Section 3: Molecular Mechanisms in Diabetes

Chapter 6

What is New in Genomics of Maturity-onset Diabetes of the Young and Neonatal Diabetes in India?

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INTRODUCTION

Monogenic diabetes consists of different subtypes of single gene disorders comprising a large spectrum of phenotypes, the dominantly inherited familial forms of diabetes called maturity-onset diabetes of the young (MODY), neonatal diabetes mellitus (NDM), and congenital hyperinsulinemic hypoglycemia (CHI). In addition, it includes rarer diabetes-associated monogenic syndromic diseases, maternally inherited diabetes and deafness (MIDD) and a few other types. All these forms are diagnosed at a very young age and are unrelated to autoimmunity.

Maturity-onset diabetes of the young was recognized initially as autosomal dominantly inherited diabetes, characterized by β-cell dysfunction and despite being diagnosed young (typically before the age of 25 years), was not insulin dependent.1,2 MODY is often misdiagnosed as type 1 or type 2 diabetes mellitus as there is significant overlap in clinical features and hence, genetic screening for MODY mutations is important. Although MODY probably represents only 3%-5% of all diabetes cases, accurate molecular diagnosis has important implications for treatment, prognosis, and risk to patients and other family members.3,5

Neonatal diabetes mellitus is a form of insulin requiring monogenic diabetes characterized by onset of hyperglycemia within the first 6 months of life. Usual clinical management includes insulin therapy to children with neonatal diabetes. However, identification of K+ channel, inwardly rectifying subfamily J, member 11 (KCNJ11) and adenosine triphosphate (ATP)-binding cassette transporter sub-family C member 8 (ABCC8) gene mutations becomes vital for further management as most of these children with ATP-sensitive K+ (KATP) channel mutations are responsive to oral sulfonylurea drugs. Sulfonylurea drugs bind to the sulfonylurea receptor 1 (SUR1) subunit of KATP channel which leads to the closure of the channel independent of ATP, with subsequent release of insulin.6

Mutation screening for the genes implicated in the monogenic diabetes should be considered in the differential diagnosis of diabetes as the genetic diagnosis of monogenic diabetes in many cases alters therapy, affects prognosis, enables genetic counseling, and has implications for screening of extended family members.7

MATUREITY-ONSET DIABETES OF THE YOUNG

Maturity-onset diabetes of the young is a heterogeneous group of diabetes caused by single gene defects in at least 13 genes affecting pancreas development and β-cell function.8,3,9 The most common MODY forms are caused by mutations in the glucokinase gene (GCK)10 and the hepatocyte transcription factor genes hepatocyte nuclear factor 1α (HNF1α) and hepatocyte nuclear factor 4A (HNF4A).11,12 Glucokinase maturity-onset diabetes of the young (GCK-MODY) (MODY2) is a mild disease manifesting as slightly elevated fasting glucose, well-controlled without medical treatment, and no risk for late diabetes associated complications.13,14 In contrast, HNF1α (MODY3) and HNF4A MODY (MODY1) typically lead to more severe forms of diabetes due to progressive β-cell dysfunction and high risk for developing diabetes related microvascular complications like retinopathy and nephropathy and patients often benefit from sulfonylurea
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HNF1B gene mutations result in a syndromic form of diabetes (MODY5), which includes renal, genital, and pancreatic malformations, and liver and renal dysfunction. Mutations in nine other genes [B lymphocyte kinase, carboxyl ester lipase (CEL), insulin gene (INS), Krueppel-like factor 11 (KLF11), neurogenic differentiation 1 (NEUROD1), paired box gene 4 (PAX4), pancreatic and duodenal homebox 1 (PDX1), KCNJ11, ABCG8] can cause inherited diabetes with a MODY phenotypes called MODY4 and MODY6-13. It is also possible that there are many other MODY genes waiting to be discovered (called MODYX).

Maturity-onset Diabetes of the Young studies in India

Earlier clinical studies reported on the high prevalence of MODY, 4.8% (using the clinical criteria of Tattersall and Fajans used at that time) in a diabetes center in Chennai. The insulin responses in MODY and the β-cell response in the offspring of MODY indicated an increased insulin resistance compared to classical Indian type 2 diabetic subjects. However, as the first MODY genes were only discovered in 1990s, it is possible that many clinically diagnosed MODY cases might have had early-onset diabetes. The mutation screening of MODY patients by us was started around 2001 and our studies during past 14 years have revealed important insights into the genetics of MODY in India.

Hepatocyte Nuclear Factor 1A (Maturity-onset Diabetes of the Young 1)

We screened 87 patients diagnosed with type 2 diabetes mellitus (T2DM) before 25 years of age and negative for glutamic acid decarboxylase antibodies, for HNF4A gene mutation (MODY1). We 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 novel variant -1009 G/C was found in four members in a MODY.

Glucokinase gene (Maturity-onset Diabetes of the Young 2)

Glucokinase maturity-onset diabetes of the young is characterized by persistent mild, asymptomatic hyperglycemia (5.5–8.0 mmol/L), good pancreatic β-cell reserve, absence of autoimmune markers of type 1 diabetes mellitus (T1DM), dominant mode of inheritance, and a potential for insulin withdrawal when the mutation is defined. Women with GCK mutations often present with gestational diabetes as their asymptomatic hyperglycemia is detected by routine testing in pregnancy. The diagnosis of a GCK mutation is very important for both mother and child.

We screened 55 patients for GCK gene (MODY2) mutations and identified two MODY2 mutations (Met251Thr, Thr206Ala) of which Thr206Ala was novel. The three diabetic members of a family carrying a known Met251Thr mutation were managed without pharmacotherapy and they have exhibited nonprogressive mild hyperglycemia over years. GCK mutation screening should be considered in patients with chronic mild early-onset hyperglycemia and this will help clinicians to classify the subtype of MODY diabetes, to predict the prognosis, and to offer correct treatment to patients.

Hepatocyte Nuclear Factor 1A (Maturity-onset Diabetes of the Young 3)

Among 96 young-onset diabetic patients screened in our center for HNF1A gene mutation, we identified nine mutations (9.6%). Thus, MODY3 is the commonest form of diabetes seen at our center. A novel HNF1A gene mutation Arg263His mutation cosegregated with diabetes in a family of 30 individuals and this mutation was not seen in nondiabetic members in the family, thus providing evidence for the mutation to be involved in causing MODY.

Insulin promoter factor 1 (Maturity-onset Diabetes of the Young 4)

Phenotype of this MODY subtype ranges from impaired glucose tolerance to overt diabetes mellitus. The homozygous or compound heterozygous mutations of insulin promoter factor 1 (IPF1) or PDX1 are known to be associated with pancreatic agenesis. In India, one mutation (Val177Met) has been reported so far.

Hepatocyte Nuclear Factor 1B (Maturity-onset Diabetes of the Young 5)

Hepatocyte nuclear factor 1B, is encoded by HNF1B gene and plays a major role in the embryogenesis of various organs, such as kidney, pancreas, liver, bile ducts, thymus, lung, gut, and genital tract. Heterozygous mutations of HNF1B gene cause a complex syndrome [renal cysts and diabetes syndrome (RCAD)], characterized by severe
abnormalities of the kidney and of the genital tracts as well as an early onset of diabetes (HNF1B-MODY or MODY5), pancreas hypoplasia, and liver dysfunction. In our study of 50 cases clinically suspected to have MODY5 based on renal abnormalities on ultrasound, such as renal cysts, horse-shoe kidney, etc., we have identified six (12%) different HNF1B gene mutations (-67C>T, Arg165His, His153Arg, IVS2nt+2insT, Asn321Asp) and whole-gene deletion (Met1_Trp557del).

**Neurogenic Differentiation Factor 1 (Maturity-onset Diabetes of the Young 6)**

Neurogenic differentiation factor 1 is an important transcription factor required for pancreatic development as well as β-cell differentiation. Mutations in NEUROD1 results in an autosomal dominant disorder which resembles T2DM (MODY6). In India, three mutations (His241Gln, Glu59Gln, c.-162G>A 5’ UTR) have been reported so far. Very recently, we have detected one case of MODY6 in our center.

**Other Maturity-onset Diabetes of the Young Subtypes**

Several other gene mutations have been discovered in recent years as rare causes for early-onset diabetes. Genome-wide screen detected mutations in the CEL gene (MODY8) to be a rare cause for diabetes and exocrine pancreatic insufficiency. The KLF11 is a transcription factor which regulates the transcription of insulin promoter factor 1 (IPF1 in humans, PDX1 in rodents), one of the crucial transcription factors involved in early pancreatic development. Recently, mutations in this gene are identified to be linked with MODY7. PAX4 and PAX6 are transcription factors involved in islet cell differentiation. In 2007, two novel mutations in the PAX4 gene were identified in two patients from a Thai population as the cause for diabetes (now termed MODY9). One MODY9 mutation (Arg31Leu) has been reported from India so far.

The search for a genetic cause for diabetes in children with T1DM with negative autoantibodies identified mutations in the INS gene as a rare cause for MODY (MODY10). Mutations in BLK gene result in β-cell dysfunction and have been shown to be associated with early-onset diabetes (MODY11). Lately, there are reports suggesting mutations in ABCC8 and KCNJ11 genes as causes for MODY (MODY12 and 13, respectively). Among the five patients screened for the ABCC8 gene mutation by us, only one MODY12 mutation has been identified till date.

**Summary of Madras Diabetes Research Foundation Maturity-onset Diabetes of the Young studies**

At the Madras Diabetes Research Foundation (MDRF), Chennai, screening of the common MODY subtypes was carried out in subjects with young-onset diabetes suspected to have MODY. The prevalence of MODY subtypes, such as MODY1, MODY2, and MODY3 among the clinically classified MODY subjects based on our study, were about 3.4%, 3.6%, and 9.6%, respectively; among those with MODY5 phenotype, 12% had MODY5. We have also identified MODY4 and MODY12 mutations in our center.

**Maturity-onset Diabetes of the Young Studies from Other Centers in India**

Recently, Chapla et al carried out MODY genetic testing among young-onset diabetic patients of Asian-Indian origin, using Ion Torrent next-generation sequencing technology. About 19% of the 56 clinically diagnosed MODY subjects carried MODY mutations in one of the MODY genes (HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, and PAX4). Aggarwal et al detected a heterozygous whole gene deletion (Met1_Trp557del) in an Indian patient with RCAD using an multiplex ligation-dependent probe amplification assay.

**Clinical Significance of Maturity-Onset Diabetes of the Young Genetic Testing**

Although MODY probably represents 3%-5% of the diabetes cases, definite molecular diagnosis has important implications both for the patients and their families. Firstly, it allows optimal therapeutic management. Genetic diagnosis of MODY is likely to have important prognostic and therapeutic implications, and will help in treatment planning enabling clinicians to avoid insulin therapy in majority of the patients with confirmed HNF1A and HNF4A mutations and changing them over to sulfonylurea agents. Secondly, a MODY diagnosis is also informative for the families of the carriers; known history of MODY does not preclude the development of another form of diabetes in a first degree relative, but will be helpful for screening purposes and prevent misdiagnosis in other family members. Furthermore, once the mutation
in the index case is known, this information can be used to quickly and readily confirm the diagnosis in affected family members. Elucidating the pathophysiology behind monogenic forms of diabetes can help unravel some of the mysteries underlying the pathogenesis of the more common T2DM.⁵

**Development of a Comprehensive Indian Maturity-onset Diabetes of the Young 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 is available regarding the phenotype and the 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, Bangalore, MDRF has recently launched a ‘monogenic diabetes genetic panel’ where all the 13 MODY subtypes can be assessed at one shot. The panel contains 27 genes which can detect mutations in any of the neonatal and MODY genes causing monogenic diabetes.

**NEONATAL DIABETES MELLITUS**

Neonatal diabetes mellitus is defined as diabetes, either isolated or with syndromic features, diagnosed within the first 6 months of life. It is a relatively rare entity and includes many clinically and genetically heterogeneous disorders that affects approximately 1:100,000 live births.⁴⁸ 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. The severe hyperglycemia 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.⁴⁹-⁵¹

Incidence of NDM was estimated to be 1 in 100,000-300,000 live births based on Slovakian and United Kingdom (UK) registries, but a slightly high incidence of 1 in 90,000 was reported in Italian population. Incidence of NDM, particularly in Indian population, is not studied so far but a number of case studies have been published from India.⁵²-⁵⁷ Our group has been the first to study the genetics of NDM in India. The most common causes of NDM are mutations in the genes encoding the two subunits of the KATP, namely KCNJ11, ABCC8, and INS genes and abnormalities in chromosome 6q24. The less common genetic causes of NDM include defects/mutations in euarkyotic translation initiation factor 2-alpha kinase 3 (EIF2AK3), GCK, solute carrier family 2 (facilitated glucose transporter), member 2 (SLC2A2), solute carrier family 19 (thiamine transporter), member 2 (SLC19A2), PDX1, pancreatic specific transcription factor 1A (PTF1A), HNF1B, forkhead box P3 (FOXP3), zinc finger protein 57 (ZFP57), GLI similar family zinc finger 3 (GLI3), GATA binding protein 6 (GATA6), NEUROD1, neurogenin 3 (NEUROG3), IER31P1, paired box 6 (PAX6) and regulatory factor X6 (RFX6) genes.⁵⁸

**Permanent Neonatal Diabetes Mellitus**

The activating mutations in these two genes—KCNJ11 and ABCC8, are responsible for over 40% of PNDM cases and are good candidates for NDM. Children with KATP mutations usually present with hyperglycemia within the first 6 months of life with the exception of a small percentage with onset between 6 and 12 months of age.⁵⁹ In our NDM study, mutations in KCNJ11, ABCC8, and INS genes were screened in a total of 98 children with PNDM, of whom 23 had mutations in one of the genes (ABCC8, KCNJ11, and INS). Some of the mutations identified are Asp212Tyr, Val86Ala, and two novel intronic variants (IVS22+71C>A and IVS28+46A>C) in ABCC8 gene; Cys42Arg and Arg201Cys in KCNJ11; and Gly32Ser in INS gene. Of the exonic mutations, Asp212Tyr and Val86Ala in ABCC8, Cys42Arg in KCNJ11 and Gly32Ser in INS were de novo in origin.⁶⁰

**Transient Neonatal Diabetes Mellitus**

Transient neonatal diabetes mellitus is defined as diabetes that has its onset within the first weeks of life and remits by 18 months, but may likely relapse in early adulthood. Incidence of TNDM is 1 in 400,000 live births based on UK registry.⁶¹ We found variants Val285Ile in KCNJ11 and Arg653Gln in ABCC8 only in two children with TNDM till date. The children are 2 and 5 years old, respectively and so far diabetes has not relapsed. Onset of diabetes was below 6 months of age in all children with KCNJ11 mutations and all of them presented with ketoacidosis.⁶⁰

**Transfer to Sulfonylurea Therapy**

Mutations in KCNJ11 and ABCC8 genes have important therapeutic implications as sulfonylurea therapy is effective in treating patients with mutations in K channel
subunits.\textsuperscript{6,50,51} We have successfully shifted the children with \textit{KCNJ11} mutations (e.g., Cys42Arg and Arg201Cys) and \textit{ABCC8} (e.g., Val86Ala and Asp212Tyr) from insulin therapy to sulfonylurea treatment.\textsuperscript{62} This is the most important translational aspect of genetic diagnosis of monogenic diabetes.

**Summary of Madras Diabetes Research Foundation Neonatal Diabetes Mellitus Studies**

In summary, among more than 150 clinically diagnosed NDM children (PNDM and TNDM) screened in our center for the common genes implicated with NDM, such as \textit{KCNJ11}, \textit{ABCC8}, and \textit{INS} genes, 19 (9.6\%) had \textit{KCNJ11} gene mutations, 38 (19.3\%) had \textit{ABCC8} gene mutations and 9 (4.6\%) had Insulin gene mutations.

**CONGENITAL HYPERINSULINEMIC HYPOGLYCEMIA**

Congenital hyperinsulinemic hypoglycemia occurs as a consequence of inappropriate and unregulated secretion of insulin by pancreatic \(\beta\)-cells. CHI typically presents in newborn babies and infants as severe and persistent hypoglycemia and is a major cause of hypoglycemia related brain injury and mental retardation. The incidence of CHI may vary from 1 in 35,000–40,000 live births to a considerably higher frequency of 1 in 2,500 in communities where consanguinity is more prevalent.\textsuperscript{63} Molar basis of CHI involves mutations in \textit{ABCC8}, \textit{KCNJ11}, glutamate dehydrogenase 1 (\textit{GLUD1}), GCK, hydroxyacyl coenzyme A dehydrogenase (\textit{HADH}), solute carrier family 16 (monocarboxylate transporter), member 1 (\textit{SLC16A1}), \textit{HNF4A}, and uncoupling protein 2 (\textit{UCP2}). These mutations result in reduced or loss of \textit{KATP} channel function which leads to constant depolarization of the cell membrane and persistent insulin secretion even at very low plasma glucose concentrations. Recessive mutations of these genes cause hyperinsulinism that is unresponsive to treatment with channel agonists like diazoxide while dominant \textit{KATP} mutations have been associated with diazoxide-responsive disease.\textsuperscript{64} CHI is heterogeneous with respect to its clinical presentation, histology, genetics, and response to treatment.\textsuperscript{65,66}

In our center, molecular abnormality was identified in 40\% (16 out of 40) of children with CHI. 14 of these mutations were identified in \textit{ABCC8} gene and two mutations were identified in \textit{KCNJ11} gene. Some of the \textit{ABCC8} gene mutations c.1delA, c.61delG, c.267delT, c.619-629delCCCGAGGACCT, Gln444*, Leu724Pro, Ala847Thr, Trp898*, Leu1454Arg, and IVS30-2A>C were novel and two were previously reported Gly111Arg, Arg598*).\textsuperscript{62}

**Treatment**

Children with CHI who had compound heterozygous and Leu1454Arg mutations responded to diazoxide partially with a dose of 12.5 and 15 mg/kg/day, respectively. A child with the IVS30-2A>C mutation was treated with a dose of 20 mg/kg/day diazoxide, and therefore, was not completely diazoxide responsive. Six children did not respond to diazoxide therapy and of these, four children (those with mutations c.61delG, Gly111Arg, Arg598*, and Leu724Pro) underwent subtotal pancreatectomy. Children with mutations c.1delA and Gln444* were treated with a combination therapy of diazoxide and octreotide to maintain normoglycemia. Children who were not responding to diazoxide were started on injection octreotide as per protocol and those children who were refractory to these medications were planned for pancreatectomy.\textsuperscript{62}

Mutations were identified in 88.9\% of diazoxide unresponsive cases and in 11.1\% of children who were treated with diazoxide. Most of the children who were not harboring 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, the timely prediction of the type of hyperinsulinemia is likely to aid in avoiding hypoglycemia related brain damage.\textsuperscript{62}

**SYNDROMIC FORMS OF DIABETES IN NEONATES AND INFANCY**

In the case of a child with Berardinelli-Seip syndrome, we found a novel homozygous mutation Val67Met and a novel variant Gly137Gly (c.411C>A) in the 1-acylglycerol 3-phosphate O-acyltransferase 2 (\textit{AGPAT2}) upon screening Berardinelli-Seip congenital lipodystrophy 2 (\textit{BSCL2}) and \textit{AGPAT2} genes. Patient with clinical features suggestive of Fanconi-Bickel syndrome, was screened for \textit{SLC2A2} gene and a novel homozygous Leu19Arg mutation was detected. Two patients have been screened for \textit{SLC2A2} gene mutation who had moon like facial features and proximal renal tubule defect suggestive of Fanconi-Bickel syndrome and identified two novel mutations (Leu19Arg, IVS5-3C>T) in \textit{SLC2A2} gene. Consanguinity is present in the families of children with syndromic forms of diabetes.\textsuperscript{60,67} Children with Wolcott-Rallison syndrome (WRS) were mainly characterized by PNDM, epiphysial dysplasia, hepatic and renal dysfunction. Two novel mutations (Trp658Ser, c.1515-1G>T) and one known mutation (Arg1065*) in EIF2AK3
gene were identified in children with WRS. One novel mutation was identified in Wolfram syndrome 1 (WS1) gene in patients with Diabetes insipidus, diabetes mellitus, optic atrophy, and deafness or Wolfram syndrome. One patient who presented with severe insulin resistance, abnormal dentition, growth retardation suggestive of Rabson-Mendenhall syndrome, was screened for insulin receptor gene (INSR) and a known mutation (Pro220Leu) in INSR was detected.

**Clinical Significance of Neonatal Diabetes Genetic Testing**

The early diagnosis and immediate medical management are important for preventing brain injury in patients with CHI. Diazoxide is the mainstay of the medical therapy, but is ineffective in children with diffuse CHI due to inactivating mutations in KATP channel genes (ABCC8 and KCNJ11) and in those children with focal CHI. Rapid genetic analysis to screen for mutations in ABCC8 and KCNJ11 genes helps in identification of the patients with diffuse disease and those with focal CHI, helping clinicians in treating this heterogeneous disease.

In India, genetic testing for monogenic diabetes is available at MDRF (www.neonataldiabetes.in). NDM, CHI, and monogenic syndromes are screened for free of cost. So far, around 300 MODY and 150 NDM children have been registered through the registry at our center and benefited from genetic testing. Our genetic diagnosis has made it possible to successfully shift some of the children with KCNJ11 and ABCC8 mutations from insulin treatment to oral sulfonylurea therapy. This is an important direct translation of genetic analysis from bench to bedside in clinical practice.

**REFERENCES**


