Genetic basis of monogenic diabetes

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Advances in the understanding of monogenic causes of diabetes and the discovery of single-gene mutations responsible for different phenotypes have greatly increased our knowledge of β-cell physiology. Such advances have had implications for the individual patient diagnosed with the specific monogenic cause of diabetes, especially in maturity onset diabetes of the young (MODY) and neonatal diabetes mellitus (NDM). Genetic diagnosis of MODY is also likely to have important prognostic and therapeutic implications in majority of the patients with confirmed *HNF1A* and *HNF4A* mutations. Genetic screening and analyses have helped several neonatal infants carrying mutations in the *KCNJ11* and *ABCC8* genes to shift from insulin treatment to oral sulphonylurea drugs. The progress in genomics of monogenic diabetic forms has helped in translating the discoveries from bench to bedside in clinical care. Therefore, there is an urgent need to incorporate genetic testing for the genes implicated in monogenic diabetes like MODY and NDM in the diabetes clinics. Discoveries in genetic research methodology and understanding of genetic etiology will have great translational implications for disease treatment and follow-up.

Keywords: Genetic screening, maturity onset diabetes of the young, monogenic diabetes, neonatal diabetes, precision medicine.

MONOGENIC diabetes encompasses relatively rare forms of non-autoimmune diabetes, caused by single gene defects that contribute to the disease phenotype affecting normal pancreatic β-cell physiology, development and differentiation resulting in insulin secretion deficiency and in moderate to very severe hyperglycaemia early in life. These mutations have a high or almost complete penetrance[1]. The different monogenic forms may be dominantly or recessively inherited or caused spontaneously by a *de novo* mutation. The common garden variety of type-2 diabetes (T2D), in contrast, is polygenic in nature with environmental factors interacting with multiple genes resulting in complex disease phenotypes.

Monogenic diabetes is subcategorized into three important types – maturity onset diabetes of the young (MODY), neonatal diabetes mellitus (NDM) or syndromic diabetes, although there are other types like mitochondrial diabetes, e.g. maternally inherited diabetes with deafness (MIDD) (Figure 1). Defects in pancreatic β-cell genes form the basis of defective insulin secretion leading to monogenic diabetes which presents with varied phenotypes such as severity of hyperglycaemia, early age at presentation of the disease and risk of complications. The main determinant of insulin secretion in pancreatic β-cells is the concentration of blood glucose. Glucose is transported into the β-cell by the GLUT-2 transporter. Following the phosphorylation of glucose by enzyme glucokinase (GCK), ATP is generated which closes the sensitive potassium channels resulting in depolarization of membrane and opening of calcium channels, which help in insulin exocytosis and finally insulin secretion. Mutations in β-cell genes disturb the coupling of blood glucose concentration and insulin secretion. Depending on the nature and position of the mutation in the gene, the biosynthesis and secretion of insulin is altered at different stages leading to various types of monogenic diabetes.

Although the prevalence of monogenic diabetes is much lower than polygenic diabetes, collectively they contribute to an important proportion of total disease burden, affecting millions of people worldwide. The majority of patients with monogenic diabetes are initially incorrectly diagnosed as having type-1 diabetes (T1D) or T2D. This is because the phenotype of monogenic diabetes is not sufficiently distinct to allow easy clinical differentiation from common forms of the disease. Accurate diagnosis is of great importance as it can predict the clinical course, the associated clinical manifestations and guide in improved treatment, as this varies with the underlying genetic defect. The purpose of this review is to highlight the importance of monogenic diabetes and its underlying molecular genetic defects. This has important clinical and therapeutic implications.

Maturity onset diabetes of the young

MODY usually presents in non-obese young individuals before the age of 25 years as a dominantly inherited familial form of non-insulin dependent, non-ketotic diabetes. To date, mutations in 14 different genes have been known to cause 14 different subtypes of MODY,

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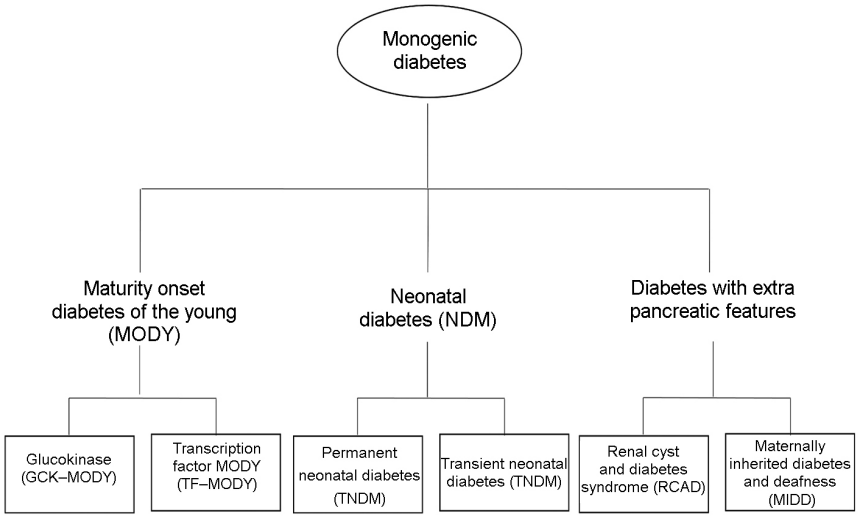

Figure 1. Types of monogenic diabetes.

Table 1. Types of maturity onset diabetes of the young

Type	Gene	Locus	Clinical features
MODY 1	*HNF4A*	20q12–q13.1	Mild–severe fasting and postprandial plasma glucose (PG). Responds well to sulphonylurea agents.
MODY 2	*GCK*	7p15–p13	Mild fasting hyperglycaemia. Less than 50% of carriers have overt diabetes mellitus (DM), and microvascular complications of diabetes are rare.
MODY 3	*HNF1A*	12q24.2	Same as MODY 1.
MODY 4	*IPF1/PDX1*	13q12.1	Pancreatic agenesis.
MODY 5	*HNF1B*	17cen–q21.3	Overt DM in association with renal and genitor-urinary abnormalities.
MODY 6	*NEUROD1*	2q32	Rare, with phenotype characterized by obesity and insulin resistance.
MODY 7	*KLF11*	2p25	Very rare; phenotype ranges from impaired glucose tolerance or impaired fasting glucose to overt DM.
MODY 8	*CEL*	9q34.3	Very rare; associated with both exocrine and endocrine pancreatic deficiency and with demyelinating peripheral neuropathy.
MODY 9	*PAX4*	7q32	Very rare. Crucial transcription factor for the development of β-cells.
MODY 10	*INS*	11p15.5	Very rare. Usually associated with neonatal diabetes. Rare <1% cases.
MODY 11	*BLK*	8p23–p22	The adapter proteins nucleate formation contributes to the qualitative and quantitative control of B-cell signalling.
MODY 12	*ABCC8*	11p15.1	Very rare. Usually associated with neonatal diabetes. Rare <1% cases.
MODY 13	*KCNJ11*	11p15.1	Very rare. Usually associated with neonatal diabetes. Rare <1% cases.
MODY 14	*APPL1*	3p14.3	Recently described. Enhances insulin-induced AKT2 activation and downstream signalling leading to insulin action and secretion.

each with differences in severity of hyperglycaemia, risk of complications, clinical presentation and treatment options between them. Table 1 presents details of the 14 MODY subtypes with the implicated phenotypes.

In select areas of world, MODY has been well studied due to availability of strong resources and clinical care research directions. The pick-up rates of MODY diagnosis and distribution of MODY subtypes vary vastly due to hyperglycaemia measurements, age at diagnosis of the patient and family history information. In populations of the UK[2] and Norway[3], the prevalence and characteristics of monogenic MODY have been well studied due to the presence and referral to centralized locations of monogenic diabetes testing. A multi-centric study has shown that MODY has an appreciable prevalence with 95% of the cases in USA being currently undiagnosed[4]. In India, age at onset of T2D is much earlier than in other parts of the world[5], and this has great implications in the prevalence of MODY. Earlier clinical studies[6,7] reported on the high prevalence of MODY (4.8%) in a diabetes clinic in Chennai[8]. However, this was in the era before the genetics of MODY was known and is therefore quite likely that many of the clinically diagnosed MODY cases at that time might indeed have had early onset diabetes. This points to the need for more studies in India on young onset T2D.

Molecular genetic basis of MODY

Molecular genetic causes of MODY are generally characterized by the presence of mutations in the gene encoding the GCK enzyme responsible for β-cell response to

changes in glycaemia or in the transcription factor genes important for β-cell function and regulation.

Glucokinase MODY

The genetic basis of MODY has been investigated since 1990s. The first MODY gene identified encodes the GCK enzyme and is commonly classified as GCK–MODY (MODY 2)[9]. GCK serves as the glucose sensor of pancreatic β-cells, catalysing glucose in the blood that is taken up at the surface of the β-cell by the glucose transporter 2 (GLUT2) to glucose-6-phosphate. GCK is predominantly expressed in hepatocytes and pancreatic β-cells and has direct control over insulin secretion. Mutations in *GCK* gene lead to reduced glycolysis, reduced ATP levels and impaired insulin secretion[10,11]. More than 600 different inactivating *GCK* mutations have been identified and these often lead to loss of function. Heterozygous loss-of-function mutations lead to mildly elevated fasting blood glucose levels, up to 6.7 mmol/l with a postprandial increase up to 8.6 mmol/l.

Patients with heterozygous loss-of-function *GCK* mutations are not diagnosed until incidental testing reveals hyperglycaemia[12]. If identified in childhood, this disease condition can be misdiagnosed as T1D; if in adult life as T2D, and if detected during pregnancy as gestational diabetes mellitus (GDM). A misdiagnosis leads to unnecessary insulin or oral hypoglycaemic agent treatment, highlighting the importance of genetic diagnosis. A distinct feature of this subtype is the relatively low risk of developing late complications of diabetes[13].

The presence of mutations in *GCK* increases only the 'set point' of fasting glucose, which is the glucose threshold needed to secrete insulin, where glucose metabolism remains well regulated with haemoglobin A1c seldom exceeding 6.5% (ref. 14). This is the reason for managing the patients with GCK–MODY solely by diet[15]. In contrast to the heterozygous loss-of-function mutations in GCK–MODY, homozygous loss-of-function mutations result in complete deficiency of this enzyme and are a rare cause of permanent insulin-requiring diabetes presenting in the neonatal period[16], while heterozygous gain-of-function mutations cause the opposite phenotype of hyperinsulinaemic hypoglycaemia[17].

It is important to diagnose GCK–MODY in pregnant women since it can save the patients from unnecessary and sometimes lifelong treatments. For foetal growth, foetal insulin secretion in response to maternal hyperglycaemia plays an important role. If the foetus does not inherit the mutation from the affected mother, its response to maternal fasting hyperglycaemia is through insulin secretion, leading to increased intrauterine growth and macrosomia[18]. However, if the foetus inherits the *GCK* mutation from the mother, it experiences the same increased set point for sensing maternal hyperglycaemia, producing normal amounts of insulin and growing normally despite maternal fasting hyperglycaemia. If the foetus inherits an inactivating heterozygous mutation from the father, the birth weight is reduced by 500 g because of reduced foetal insulin[18,19]. Thus, this underscores the importance of *GCK* genotype of the baby as the best predictor of foetal weight during pregnancy.

Transcription factor genes MODY

β-cell transcription factor genes play an important role in the etiology of T2D[20]. Both rare, severe changes in transcription factor gene sequences (mutations), and more common, less severe changes (polymorphisms) contribute to different forms of diabetes. Importantly, mutations in the MODY genes result in autosomal dominant diabetes, demonstrating that the protein which they code for is a key biochemical factor and cannot be compensated for, while common variants in the MODY genes are strong candidates in T2D and related traits, because changes in the activity or expression of one of these genes is likely to result in elevated blood sugar levels. In contrast to mutations in the Glucokinase gene, mutations in transcription factor genes, viz. *HNF1A*, *HNF4A*, *IPF1* and *Neuro D* cause progressive, severe β-cell dysfunction[21]. Proteins encoded by these genes are responsible for regulation of gene expression mainly in the liver, pancreatic islets, kidneys and genital tissues[18]. In pancreatic β-cells where insulin secretion takes place, these factors regulate the expression of the insulin gene and genes involved in glucose transport and metabolism[19]. In the liver, they regulate lipoprotein biosynthesis[22]. Mutations in these transcription factor genes result in an alteration of gene expression of proteins that are involved in glucose transport and glucose metabolism and increase apoptosis of the β-cells[23].

Hepatocyte nuclear factor 1A MODY (HNF1A-MODY; MODY 3)

This is the most common form of monogenic diabetes in the world. More than 400 mutations of *HNF1A* gene are associated with the disease[24]. Insulin secretion in response to glucose is severely reduced in MODY 3 patients. The clinical presentation of the disease is highly variable from one family to another and even within the same family[25]. This is related in part to the type and nature of the mutations present. Truncation mutations or missense mutations of the dimerization/binding domain of *HNF1A* result in a 10-year-earlier onset of diabetes than missense mutations of the transactivating domain. Obesity is another factor modulating the age at diagnosis of diabetes[26]. The disease usually occurs after puberty, at a median age of 21–25 years. In 25% of the patients, diabetes is revealed by clinical symptoms that may suggest T1D, although ketoacidosis is rare[27], or as young-onset T2D, with no features of insulin resistance. The

prevalence of diabetic retinopathy and nephropathy is high, and macrovascular complications are also common[28]. Differential diagnosis of T2D may be difficult because of the epidemic of the disease in young individuals and that of body-weight excess, which is also present in 30% of patients with HNF1A-MODY. Moreover, HNF1A-MODY may be seen in subjects older than previously described, that is over 25 years of age, proving that the classical criteria for MODY diagnosis are not specific enough, warranting genetic diagnosis as the best method of choice for identifying the subtype of diabetes.

Hepatocyte nuclear factor 4A MODY (HNF4A-MODY; MODY 1)

This is a less common MODY subtype, accounting for 5–10% of the cases[2,29]. Its pathophysiology, clinical presentation and sensitivity to sulphonylureas are similar to those of HNF1A-MODY[22,30].

All patients with these two MODY subtypes require pharmacological treatment. Pearson *et al.*[31] showed in a randomized trial that *HNF1A* patients had a fourfold greater response to the SU gliclazide than T2D patients due to greater insulin secretion in response to the drug. This has clear practical implications. In T2D, metformin is usually preferred. However, if a patient is known to have a *HNF1A* mutation, use of sulphonylurea as the first-line of treatment is preferred. This is due to the marked sensitivity to sulphonylurea exhibited by the *HNF1A* patient[32]. Therefore knowing that someone has an *HNF1A* mutation should prompt transition from insulin to sulphonylureas. This is the first robust evidence for genetics impacting on the clinical therapeutic management of diabetes[33].

Pancreatic and duodenal homeobox 1 MODY (PDX 1 MODY; MODY 4)

Also known as insulin promoter factor 1 (IPF 1), is a homeodomain transcription factor expressed in β cells[34]. PDX 1 is essential for the development of pancreas, β-cell differentiation and the maintenance of mature β-cell function by regulating the expression of key islet-specific genes such as *INS*, *GCK* and *SLC2A2*, causing MODY 4 (ref. 35). Stoffers *et al.*[36] identified that heterozygous single cytosine deletion mutation Pro63fsdelC in *PDX 1* gene causes MODY 4, while homozygous single cytosine deletion mutation within the same codon (Pro63fsdelC) is responsible for permanent neonatal diabetes mellitus (PNDM) syndrome resulting in pancreatic exocrine insufficiency. Deletion C at codon 63 leads to a frame shift resulting in a truncated protein with 59 aberrant codons that locks transactivation domains at the C terminals. In the case of patients with homozygous mutation, the protein fails to transactivate the *INS* gene transcription leading to PNDM, and in the case of heterozygous mutation, there is a partial reduction in transactivating activity on the *INS* gene, thus resulting in MODY 4 subtype. A few other PDX 1 point mutations were seen to lead to reduced binding of the mutant protein to the *INS* gene promoter and decreased *INS* gene transcription resulting in insulin secretion impairment. Thus *PDX 1* gene mutations underlie both MODY 4 and PNDM.

Hepatocyte nuclear factor 1B MODY (HNF1B-MODY; MODY 5)

This is a less common MODY subtype (5–10% of cases). Its expression is seen in the kidney, pancreas, liver, biliary and genital tract, which explains its wide phenotype that is generally seen in patients with the molecular abnormalities of HNF1B[37–39].

Heterozygous mutations of *HNF1B* gene cause a more complex syndrome (renal cysts and diabetes syndrome; RCAD), characterized by severe abnormalities of the kidney and genital tracts as well as an early onset of diabetes, pancreas hypoplasia and liver dysfunction[40–42]. HNF1B-MODY should be suspected in children or young adults with diabetes and non-diabetic renal disease even in the absence of relevant family history, as spontaneous *de novo HNF1B* gene mutations occur relatively frequently[43].

Physiological studies have suggested that suppression of endogenous glucose production by insulin is impaired in patients with *HNF1B* mutations while peripheral insulin sensitivity is preserved[44]. In contrast to those with HNF1A-MODY, these patients are not sensitive to sulphonylureas. Patients with diabetes due to *HNF1B* mutations frequently require early treatment with insulin therapy.

Neurogenic differentiation factor 1 MODY (NEUROD1/Beta 2; MODY 6)

The basic helix–loop–helix (HLH) transcription factor *NEUROD1*, also called BETA 2, plays a crucial role in pancreatic β-cell maturation and maintenance. The importance of *NEUROD1* was realized from studies in mice. Pancreatic β-cell-specific *NEUROD1*-deficient mice exhibit severe glucose intolerance with greatly reduced insulin secretion. They fail to develop mature islets due to enhanced apoptosis, leading to neonatal diabetes and death within a few days of life[45]. In humans, heterozygous loss-of-function mutations in *NEUROD1* produce a very rare type of MODY 6 with very few families reported to date[46]. A few missense mutations reported in different ethnic groups such as Icelanders, Chinese, Czech and Polish, have been implicated to result in MODY 6. Homozygous deletion, duplication and recessive mutations have been seen to result in PNDM with neurological abnormalities[47]. These mutations produce proteins without the activation domain at the C-terminus. Mutations in the basic portion of HLH domain eliminate

E box binding activity of *NEUROD1* compromising insulin gene transcription in pancreatic β-cells leading to diabetic phenotype.

Kruppel-like factors MODY (KLF MODY; MODY 7)

KLF is a member of zinc finger regulatory proteins whose functions include β-cell insulin gene expression[48]. KLF11 activates PDX1 through the conserved domains which are the principal control domains for islet β-cell expression. The KLF family of transcriptional regulators have been strongly associated with diabetes, obesity and insulin resistance. An important mutation A347S of the *KLF* gene is shown to impair the regulation of metabolic gene networks.

Carboxyl ester lipase MODY (CEL MODY; MODY 8)

CEL MODY is a monogenic form of diabetes and pancreatic exocrine dysfunction due to mutations in the *CEL* gene. A few families have been reported with mutations in the gene with pancreatic exocrine dysfunction[49]. Subjects with CEL MODY develop multiple cysts in the pancreas and diabetes at a young age. Very few CEL MODY studies have been conducted so far[50].

B-Lymphocyte kinase MODY (BLK MODY; MODY 9)

The *BLK* gene as MODY 9 was identified in a family[51], where it was shown to be in linkage at the loci of the gene. Mutations in the gene were also shown to segregate with the disease in the family. It was also shown that *BLK* is a modulator of insulin synthesis and secretion, which enhances the expression of key β-cell transcription factor such as PDX 1.

Insulin MODY (INS MODY; MODY 10)

Insulin gene mutation is a very rare form of MODY, representing MODY 10. Mutations in the *INS* gene that produce structurally defective insulin result in MODY 10, hyperinsulinaemia, neonatal diabetes and T1D, and depending on the position and nature of the mutation, the disease phenotypes present in different ways. The position of the mutations on the insulin gene determines the severity of the disease, that is, mutations causing neonatal diabetes are located in proinsulin folding and most of the mutations causing later-onset diabetes are located in the signal peptide of preproinsulin, suggesting the involvement of different pathophysiological mechanisms[52]. The less severe *INS* mutations were reported in patients with MODY 10 (ref. 53). The mutation *R45Q* was found in a familial case and was known to disrupt a critical hydrogen bond formation, which impairs the stability of the insulin molecule. Recently, in a MODY family, a novel heterozygous single nucleotide deletion (c. 233 delA) leading to a frameshift mutation has been identified. This mutation produces an aberrant proinsulin that lacks the native structures of C-peptide and alpha-chain[54]. While these mutations that cause MODY are because of dominant heterozygous nature, recessive mutations cause PNDM and transient neonatal diabetes mellitus (TNDM) which will be dealt later in text.

Paired box protein 4 MODY (PAX4 MODY; MODY 11)

Paired homeodomain TF functions mainly as a transcription repressor[55]. PAX4 is expressed in human islets. Mutations in *PAX4* gene, although rare result in MODY 9 subtype. *PAX4* is known to act on α- and β-cells of the pancreas, being predominant in α-cells and less so in β-cells. It is known to repress the glucagon promoter activity in α-cells, and inhibit insulin promoter activity in β-cells. Plengvidhya *et al.*[56] screened *PAX4* coding sequences of the gene in 46 clinical MODY patients in Thai and found two possible pathogenic mutations of *PAX4*, namely R164W and IVS7-IG->A (splice acceptor of intron 7). It is believed that these mutations may cause insulin deficiency possibly through the disruption of β-cell development. A few recent studies have shown the efficacy of GLP-1 receptor (GLP-1R) agonists[57,58] and dipeptidyl peptidase-IV (DPP IV) inhibitors in these patients. It is possible that these two drugs are efficacious in the treatment of patients with *PAX4* mutations, through their action on glucagon inhibition[59].

ATP-binding cassette subfamily C member 8 MODY (ABCC8; MODY 12) and KCNJ11 potassium voltage-gated channel subfamily J member 11 (KCNJ11; MODY 13)

ABCC8 and *KCNJ11* MODY gene mutations are thought to be rare causes of MODY 12 and MODY 13 respectively, and a predominant cause of PNDM[60]. However in our study with targetted gene sequencing and exome sequencing *ABCC8* MODY 12 is seen more often than other types of MODY.

Adaptor protein and leucine zipper 1 MODY (APPL1 MODY; MODY 14)

This is a recently described *MODY 14* gene described in two families[61]. *APPL1* binds to *AKT2*, a key molecule in the insulin signalling pathway, thereby enhancing insulin-induced AKT2 activation and downstream signalling leading to insulin action and secretion. Mutations in this gene cause *APPL1* loss-of-function leading to diabetes phenotype.

Neonatal diabetes mellitus

Presentation of diabetes within the first 6 months of life is called neonatal diabetes mellitus and that between 6 months to one year is called infantile onset diabetes. These are non-autoimmune in nature in contrast to T1D, which usually does not present before the first year. The condition is rare and its frequency is about two cases per 50,000–100,000 infants. Based on the course of the disease, NDM is stratified as transient (TNDM) and permanent (PNDM). In TNDM, diabetes remits and may likely resolve or relapse later in the course of life. A delayed maturation of pancreatic islets and β-cells results in deficiency in insulin output in the beginning, and this has been implicated due to disorder of imprinting in chromosome region 6q24, where only the paternal allele is actively expressed while the maternal allele remains silent. Around 70% of the TNDM cases are due to loss of imprinting in 6q region[62]. Children with TNDM present with intrauterine growth retardation, hyperglycaemia and absence of ketosis. Genetic testing alone helps in differentiating the TNDM and PNDM children in the early age as clinical differentiation is not possible or accurate.

PNDM, which is also characterized by early hyperglycaemia, has no period of remission and must be treated lifelong. NDM is genetically heterogeneous with many genetic abnormalities as the cause behind the condition. Genes involving β-cell function or development such as glucokinase, β-cell ATP sensitive potassium (K^{ATP}) channel, or insulin itself lead to insulin secretary defects. So far 20 genes have been implicated to cause PNDM[63], but the most common causes of mutations in *ABCC8*, *KCNJ11* and *INS*.

K^{ATP} channel mutations (KCNJ11 and ABCC8 mutations)

Patients with NDM were originally believed to require lifelong insulin treatment as they have apparently very little or no endogenous insulin. The identification of K^{ATP} channel mutations in these patients revolutionized the treatment of NDM[64]. Mutations in the K^{ATP} channels encoded by the *KCNJ11* and *ABCC8* genes are a common cause of PNDM. These mutations are either activating or inactivating. Activating mutations in the K^{ATP} channel complex lead to increased channel opening, resulting in a suppression of insulin secretion and subsequent hyperglycaemia[65–67], while inactivating mutations decrease channel activity causing over-secretion of insulin that is poorly coupled to plasma glucose levels[65] resulting in persistent hyperinsulinaemia that presents as hypoglycaemia in infancy (HI)[68,69].

Children with K^{ATP} mutations usually present with hyperglycaemia within the first 6 months of life, with the exception of a small percentage with onset between 6 and 12 months of age[70]. Children with *KCNJ11* and *ABCC8* mutations generally have reduced birth weights, i.e. small for gestational age (SGA), which is indicative of reduced *in utero* insulin secretion. Ketoacidosis is generally seen in children with *KCNJ11* mutations, but not so commonly seen in those with *ABCC8* mutations.

It is important to note that various regions (domains) in the gene structure are responsible for different functions of the protein having direct effect on the phenotypes of the patients. For example, two domains, namely ATP-binding domain and pore forming domain are important in the *KCNJ11* gene. Most of the mutations causing isolated diabetes are present in the ATP-binding site and those causing syndromic diabetes are present in the pore forming domain[71]. In case of *ABCC8*, mutations causing PNDM are located throughout the gene.

The hyper- and hypo-secretion of insulin in response to the cellular ATP concentration have been shown to be due to mutations at adjacent residues in the *KCNJ11* gene[72] causing opposite phenotypes, namely iDEND syndrome and recessive hyperinsulinaemic hypoglycaemia. The K^{ATP} channels are expressed in skeletal, cardiac muscle, kidney and brain, and the mutated channels in the brain are responsible for the neurological symptoms. A similar situation is also seen in amino acid positions 59 and 201, where mutations in the former residue result in syndromic form of diabetes while those in residue 201 result in isolated PNDM. This is proof of the fact that genetic testing is critical, since the severity of disease phenotype varies based on the position of mutation. It is important to realize that children with some of the K^{ATP} mutations are responsive to oral sulphonylurea drugs and hence can be transitioned from insulin to oral sulphonylurea[73]. Therefore, identification of *KCNJ11* and *ABCC8* mutations becomes important for clinical management as most of these sulphonylurea drugs bind to SUR1 subunit of K^{ATP} channel leading to the closure of the channel independent of ATP, with subsequent release of insulin. It has also been shown that the dosage of sulphonylurea required is less in the case of children with *ABCC8* mutations compared to those with *KCNJ11* mutations[74]. This is likely due to the underlying molecular mechanisms of mutations that alter K^{ATP} channel function. It is one of the dramatic impacts and applications of genetic findings to clinical care. Although insulin therapy may control glucose homeostasis in NDM patients with mutant K^{ATP} channels, it does not restore the normal K^{ATP} channel activity in non-pancreatic tissues such as the brain and skeletal muscle. On the other hand, sulphonylureas can inhibit K^{ATP} channels in many tissues such as the central nervous system, and improve the neurological dysfunction often seen in neonatal diabetes.

In clinical practice, the two major treatments for NDM patients are insulin therapy and oral sulphonylureas, and treatment for individual patients varies depending on the genetic cause of NDM[75]. In most of the PNDM and TNDM patients carrying mutant K^{ATP} channels, sulphonylurea

therapy is an attractive alternative to insulin therapy. However, for other PNDM patients carrying mutations in *PTF1A*, *EIF2AK3*, *FOXP3* and 80% of TNDM patients carrying mutations in chromosome 6q24 (e.g. *ZAC/HYMAI*), sulphonylurea responsiveness is minimal and insulin therapy is the only option[76].

It is at this juncture that genetic testing becomes imperative not only for correct diagnosis but also for optimization of treatment in PNDM and TNDM with mutations in K^{ATP} channel[77].

Studies on MODY in India

Molecular genetic studies of monogenic diabetes in India have been conducted by us at the Madras Diabetes Research Foundation (MDRF), Chennai for over 20 years. Our initial genetic studies and screening were performed using Sanger sequencing method choosing clinically suspected MODY patients. Among 96 young onset diabetic patients screened for *HNF1A* gene mutation, we identified nine mutations (9.6%). A novel *HNF1A* gene mutation Arg263His co-segregated with diabetes in a family of 30 individuals; it was not seen in non-diabetic members in the family, thus providing evidence for the mutation to be involved in MODY[78]. This mutation was further evaluated for pathogenicity using functional genomic assays which demonstrated that the mutant acts as dominant negative suppressor and causes MODY 3 in the affected family, due to reduced HNF1A transactivation potential, DNA binding to HNF1A targets and improper nuclear translocation[79].

We screened 87 patients diagnosed with T2D before 25 years of age and negative for GAD antibodies, for *HNF4A* gene mutation (MODY 1) and identified three mutations in the *HNF4A* gene (3.4%). Three novel variants, namely –129 T/C, –1009 G/C and –79 C/T in the region of P2 promoter were identified in a family. The variant –1009 G/C was found in four members in a MODY[80]. Next we screened 55 patients for *GCK* gene (MODY 2) mutations and identified two MODY 2 mutations – Met251Thr and Thr206Ala – of which Thr206Ala was novel. The three diabetic members of a family carrying a known Met251Thr mutation were managed without pharmacotherapy and they show non-progressive mild hyperglycaemia over the years[81].

In our study of 50 cases, clinically suspected to have MODY 5 based on renal abnormalities on ultrasound such as renal cysts, horse-shoe kidney, etc. we have identified six (12%) different *HNF1B* gene mutations and whole-gene deletion[82].

Recently, Chapla *et al.*[83] carried out MODY genetic testing among young onset diabetic patients of Asian-Indian origin, using Ion Torrent next-generation sequencing (NGS) technology. About 19% of the 56 clinically dignosed MODY subjects carried MODY mutations in one of the MODY genes (*HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1* and *PAX4*). Aggarwal *et al.*[84] detected a heterozygous whole-gene deletion (Met1_Trp557del) in an Indian patient with renal cysts and diabetes syndrome (RCAD). Very recently, Doddabelavangala Mruthunjaya *et al.*[85], screened 50 pregnant women with diabetes for the presence of 13 MODY genes using NGS. Of these subjects, 18% (9/50) were positive for definite or likely pathogenic or uncertain MODY variants. The majority of these variants were identified in subjects with autosomal dominant family history.

Development of a comprehensive Indian monogenic diabetes gene panel

Mutations in other MODY genes have been reported sporadically, but their total contribution to the prevalence of MODY is not substantial. These genes are involved in β-cell function and pancreas organogenesis. Since they are rare, limited data are available regarding the phenotype and clinical progress of diabetes. They are not usually routinely screened in molecular testing for MODY, but performed when the common genes have been negated and there is high clinical suspicion of MODY. In collaboration with MedGenome Labs, Bengaluru, we have launched an MDRF–Medgenome Monogenic Diabetes Genetic Panel, where all the 14 MODY subtypes can be assessed simultaneously. The panel contains 32 genes which can detect mutations in any of the neonatal or MODY genes identified so far. Using this targetted gene panel we have identified 125 mutants/variants implicated in monogenic diabetes, spanning all the MODY subtypes, except MODY 10. Very recently, we have used exome and whole genome strategy to analyse 153 clinically confirmed MODY patients from South India and have discovered a novel MODY gene loci in them (unpublished). Describing novel subtypes of MODY thus helps in understanding disease etiology better. This is an advantage of the using NGS technology.

Studies on neonatal diabetes in India

Incidence of NDM in Indian population has not been studied so far, but a few case studies have been published from the country[86–91].

Our group has studied the genetics of NDM in India. A total of 150 clinically diagnosed NDM children (PNDM and TNDM) were screened for the common genes implicated in neonatal diabetes such as *KCNJ11*, *ABCC8* and *INS*; 19 (9.6%) had *KCNJ11* gene mutations, 38 (19.3%) had *ABCC8* gene mutations and 9 (4.6%) had insulin gene mutations[92].

We have successfully shifted the children with *KCNJ11* mutations (e.g. Cys42Arg and Arg201Cys) and *ABCC8* (e.g. Val86Ala and Asp212Tyr), from insulin therapy to sulphonylurea treatment[93]. This is the most

important translational aspect of genetic diagnosis of monogenic diabetes.

Congenital hyperinsulinaemic hypoglycaemia (CHI) occurs as a consequence of inappropriate and unregulated secretion of insulin by the pancreatic β-cells. CHI typically presents in newborn babies and infants as severe and persistent hypoglycaemia, and is a major cause of hypoglycaemia-related brain injury and mental retardation. Molecular basis of CHI involves mutations in ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, HNF4A and UCP2, and hence response to treatment heavily depends on genetics[94,95].

In one of our studies, molecular abnormality was identified in 40% (16 of 40) of children with CHI. Fourteen of these mutations were identified in *ABCC8* gene and two in KCNJ11 gene[93]. Children with CHI who had compound heterozygous mutations responded to diazoxide, but others did not. Children who did not respond to diazoxide were started on injection octreotide and those who were refractory to these medications were planned for pancreatectomy[93]. Most of the children who were not harbouring mutations in any of the genes studied responded well to pharmacological therapy, whereas true diazoxide responsiveness was not seen in children with mutations. Genetic testing assists in understanding the nature of the molecular abnormality and in most cases timely identification of the type of hyperinsulinaemia is likely to aid in avoiding hypoglycaemia related brain damage.

Our genetic diagnosis has made it possible to successfully shift some of the children with *KCNJ11* and *ABCC8* mutations from insulin treatment to oral sulphonylurea therapy[93]. This is an important direct translation of genetic analysis from bench to bedside in clinical practice.

Conclusion

Our understanding of monogenic diabetes has advanced tremendously in the past 20 years, and it will continue to progress in the future. Genetics of MODY and NDM exemplifies the need to bring the study of genomics of diabetes to the diabetic clinic. Careful interpretation of sequence data taking into account molecular function in relevant experimental systems, clinical and biochemical phenotypes, allelic penetrance and familial segregation will collectively assist in making more reliable assessments of variant pathogenicity. This would be the greatest challenge for genomics research in the coming years. Developing such a contextual understanding of mutation will be of utmost importance to deliver on precision and personalized medicine.

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ACKNOWLEDGEMENTS. We thank ICMR, New Delhi for financial support awarded to R.V. through the projects ‘ICMR Advanced Centre for Genomics of Type 2 diabetes’, ‘Genetic Analysis of Maturity Onset Diabetes of Young (MODY) and Neonatal Diabetes in India’ and ‘Changing treatment profiles in monogenic forms of diabetes such as Neonatal Diabetes and Maturity Onset Diabetes of the Young (MODY) by translational Genomics Research’.

doi: 10.18520/cs/v113/i07/1277-1286