (apolipoproteins and others). Although the levels of apolipoproteins A-II and C-III, lipoprotein (a) and...
triglycerides are significantly decreased in HNF4A mutation carriers, a substantial overlap between patients groups may limit their usefulness as biomarkers.

More recently, genome-wide association studies (GWAS) have revealed that common variants mapping near the HNF1A gene on chromosome 12q24 are associated with alterations in serum C-reactive protein (CRP) levels in healthy adults. The presence of HNF-1x binding sites in the CRP promoter suggests that the effect on CRP is mediated through altered regulation of HNF1A expression. Serum high-sensitivity CRP (hsCRP) levels were found to be significantly lower in HNF1A-MODY than in other forms of diabetes. This finding was confirmed in a larger dataset of MODY participants and T2D individuals recruited from across seven European centres, with a ROC-derived C-statistic for distinguishing HNF1A-MODY patients from young adult-onset T2D ranging from 0.79 to 0.97. In this study, lower hsCRP levels were not observed in HNF4A-MODY patients. Along with increased birth weight for HNF4A-MODY, hsCRP could be a useful biomarker to guide molecular genetic testing in patients with features of transcription factor-related MODY.

Blood C-peptide may also aid in the diagnosis of MODY, but practical reasons limit its widespread use. Urinary C-peptide creatinine ratio (UCPCR) is a stable measure of endogenous insulin secretion, that was found to be lower in type 1 diabetes than HNF1A/4A MODY (area-under-curve [AUC]: 0.98 with a cut-off UCPCR $\geq 0.2$ nmol/mmol for differentiating HNF1A/4A MODY from type 1 diabetes). Therefore, UCPCR may be another non invasive tool for discriminating HNF1A/4A MODY from type 1 diabetes and for prioritizing gene screening in the patients.

**Similar genetic defects may cause different phenotypes: a continuum of same or various disease(s)?**

The genetic dissection of many subtypes of monogenic early-onset diabetes along with the identification of major genes involved in the pathophysiology of insulin secretion provided evidence that various mutations affecting a same gene may cause a wide spectrum of clinical phenotypes ranging from NDM to inherited diabetes with a lower penetrance appearing in childhood or adulthood. There is also rising evidence that common polymorphisms in a number of genes implicated in monogenic diabetes can modestly increase the risk of common adult T2D, as outlined below (Table 1).

Indeed, several studies reported an association between adult-onset diabetes and $K_{\text{ATP}}$ channel mutations (especially in ABCC8/SUR1), even in the absence of a history of diabetes or hyperinsulinism in the neonatal period. The first demonstrations came from the study of several extended families, in which an ABCC8 mutation responsible for NDM (or neonatal hypoglycaemia) was inherited from a diabetic parent diagnosed later in life. By screening an adult cohort of T2D patients diagnosed before the age of 35–40 years, we found four ABCC8 missense mutations in four sporadic T2D patients, one of them having been treated with glibenclamide for several years. We also further evaluated the adult-onset diabetes phenotype associated with ABCC8 mutations, showing an impaired insulin secretion capacity that was restored after a 4-week SU trial in two patients. Although of rare occurrence, recognition of adult-onset ABCC8-associated diabetes has significant clinical implications, since a good metabolic control may be achieved with sulfonylurea treatment alone. A few KCNJ11/Kir6.2 mutations responsible of TNDM were also found in relatives with later-onset diabetes, showing variable clinical phenotypes at diagnosis.

A subgroup of INS missense mutations (p.R6H, p.R6C, p.L30M, p.R46Q and p.R55C) was also found exclusively in patients diagnosed with diabetes outside early-infancy and childhood. These patients may fulfil classical MODY criteria as they are non-obese, generally diagnosed before age 25, with a family history of diabetes consistent with autosomal dominant inheritance, and diabetes is non-ketotic. They are treated with diet, oral hypoglycemic agents or insulin, and most of them have residual $\beta$-cell function as evidenced by detectable C-peptide levels. The extent of $\beta$-cell failure could also be progressive, as some patients had declining C-peptide levels over time. It is to note that in the early 1980s, familial hyperproinsulinaemia with normal to mildly impaired glucose tolerance in adults was reported to be caused by INS mutations at residues R89P/H and H34D.

Several factors may explain this marked clinical variability including the type and location of the mutation itself (or even seen between carriers of a same mutation), the behaviour of the mutated protein, as well as other likely modifier genetic or epigenetic events, and superimposed environmental factors.
Implications of monogenic diabetes genes in adult polygenic type 2 diabetes

Taken together the research efforts in identifying rare, highly penetrant mutations responsible for monogenic forms of diabetes and the more recent findings from GWAS for common T2D, there is strong evidence that frequent polymorphisms in or near some of the genes implicated in monogenic diabetes may also be involved in susceptibility to polygenic/multifactorial forms of adult T2D. Indeed, more subtle genetic changes affecting the protein structure or gene expression might play a role in determining susceptibility to late-onset T2D. Although these genetic variants contribute very modestly to the risk of T2D in adulthood, remarkable examples of loci implicated in monogenic and multifactorial forms of diabetes were shown to reach genome-wide significance.

Common single nucleotide polymorphisms (SNPs) in and upstream of the HNF1A gene influence transcriptional activity and insulin secretion in vivo. A non-coding HNF1A variant was significantly associated with the risk of T2D (odds ratio [OR] of 1.05–1.14) from the large-scale DIAGRAM meta-analysis. Moreover, a population-specific variant (p.G319S) was shown to influence T2D risk in the Oji-Cree native Canadian population, that is characterized by a very high risk for developing T2D. The p.G319S HNF1A mutation was found in ~40% of diabetic patients and accelerates the onset of T2D by seven years. Common variants in HNF4A may also be associated with T2D risk, at least in some populations.

Several frequent non-coding SNPs (with minor allele frequency [MAF] >5%) in HNF1B were found to contribute to T2D risk with yet modest effects (allelic OR <1.25), through possibly different mechanisms than the molecular defects involved in HNF1B-MODY patients. Independently, a GWAS of prostate cancer risk demonstrated that two intronic HNF1B variants confer protection against T2D (OR of 0.91) in individuals of European, African and Asian descent, although these effects might be independent. An inverse relationship between T2D and the risk of prostate cancer was also reported, and a meta-analysis estimated the relative risk of prostate cancer to be 0.84 among diabetic patients.

A common polymorphism in KCNJ11 (encoding Kir6.2), p.E23K, was shown to be associated with an increased risk of developing T2D in European populations. Large-scale association studies and meta-analyses in several populations of this coding variant have further replicated a modest size effect of the K23 allele on T2D risk (OR of ~1.2). Because approximately 60% of the population carry at least one K23 allele, it is likely to have a substantial effect on population-attributable risk. In European, West African and East Asian populations, p.E23K is in perfect linkage disequilibrium with a second non-synonymous variant (p.A1369S) within the adjacent gene ABCC8. Interestingly, a relationship between KCNJ11(E23K)/ABCC8(A1369S) genotypes and sulfonylurea response was reported: a greater decrease in fasting plasma glucose following gliclazide treatment was observed amongst at-risk haplotype carriers (that is genotypes KCNJ11-K23/ABCC8-S1369). This enhanced hypoglycaemic effect was explained by a 3.5-fold increased sensitivity of the K_ATP channel variant to inhibition by gliclazide, that is a sulfonylurea drug of the site A-binding class. This finding may provide a novel pharmacogenomic therapeutic approach for T2D patients who are homozygous for both KCNJ11/ABCC8 at-risk alleles.

Genetic variation in WFS1 not only results in a rare syndrome characterized by early-onset non-autoimmune diabetes with optic atrophy and deafness (namely the Wolfram syndrome) but is also associated with susceptibility to adult T2D. In a pooled case-control analysis comprising >20,000 individuals, several SNPs in WFS1 (including a non-synonymous SNP, p.R611H) were shown to modulate the diabetes risk (with ORs of ~0.92 for a MAF of ~40%, with a population-attributable fraction of 9%). Such findings suggest that WFS1, encoding wolframin that has an essential role in the endoplasmic reticulum stress response in insulin-producing pancreatic β-cells, contributes to the risk of common T2D.

The −30G/A polymorphism in the β-cell specific promoter of GCK (encoding glucokinase) was found to modulate diabetes risk (OR of 1.08 from a meta-analysis of association results for the A-allele with T2D) in mostly European origin populations. A significant impact on the modulation of fasting glycaemia and on a surrogate estimate of β-cell function (HOMA-B, derived from fasting glycaemia and fasting insulinemia by homeostasis model assessment) was also observed in a French prospective study. A constant effect of the GCK [-30]-A allele on fasting glucose was reported throughout the lifespan in several groups of normoglycemic subjects whose median age varied from 8 to 72 years, although insulin secretion is known to decrease with age in the general population.

In contrast, most of the novel T2D-associated genes (e.g. TCF7L2, HHEX or SLC30A8) with (very) modest effects of at-risk alleles, or genes strongly associated with fasting plasma glucose (like G6PC2, ABCC8, and K CNJ 11).
MTNR1B or ADCY5)\textsuperscript{107,108} (Fig. 2), that have arisen from the latest GWAS meta-analyses, do not contribute to monogenic forms of early-infancy or young-onset diabetes.\textsuperscript{81,90}

Concluding remarks

The long-standing exploration of various subtypes of monogenic diabetes and their genetic dissection have greatly improved our understanding of the β-cell physiology and regulation of insulin secretion in humans, with outstanding therapeutic and pharmacogenetics consequences both in infants and in adults. Early-onset monogenic diabetes still represents an invaluable model to identify new targets and mechanisms of β-cell dysfunction. With the advances and decreasing cost of the latest genetic and genomic technologies (like WES and whole-genome sequencing), this area of genomic research is likely to provide novel genetic aetiologies of primary pancreatic defects in the next future, which will translate into novel options of genetic diagnosis, diabetes treatment and patients care management.

Summary

Advances in molecular genetics helped in the identification of the genetic aetiologies of many clinically defined subgroups of monogenic diabetes ranging from NDM, either isolated diabetes or associated with syndromic features, to early-infancy diabetes and familial forms of young-onset diabetes. Knowledge of the genetic causes allowed to explain clinical heterogeneity, to depict various causal molecular mechanisms and to highlight key targets of the pancreatic β-cell physiology. The concept of genomic or personalized medicine in young patients knowing their mutation has emerged,
as it was demonstrated in the subgroup of patients carrying $K_{\text{ATP}}$ channel mutations, having a great impact on diabetes care and quality of life. Molecular genetics is now routinely used to define the diagnosis and treatment of children presenting with non auto-immune diabetes. Consequently, genetic counselling and information on risk prediction can be offered to the patients and their families. Currently used next-generation sequencing approaches should inform us on novel genetic aetiologies of pancreatic $\beta$-cell dysfunction and early-onset diabetes, with new insights on diagnosis, prevention and therapeutic policies. The expanding field of genomic research aiming to elucidate atypical forms of diabetes will likely yield a broader translational research that will foster biomedical discoveries for the polygenic forms of diabetes.

**Practice points**

A genetic test for monogenic diabetes genes should be considered in the following conditions:

1/ in any patient diagnosed with diabetes before 6 months of age:
- if isolated diabetes $\pm$ neurodevelopmental disabilities: i/ $\text{KCNJ11}$ and $\text{INS}$, ii/ $\text{ABCC8}$, if negative iii/ $\text{GCK}$ in case of family history of diabetes;
- if TNDM or in the neonatal period before a possible remission (in case of very low birth weight): testing for 6q24 anomalies;
- if syndromic diabetes and/or suspected consanguinity: the genes that should be tested depend on the clinical features, as described in Table 1.

2/ in any patient diagnosed with diabetes between 6 and 12 months or older, and autoantibody negative: i/ $\text{INS}$ and $\text{KCNJ11}$, ii/ $\text{ABCC8}$, then iii/ other likely causes in the context of a family history.

3/ in case of a MODY phenotype (autosomal dominant family history of diabetes, young ages at diagnosis, antibody-negative): $\text{GCK}$ (especially if there is a consistent mild hyperglycaemia), $\text{HNF1A}$, $\text{HNF4A}$, $\text{HNF1B}$ (especially in presence of renal cysts/developmental anomalies). Direct sequencing and analysis for deletions have to be used. If negative, $\text{INS}$, $\text{ABCC8}$ and $\text{KCNJ11}$ (especially in case of NDM in the family); more rarely $\text{IPF1}$ or $\text{NEUROD1}$.

Usefulness of genetic counselling in monogenic early-onset diabetes:

- A genetic counselling can be provided to a family (parents) when a specific mutation in a known gene is identified with a clear link to the disease; this is more important when the disease develops earlier and/or with a severe phenotype (e.g. PNDM either isolated diabetes or associated with neurological and developmental abnormalities, and also in MODY).
- The risk of disease recurrence in NDM/MDI families differs according to the subtype of disease and molecular aetiology (e.g. de novo or transmitted mutation in an NDM case).

Examples of personalized medicine practice in monogenic diabetes:

- The $\text{GCK}$-MODY subtype is usually responsive to diet and leads to few complications.
- The $\text{HNF1A}$-MODY patients are generally very sensitive to the hypoglycemic effect of sulfonylureas (SU), because the $\beta$-cell defects are in glucose metabolism and can be bypassed by SU which act on the $K_{\text{ATP}}$ channel to stimulate insulin release. Very low doses (e.g: 20–40 mg gliclazide) are recommended as the first-line pharmacological treatment in $\text{HNF1A}$-MODY patients. The patients on other oral agents or insulin may have a trial with SU.
- PNDM patients with a $K_{\text{ATP}}$ channel mutation (either in $\text{KCNJ11}$ or in $\text{ABCC8}$) can be transferred from insulin injections to oral SU therapy, as they will experience long-lasting improved glycemic control without increases in hypoglycaemia. Even in the most severe DEND phenotype, some degree of improvement in neurological function and developmental delay was achieved on SU treatment.
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References


Research agenda

- The many subtypes of monogenic diabetes can still offer an opportunity to elucidate key targets of β-cell function and mechanisms of its dysfunction.
- ~30% of young-onset dominant MODY cases (those diagnosed before age 25) have an unexplained genetic aetiology. Current research using state-of-art next-generation sequencing (e.g. whole-exome sequencing) should provide novel aetiologies and novel mechanisms of β-cell functional defects.
- State-of-art next-generation sequencing approaches should be applied to the molecular genetic diagnosis of monogenic diabetes (NDM/MDI, MODY), so as to expand the analyses to further genes.
- Further molecular and clinical studies are required to better understand the pathological mechanisms underlying the progression of diabetes from a young age to adulthood, especially in transient forms of neonatal diabetes with still unexplained remission and later relapse of diabetes.


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