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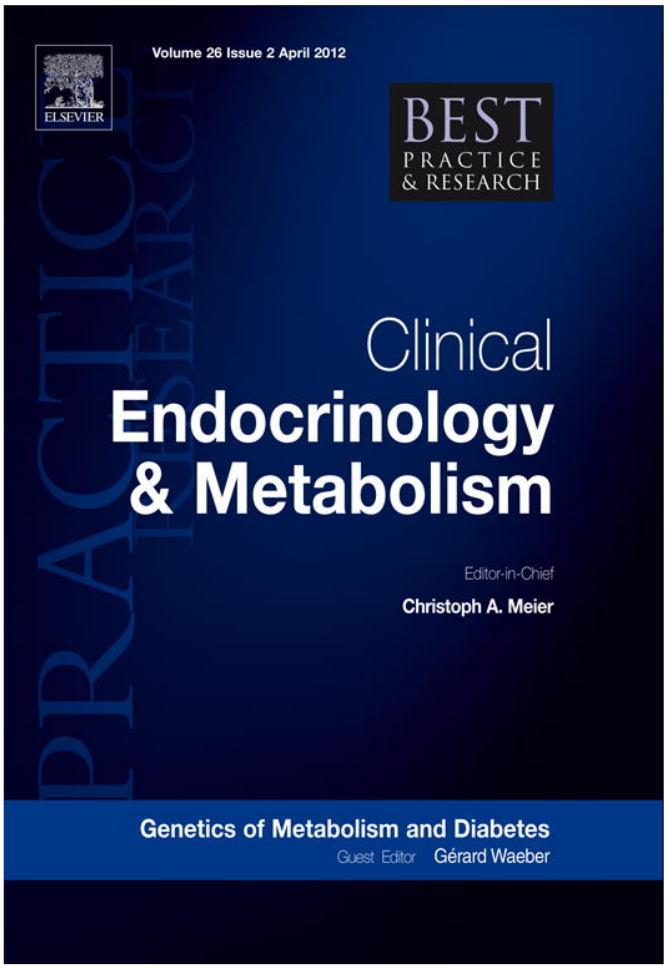

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The lessons of early-onset monogenic diabetes for the understanding of diabetes pathogenesis

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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 β-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 β-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 ~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.[1,6] 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 β-cell expressed ATP-sensitive potassium (K_{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 β-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 >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 ~2/3 of patients.[1,6] 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 β-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.[1,17] 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/ paternally 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.[6,18] In some patients, diabetes may be the initial

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Table 1
Subtypes of monogenic diabetes: Neonatal diabetes (NDM/MDI), with or without syndromic features, and MODY.

Gene name and locus	Protein/Function	Inheritance	Phenotypes/syndromes	OMIM	Impact in adult T2D (ref.)
NDM/MDI					
PLAGL1 6q24.2	Pleiomorphic adenoma gene-like 1, zinc-finger protein or ZAC tumour suppressor	UPD6 (*de novo*), paternal duplication (may be inherited), maternal methylation defect	TNDM; Chromosome 6q structural anomalies with imprinting mechanisms (methylation defects)	601410 603044	
ZFP57 6p22.1	Zinc-finger protein 57, role in maintenance of imprinted DNA methylation	Autosomal recessive	TNDM with macroglossia, developmental delay, umbilical defect, visual impairment, CHD	601410 612192	
KCNJ11 11p15.1	Kir6.2 Inward rectifier K+ channel pore-forming subunit	Spontaneous (80%) or autosomal dominant	PNDM (more often), TNDM (less often), DEND (rarely)	606176 600937	+ (97–99, 102)
ABCC8 11p15.1	Sulfonylurea receptor-1 (SUR1) subunit of K_{ATP} channel	Spontaneous (80%) or autosomal dominant	TNDM (more often), PNDM (less often), DEND (very rare)	606176 600509	+ (35,82, 99,101)
INS 11p15.5	Preproinsulin, insulin Hypoglycaemic hormone, effect on anabolism	Spontaneous (80%), autosomal dominant or recessive (rarely)	PNDM and early-infancy diabetes; Heterozygous mutations in *INS* coding regions, homozygous mutations in *INS* regulatory regions	606176 176730	
GCK 7p13	Glucokinase Glycolytic enzyme	Autosomal recessive	PNDM (homozygous or compound heterozygous mutations)	606176 138079	**+** (93,105, 106)
NDM/MDI associated with developmental anomalies and/or extra-pancreatic features					
PDX1 13q12	Pancreatic duodenal homeobox protein 1 (PDX1)or IPF1 Transcription factor	Autosomal recessive	PNDM with pancreas agenesis/hypoplasia for homozygous mutations	260370 600733	
EIF2AK3 2p12	Eukaryotic translation initiation factor 2-α kinase 3, or pancreatic eIF2-α kinase (PERK)	Autosomal recessive	Wolcott Rallison syndrome (diabetes associated with epiphyseal dysplasia)	226980 604032	
PTF1A 10p12	Pancreas transcription factor 1, Subunit α	Autosomal recessive	PNDM with pancreatic and cerebellar hypoplasia	609069 607194	
GLIS3 9p24.2	GLI-similar family zinc-finger 3 transcription factor	Autosomal recessive	PNDM with congenital Hypothyroidism, glaucoma (NDH syndrome)	610199 610192	
FOXP3 Xp11.23	Forkhead box protein P3 (FoxP3) or Scurfin Transcription factor	X-linked recessive	X-linked IPEX syndrome (Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) with diffuse auto-immunity	304790 300292	
NEUROD1 2q31.3	NeuroD1 or Beta2 Transcription factor	Autosomal recessive	PNDM with cerebellar hypoplasia, sensorineural deafness, visual impairment and developmental delay	601724	
NEUROG3 10q22.1	NeuroG3 or NGN3 Transcription factor	Autosomal recessive	PNDM with severe diarrhoea, absence of intestinal enteroendocrine cells	610370 604882	

(*continued on next page*)

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Table 1 (*continued*)

Gene name and locus	Protein/Function	Inheritance	Phenotypes/syndromes	OMIM	Impact in adult T2D (ref.)
PAX6 11p13	Paired box 6 containing Transcription factor	Autosomal recessive	PNDM with brain malformations, microcephaly, microphtalmia, anhypopituitarism	165550 607108	
HNF1B 17q21	HNF-1β Transcription factor	Spontaneous or autosomal dominant	TNDM/PNDM with pancreatic atrophy and/or renal abnormalities (GCKD: renal cysts) or renal dysplasia	189907	+ (91,93,95)
RFX6 6q22.2	Regulatory factor X, 6 Transcription factor	Autosomal recessive	PNDM with pancreatic hypoplasia, intestinal atresia, gall bladder hypoplasia or aplasia, diarrhoea	601346 612659	
WFS1 4p16.1	Wolframin Membrane glycoprotein	Autosomal recessive	Wolfram syndrome (DIDMOAD) Diabetes insipidus, diabetes mellitus, optic atrophy, deafness	222300 606201	+ (103,104)
IER3IP1 18q21.1	Immediate early response 3 interacting protein 1	Autosomal recessive	PNDM with microcephaly, severe infantile epileptic encephalopathy	614231	
SLC19A2 1q24.2	Thiamine transporter 1 Solute carrier family 19 member	Autosomal recessive	TRMA syndrome (megaloblastic anaemia) Sensorineural deafness, occasional heart defect, NDM or later-onset diabetes	249270 603941	
SLC2A2 3q26.2	GLUT2 Facilitated glucose transporter	Autosomal recessive	Fanconi-Bickel syndrome, NDM or IGT or diabetes in infancy/ childhood	227810 138160	
MODY					
HNF4A 20q12	HNF-4α Transcription factor	Autosomal dominant	MODY1, in adolescence or early adulthood (and neonatal hyperinsulinism)	125850 600281	+ (51,93)
GCK 7p13	Glucokinase Glycolytic enzyme	Autosomal dominant	MODY2, mild hyperglycemia (onset in early-childhood, and lifelong) [frequent]	138079 125851	+ (93,105, 106)
HNF1A 12q24.2	HNF-1α Transcription factor	Autosomal dominant	MODY3, in adolescence or early adulthood [frequent]	600496 142410	+ (91–94)
PDX1 13q12.1	IPF1 Transcription factor	Autosomal dominant	MODY4, in early adulthood (similar to *HNF1A* but rare)	606392 600733	
HNF1B 17q21	HNF-1β Transcription factor	Autosomal dominant	MODY5, in early adulthood, renal cysts and diabetes (RCAD)	137920 189907	+ (91,93,95)
NEUROD1 2q31.3	NeuroD1 or Beta2 Transcription factor	Autosomal dominant	MODY6, in early adulthood (similar to *HNF1A* but rare)	606394 601724	
INS 11p15.5	Preproinsulin, insulin Hypoglycaemic hormone, effect on anabolism	Autosomal dominant	MODY7, in childhood and early adulthood	613370 176730	

The OMIM (Online Mendelian Inheritance in Man) numbers depict phenotype and/or gene MIM numbers. CHD, congenital heart defect; DEND, developmental delay, epilepsy, neonatal diabetes; DIDMOAD, diabetes insipidus, diabetes mellitus, optic atrophy, deafness; GCKD, glomerulocystic kidney disease; IGT, impaired glucose tolerance; K_{ATP}, 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.

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.

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Neonatal diabetes due to ATP-sensitive potassium channel mutations and therapeutic consequences

Activating mutations of the K_{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_{ATP} channels couple cellular glucose metabolism to membrane excitability by regulating potassium flux in many cell types.[22] The pancreatic β-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_{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_{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_{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_{ATP} current and prevents β-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_{ATP} channel.[10,24] Patients with K_{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_{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

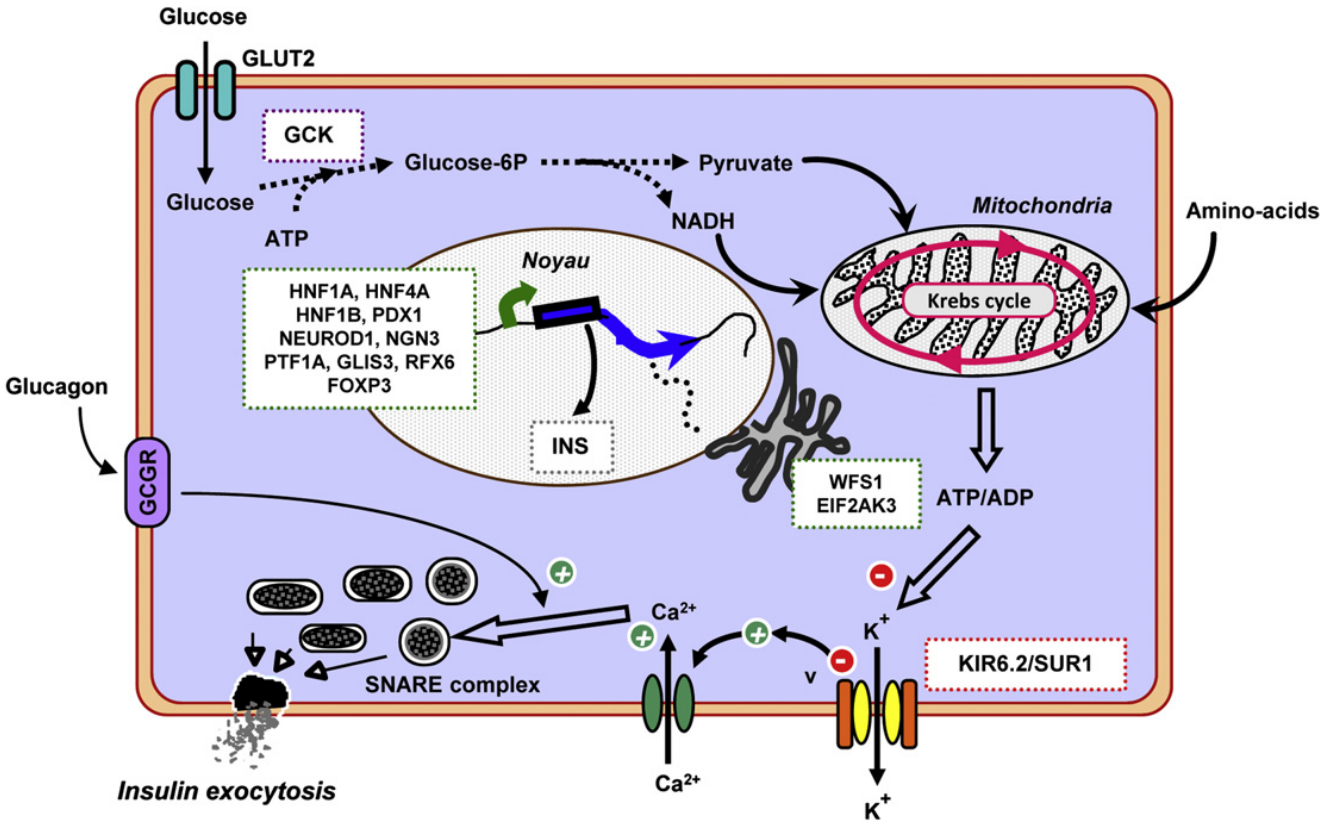

Fig. 1. Pancreatic β-cell genes known as targets of monogenic diabetes. Human genes (or gene products) identified as responsible for monogenic diabetes are indicated within a white square. EIF2AK3, eukaryotic translation initiation factor 2-alpha kinase 3; GCG-R, glucagon receptor; GCK, glucokinase; Glucose-6P, glucose-6 phosphate; GLUT2, glucose transporter 2; FOXP3, forkhead box protein P3; GLIS3, GLIS family zinc-finger 3; HNF, hepatocyte nuclear factor; NEUROD1, neurogenic differentiation factor 1; NGN3, neurogenin 3; PDX1, pancreas/duodenum homeobox protein 1; PTF1A, pancreas-specific transcription factor 1a; RFX6, regulatory factor X, 6; WFS1, wolframin transmembrane protein.

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severe phenotype, termed DEND for "developmental delay, epilepsy and neonatal diabetes syndrome" is linked to some PNDM-*KCNJ11* mutations (very rarely to *ABCC8* mutations).[26–28] An intermediate DEND syndrome (iDEND) with less severe symptomes, usually without seizures, has been described in several cases with *KCNJ11* mutations.[28] Other discrete neurological abnormalities, as minor dystonia and dyspraxic features, were also reported in NDM patients with various *ABCC8* mutations.[21,28] The clinical variability potentially reflects the magnitude of the changes in K_{ATP} current (the larger the K_{ATP} current, the more severe the disease phenotype) and also the differential expression of *ABCC8*/SUR1 in endocrine cells and several populations of neurons and *KCNJ11*/Kir6.2 in both neuroendocrine and skeletal muscle cells.[2,20,25] For example, the most prevalent p.R201H/Kir6.2 mutation produces diabetes only, whereas the more severe p.V59M/Kir6.2 mutation causes DEND.[2,25] In the latter case, the reduced motor function originates in the central nervous system rather than in muscle or peripheral nerves.[29]

The most striking clinical implication of molecular diagnosis of a K_{ATP} channel mutation is the radical change from insulin injections to an oral sulfonylurea (SU) drug to treat diabetes.[10,11,30] SU drugs (e.g. glibenclamide, glipizide or gliclazide) specifically bind the β-cell expressed high-affinity SUR1 receptor and close the K_{ATP} channels, and directly stimulate insulin secretion.[2,22] Most of the patients carrying a Kir6.2 or SUR1 mutation retain sensitivity to SU,[10,11,30] but the effectiveness of oral SU treatment is determined by the nature of the activating K_{ATP} channel mutations.[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 *KCNJ11*-PNDM, of whom 44 (90%) were able to stop insulin injections.[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).[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 *KCNJ11* mutations p.V59M, p.G53D and p.H46L) following SU therapy was reported.[28,31] Interestingly, glibenclamide therapy in 11 PNDM patients with *KCNJ11* mutations (including five iDEND/DEND cases with the p.V59M mutation) retained its efficacity after a median of 68 months (mean HbA1c: 6 ± 0.39%) with a progressive reduction of SU dose in all cases.[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.[11,32,33] Thus, patients for whom a K_{ATP} channel mutation is identified in childhood or young adulthood may also benefit from a therapeutic switch.[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.[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 K_{ATP} channel mutation was recently evaluated by Greeley et al.[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.[36]

Impaired insulin biosynthesis as a cause of permanent early-infancy diabetes

Heterozygous missense mutations in the preproinsulin (*INS*) gene are responsible for permanent NDM/MDI diagnosed before or after the age of 6 months.[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.[37,38,40–42]

Most of the *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.[39] Three mutations reported in humans (p.C95Y[or A6], p.C96Y[or A7], p.C43G[or B19])[39] affect the formation of disulfide bonds linking the

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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.[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.[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.[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.[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).[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.[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.[46] Importantly, mice deleted for *Klf11* recapitulate the disruption in insulin production and the phenotype observed in the patients.[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.

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.[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.[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.

Lessons from the MODY subtypes of diabetes

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.[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.[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.

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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.[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 ~100 cases per million.[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),[54] the transcription factors HNF-1α (*HNF1A*), -1β (*HNF1B*), -4α (*HNF4A*), *PDX1* and *NEUROD1*,[51,52] or the preproinsulin (*INS*),[41] each having a crucial role in the development and/or function of the pancreatic β-cells (Fig. 1). A sub-classification of the MODY subtypes according to the gene involved was then proposed,[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,[50,54] more than 600 *GCK* mutations distributed throughout the gene have been reported worldwide in more than 1400 families.[55] A growing number of heterozygous activating *GCK* mutations causing hypoglycaemia have also been reported.[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.[16] Heterozygous loss-of-function *GCK* mutations result in alterations of both glucose-stimulated insulin secretion and hepatic glycogen synthesis, which cause mild fasting hyperglycemia (5.5–8.0 mmol/l) with little deterioration with age (<50% of affected individuals having overt diabetes).[56–58] The kinetic properties and relative enzymatic activity of the mutant GCK proteins are impaired, entailing a decreased glycolytic flux in pancreatic β-cells. These defects translate *in vivo* as a glucose-sensing defect leading to an increase in the blood glucose threshold that triggers insulin secretion.[56,57] Decreased storage of hepatic glycogen and increased hepatic gluconeogenesis following standard meals contribute to the post-prandial hyperglycemia in GCK-deficient subjects.[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 hyperglycemia during foetal life.[59]

More than 200 different *HNF1A* mutations located in the promoter and coding regions have been described in ~370 families of various ethnic origins, whose the most common, p.P291fsinsC, was reported in 65 families.[60] *HNF4A* mutations are rarer with at least 31 mutations reported in 40 families.[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-1α and HNF-4α forming part of a regulatory network in the pancreatic β–cell.[61] A key, unanticipated role of HNF-4α was shown in determining foetal birth weight and hyperinsulinemia *in utero* and at birth evolving to decreased insulin secretion and diabetes later in life.[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.[62] Thus, *HNF4A* mutations can be viewed as a cause of neonatal hypoglycaemia. Macrosomia and neonatal hypoglycaemia have not been associated with *HNF1A* mutations,[62] raising the question as to which genes are regulated by HNF-4α but not by HNF-1α in early stages of β-cell development.

Mutations in *HNF1B*/TCF2 were at first associated with MODY in a few families.[51] In addition to have an important role in early pancreas development, HNF-1β 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.[63,64] This has been recognized as a discrete clinical syndrome, called RCAD for renal cysts and diabetes syndrome.[63] Other extra-renal phenotypes include uro-genital tract malformations (affecting the Mullerian tract), abnormal liver and biliary function, gout and hyperuricemicia.[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).[65,66] Although *HNF1B* was

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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-1β 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 results[52,71] (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 hygoglycaemic 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).[51,72] 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 K_{ATP} channel.[74] According to animal and cellular models of HNF-1α deficiency, the pancreatic β-cell defect is indeed upstream of the K_{ATP} 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 (RO0281675), 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

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triglycerides are significantly decreased in *HNF4A* mutation carriers,[50,52] 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.[77] The presence of HNF-1α 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.[78] 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.[79] 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).[80] 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).[81]

Indeed, several studies reported an association between adult-onset diabetes and K_{ATP} channel mutations (especially in *ABCC8*/SUR1), even in the absence of a history of diabetes or hyperinsulinism in the neonatal period.[10,82,83] 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.[10,84–86] 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.[82] 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.[35] 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.[87,88]

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.[39,41,42] 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.[41,42] They are treated with diet, oral hypoglycemic agents or insulin, and most of them have residual β-cell function as evidenced by detectable C-peptide levels. The extent of β-cell failure could also be progressive, as some patients had declining C-peptide levels over time.[39] 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.[89]

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[35,41]), the behaviour of the mutated protein, as well as other likely modifier genetic or epigenetic events, and superimposed environmental factors.

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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.[81,90] 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.[91]

Common single nucleotide polymorphisms (SNPs) in and upstream of the *HNF1A* gene influence transcriptional activity and insulin secretion *in vivo*.[92,93] 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.[91] 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.[94] The p.G319S *HNF1A* mutation was found in ~40% of diabetic patients and accelerates the onset of T2D by seven years.[94] Common variants in *HNF4A* may also be associated with T2D risk, at least in some populations.[91]

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.[91,93] 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,[95] 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.[96]

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.[97,98] 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).[98,99] 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*.[100] 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).[101] 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.[102] 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.[103] 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%.[103,104] 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.[93] 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 insulinaemia by homeostasis model assessment) was also observed in a French prospective study.[105] 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[106]; 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*,

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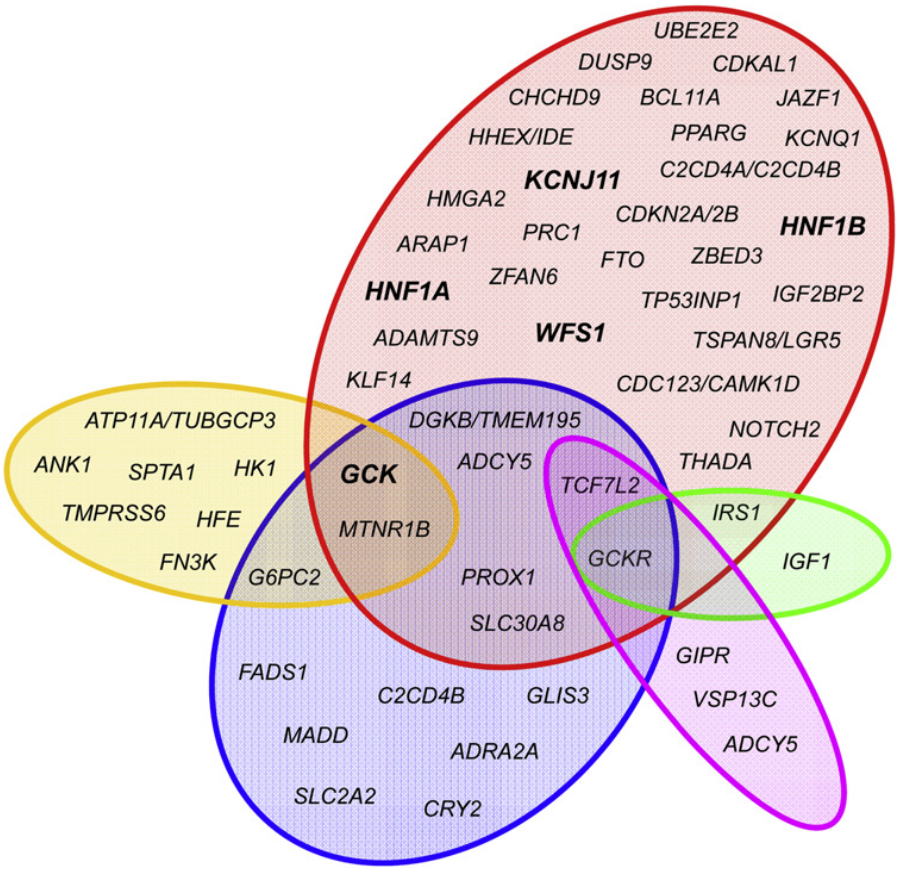

Fig. 2. Loci associated with T2D risk and related quantitative traits from GWAS data. The red circle indicates loci associated with T2D risk, the blue with fasting glycaemia and/or HOMA-B index, the green with fasting insulinaemia and/or HOMA-IR index, the pink with glycaemia 2-h after a glucose challenge (2-h glucose) during an oral glucose tolerance test, and the yellow one with glycated haemoglobin (HbA1c). The genes/loci involved in both monogenic diabetes and polygenic/multifactorial forms of adult T2D are shown in bold.

MTNR1B or *ADCY5*)[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.[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,

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as it was demonstrated in the subgroup of patients carrying K_{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 β-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 ± neurodevelopmental disabilities: i/ *KCNJ11* and *INS*, ii/ *ABCC8*, if negative iii/ *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/ *INS* and *KCNJ11*, ii/ *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): *GCK* (especially if there is a consistent mild hyperglycaemia), *HNF1A*, *HNF4A*, *HNF1B* (especially in presence of renal cysts/developmental anomalies). Direct sequencing and analysis for deletions have to be used. If negative, *INS*, *ABCC8* and *KCNJ11* (especially in case of NDM in the family); more rarely *IPF1* or *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 *GCK*-MODY subtype is usually responsive to diet and leads to few complications.
- The *HNF1A*-MODY patients are generally very sensitive to the hypoglycemic effect of sulfonylureas (SU), because the β-cell defects are in glucose metabolism and can be bypassed by SU which act on the K_{ATP} channel to stimulate insulin release. Very low doses (eg: 20–40 mg gliclazide) are recommended as the first-line pharmacological treatment in *HNF1A*-MODY patients. The patients on other oral agents or insulin may have a trial with SU.
- PNDM patients with a K_{ATP} channel mutation (either in *KCNJ11* or in *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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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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