

Molecular diagnosis of maturity onset diabetes of the young in India

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ABSTRACT

Diabetes is highly prevalent in India and the proportion of younger patients developing diabetes is on the increase. Apart from the more universally known type 1 diabetes and obesity related type 2 diabetes, monogenic forms of diabetes are also suspected to be prevalent in many young diabetic patients. The identification of the genetic basis of the disease not only guides in therapeutic decision making, but also aids in genetic counselling and prognostication. Genetic testing may establish the occurrence and frequency of early diabetes in our population. This review attempts to explore the utilities and horizons of molecular genetics in the field of maturity onset diabetes of the young (MODY), which include the commoner forms of monogenic diabetes.

Key words: Consanguinity, epigenetics, maturity onset diabetes of the young, molecular diagnosis, monogenic diabetes, neonatal diabetes

INTRODUCTION

According to the International Diabetes Federation (IDF) Atlas 2011, India stands second in the world with regards to the prevalence of diabetes mellitus. Nearly sixty two million people are affected at present and the number is expected to rise to more than hundred million by 2030.^[1] Type 2 diabetes is the commonest cause for this disorder followed by type 1 diabetes; both of which account for more than 95% of cases. Less frequent causes include pancreatic diabetes, monogenic diabetes and secondary forms of diabetes mellitus. The proportion of young people affected with type 2 diabetes is on the increase which parallels the obesity epidemic worldwide with an impact on developing countries as well.^[2] There is a shift to the left in the age of onset of type 1 diabetes as shown by studies across the world,^[3] though no data has been published from India in

this regard. Since diabetes is a silent killer and India being one of the heavily affected countries, more focus needs to be placed on patient care as well as research. Diagnosis of the etiology of young onset diabetes is sometimes a puzzle. Overlapping or atypical features often lead to misdiagnosis. A single clinical characteristic or lab finding may not help reach the diagnosis in such cases. The algorithm [Figure 1] provided below is an attempt to approach a young patient with diabetes.

Monogenic diabetes

Monogenic diabetes is defined as diabetes that occur due to a mutation of a single gene affecting β -cell function. These mutations involve genes coding for transcription factors involved in β -cell development, differentiation and survival; as well as enzymes like glucokinase (GCK) and carboxy ester lipase (CEL). Neonatal diabetes mellitus (NNDM) and Maturity onset diabetes of the young (MODY) are the 2 main types of monogenic diabetes that result from β -cell dysfunction. Other forms of monogenic diabetes result in defective insulin action or insulin receptor dysfunction.

NEONATAL DIABETES

Neonatal diabetes mellitus (NNDM) is a rare disorder with a reported incidence of 1 in 2 60 000 live births in

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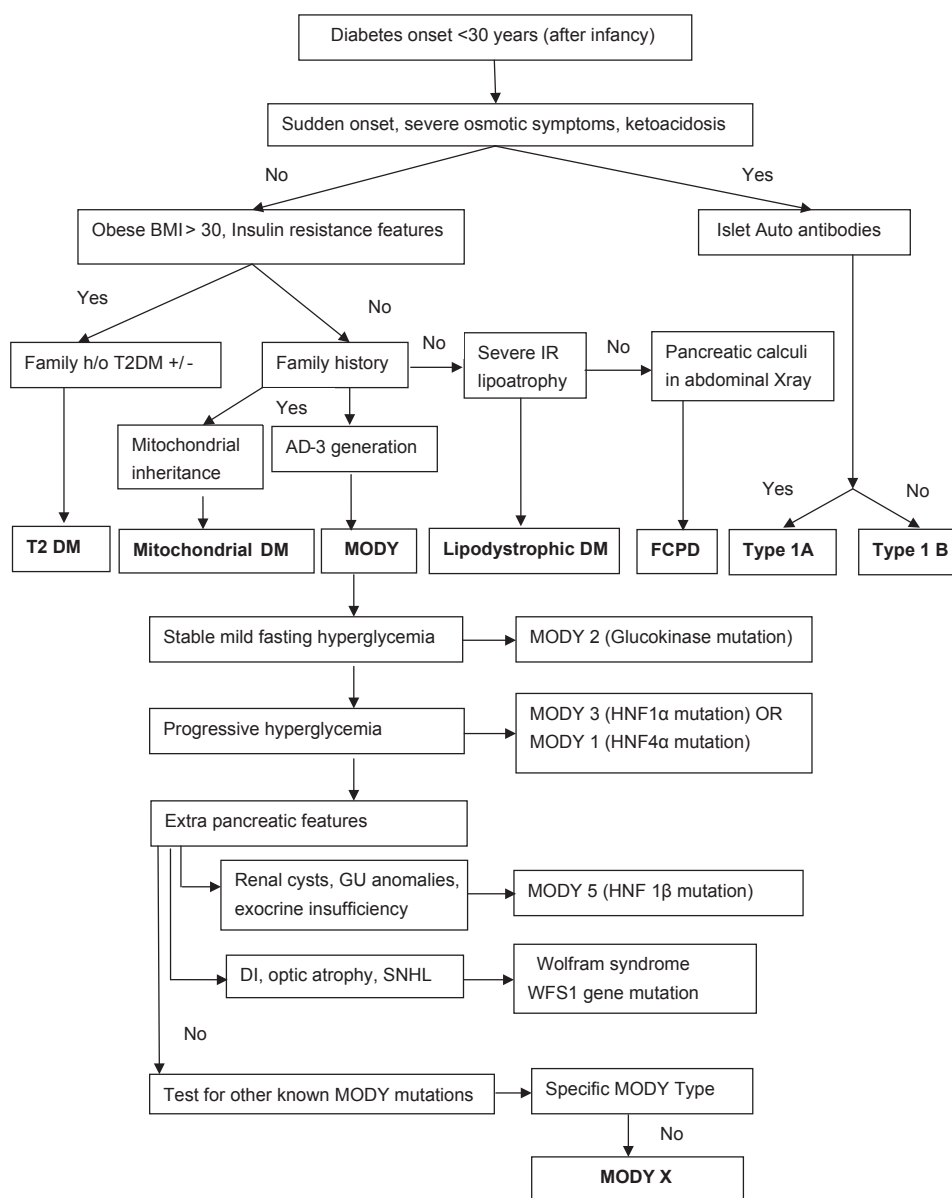


Figure 1: Diagnostic approach to young onset diabetes

European population.^[4] By definition, neonatal diabetes mellitus, presents within the first 6 months of life with failure to thrive, dehydration and sepsis with or without ketoacidosis. Based on its clinical course, 2 forms have been recognised-transient and permanent. In short, approximately half of these cases have a transient course which resolves in the first few months of life, while others might require lifelong insulin therapy.

Transient neonatal diabetes

Transient neonatal diabetes mellitus (TNDM) results from a developmental defect in β -cell maturation which resolves spontaneously during the postnatal period. It manifests as insulin requiring hyperglycemia within the first week of life. Affected babies are generally growth retarded as insulin is

a major foetal growth factor. Ketoacidosis rarely occurs in TNDM. The condition usually resolves within the first year of life. Infants born with TNDM harbour a greater risk of developing type 2 diabetes later in the life. The persistence of the underlying pancreatic defect predisposes to a heightened risk, especially during periods of increased demand like puberty and pregnancy.

The genetics of transient neonatal diabetes

The molecular genetic mechanism resulting in TNDM has been elucidated in a majority of cases. Two third of cases of TNDM results from an imprinting defect on chromosome 6q24.^[5] Mutations in the KCNJ11 and ABCC8 genes which encode for Kir6.2 and SUR1 subunits of the ATP sensitive potassium channel, usually result in permanent neonatal

diabetes mellitus (PNDM), though transient forms have been reported.^[6] Heterozygous mutations in HNF-1 β gene have also been shown to result in TNDM though the typical presentation is later in the young adult as MODY5.^[7] Transient neonatal diabetes is a well established example of a genomic imprinting disorder. Genomic imprinting is a phenomenon where the phenotypic expression of a particular genetic allele in an individual depends on the parent from which the allele is transmitted. In other words, the allele from one parent is 'silenced' through epigenetic mechanisms as methylation and histone modification which results in a monoallelic gene expression. Mutations affecting this single allele have a greater chance of affecting the gene function which results in disease. Abnormalities in the imprinted region of chromosome 6q24 results in the majority of cases of TNDM.^[8,9] In the normal foetus only 1 allele of chromosome 6q24 is expressed and this is of paternal origin; the maternal allele being silenced by methylation. Over expression of the paternal allele results from uniparental di-somy or duplication of the 6q24 region of the paternal chromosome. Activation of the maternal allele can occur as a result of hypomethylation of the maternal differentially methylated region (DMR). This results in TNDM as the imprinted region has been shown to contain genes which regulate apoptosis, insulin secretion and foetal growth namely PLAGL1 (Pleomorphic adenoma gene like-1 (also known as ZAC)) and HYMAI- (Hydatidiform mole associated and imprinted).^[8] Studies in animal models have proven that over expression of genes in the 6q24 locus adversely affects islet cell development.^[10] However, the cellular mechanism of hyperglycemia and its spontaneous resolution in the postnatal period are not well understood. The ZAC gene is also shown to regulate the imprinted region on chromosome 11p15.5 near the Beckwith-Weidmann syndrome locus and 2 other growth regulatory genes namely *igfr2* and *igfr2r* which possibly explain the sharing of associated features such as macroglossia and umbilical hernia in both conditions.^[11]

Permanent neonatal diabetes

Permanent neonatal diabetes mellitus (PNDM) is characterised by the early onset of persistent hyperglycemia requiring lifelong treatment. All cases of PNDM result from mutations affecting genes regulating pancreatic development, β -cell function, apoptosis or the insulin molecule as such. Approximately half the cases of PNDM in the Caucasian population have been shown to involve defects in the genes transcribing the 2 subunits of K⁺ ATP channel Kir6.2 and SUR1, which regulate insulin release from the β -cell.^[12,13] These subunit proteins are transcribed by KCNJ11 and ABCC8 genes respectively. Other genes implicated in PNDM include PTF-1 α , IPF-1 (Pdx-1), EIF-2AK3, Glucokinase, FOXP3, insulin and GLIS3.

PNDM due to activating mutations in potassium sensitive channels

Glucose sensing by the β -cell and subsequent insulin release is a complex cascade involving multiple receptors and channels. The glucose molecule that is transported into the β -cell through the GLUT receptor undergoes hydrolysis after phosphorylation. The pyruvate molecule that is generated is then metabolised inside the mitochondria to CO₂ and H₂O to generate ATP molecules. The increased ATP/ADP ratio inside the β -cell closes the potassium channels on the cell membrane resulting in depolarization which subsequently activates the voltage gated calcium channels. The influx of calcium ions causes release of insulin from the secretory granules. The potassium sensitive channel on the β cell membrane has two kinds of subunits-Kir6.2 (Potassium inward rectifying) and SUR1 (sulphonylurea receptor 1) which are encoded by 2 different genes – KCNJ11 (Potassium Channel, subfamily J, member 11) and ABCC8 (ATP Binding Cassette transporter subfamily C, member 8) respectively. Activating mutations in these genes result in persistent opening of the potassium channels preventing insulin release resulting in impaired glucose sensing and hence diabetes. However, the mutant channels retain the ability to bind and respond to sulphonylureas. These potassium channel subunits are expressed in non pancreatic tissues as well; SUR1 being found in neurons while Kir6.2 in the brain, heart and skeletal muscles. This explains the occurrence of associated features like developmental delay, epilepsy and muscle weakness (DEND – Developmental delay, Epilepsy and Neonatal Diabetes- syndrome) in infants with NNDM due to these mutations.^[14]

PTF-1 α (Pancreatic transcription factor 1, α subunit)

PTF-1 α is a transcription factor with critical roles in foetal pancreatic development and maintenance of mature pancreatic function. It has also been found to regulate cerebellar development. Homozygous mutations in gene encoding PTF-1 α results in PNDM characterised by cerebellar hypoplasia, pancreatic agenesis, intrauterine growth retardation, respiratory distress and microcephaly as described initially in a consanguineous Pakistani family.^[15]

IPF-1 (Insulin promoter factor-1) or PDX-1 (Pancreatic and duodenal homeobox 1)

IPF-1/PDX-1 is one of the early transcription factors regulating the development of both exocrine and endocrine pancreas in the foetus. Homozygous loss of function mutation in the gene encoding this factor results in PNDM with exocrine deficiency due to pancreatic agenesis.^[16] Heterozygous mutations in the same gene has been shown to result in early onset diabetes in the young (MODY4) due to impaired β -cell function.

Glucokinase (GCK)

The glucokinase enzyme is the glucose sensor of the β -cell. It phosphorylates the glucose molecules entering the cell and initiates subsequent pathways leading to insulin release. Homozygous mutations in the GCK gene results in complete loss of glycolytic activity in the β -cell resulting in PNDM. In the heterozygous state it results in mild impairment of insulin release manifesting as maturity onset diabetes of the young (MODY2) or as gestational diabetes.^[17]

Eukaryotic translation initiation factor-2 kinase-3 (EIF2AK3)-wolcott-rallison syndrome

Wolcott Rallison syndrome is an autosomal recessive disorder characterised by infantile onset diabetes, multiple epiphyseal dysplasia, growth retardation, exocrine pancreatic dysfunction, osteopenia, developmental delay, hypothyroidism, acute liver failure, renal failure, cardiomegaly, cerebellar cortical dysplasia and early death. The molecular defect is localised to the EIF-2AK3 gene on chromosome 2p12 which regulates protein synthesis and folding. The resultant misfolded proteins increase the stress in the endoplasmic reticulum of various tissues leading to cell death by apoptosis which explains the various phenotypic features.^[18]

FOXP3 gene mutation (Immune dysregulation, polyendocrinopathy and enteropathy, X-linked-IPEX syndrome)

The IPEX syndrome is an X-linked disorder characterised by the combination of PNDM, intractable diarrhoea with villous atrophy, exfoliative dermatitis, autoimmune hypothyroidism, haemolytic anaemia, recurrent infections due to immune deficiency. The condition is fatal and often results in death before the first birthday. A genetic defect has been identified in the FOXP3 gene on the X chromosome which codes for a forkhead domain-containing protein known as 'scurfin' that is required for immune homeostasis.^[19] Studies in the mouse model have shown over proliferation of CD⁴⁺/CD⁸⁻ T-lymphocytes with infiltration of multiple organs.^[20] There is an increased predilection for autoimmunity in this condition. The diabetes mellitus in this disorder is due to autoimmune destruction of the β -cells as suggested by the presence of a number of islet cell autoantibodies.^[19]

Insulin gene mutations

Mutations in the insulin gene have been shown to affect the normal folding of the insulin molecule inside the endoplasmic reticulum resulting in reduced insulin release. The abnormal/misfolded protein increases the intracellular stress leading to apoptosis and cell death.^[21] The age of presentation and severity of diabetes in this condition varies widely. It ranges from severe early onset neonatal diabetes

to a mild form of MODY and gestational diabetes. Absence of extrapancreatic features differentiates this disorder from that due to the EIF-2AK3 mutation.

GLIS3 – (GLI subfamily of Kruppel-like zinc finger protein-3)

Mutations in GLIS3, yet another transcription factor gene, have been shown to be associated with PNDM with multisystem involvement.^[22] The affected infant presents with intrauterine growth retardation, neonatal onset diabetes, congenital hypothyroidism, congenital glaucoma, renal cysts and dysmorphic facies. The protein encoded by the GLIS3 gene plays an important role in various cellular processes during foetal development including that of pancreatic β -cells.

SLC19A2 (Soluble carrier family 19, member 2) – Thiamine transporter mutations

Recessive SLC-19A2 mutations have been shown to result in early onset diabetes, thiamine responsive megaloblastic anaemia and deafness. Neurological deficits, cardiac abnormalities and visual problems are also reported in some of these cases.^[23]

Table 1 summarizes the important mutations that cause neonatal diabetes and their characteristics features.^[24,25] Identification of the genetic defect in neonatal diabetes helps in tailoring a treatment strategy. Neonatal diabetes due to mutations in the ABCC8 and KCNJ11 genes generally respond well to oral sulfonylureas.^[26,27] This allows a shift from insulin therapy to oral drugs which gives immense logistic as well as a psychological advantage to the parents of the patients. Sulfonylurea therapy has been found to ameliorate the associated neurological dysfunction and hence early initiation of the drug is of utmost importance for an improvement in long term prognosis.^[28]

So far, mutations in more than ten genes have been identified as a cause for neonatal diabetes. Recently a very high incidence of PNDM is reported from Saudi Arabia from a highly consanguineous population (1 in 21, 196 live births). Fourteen out of seventeen PNDM cases had an identifiable genetic aetiology of which 50% were due to the autosomal recessive Wolcott Rallison syndrome. Interestingly none of the patients had a mutation in KCNJ11, ABCC8 or INS genes.^[29] There are case reports on several individual mutations causing NNDM from different parts of India localised on KCNJ11,^[30] insulin gene,^[31] and a novel mutation in the ABCC8 gene.^[32] However no population based study has been published till now. Hence the true prevalence of each mutation is not known in our population. A Neonatal Diabetes Registry has been established in India where facilities are available to sequence KCNJ11, ABCC8 and insulin genes.

Table 1: Genetic mutations causing neonatal diabetes and special characteristics^[24,25]

Gene	PNDM/TNDM	Inheritance	Extrapaneacreatic features	Treatment of DM
Imprinting defect on 6q24 KCNJ11 (Kir6.2)	TNDM PNDM TNDM	AD	Macroglossia, umbilical hernia Developmental delay epilepsy	Insulin until remission Can be treated with sulfonyl ureas
ABCC8 (SUR1)	PNDM TNDM	AD	Developmental delay	Can be treated with sulfonyl ureas
EIF-2Ak3 (Wolcott and Rallison syndrome)	PNDM	AR	Epiphyseal dysplasia, osteopenia, acute liver failure, developmental delay hypothyroidism	Insulin
FOXP3 (IPEX syndrome)	PNDM	X-linked	Chronic diarrhoea with villous atrophy, pancreatic and thyroid autoantibodies, thyroiditis, eczema, anemia	Insulin
INS	PNDM		None	Insulin
GCK	PNDM	AR	Heterozygous parents	Insulin
IPF-1	PNDM	AR	Heterozygous parents	Insulin
PTF-1A	PNDM	AR	Cerebellar hypoplasia, neurological dysfunction	Insulin

IPEX: Immune dysregulation, polyendocrinopathy, enteropathy, X-linked, INS: Insulin gene, GCK: Glucokinase, IPF-1: Insulin promoter factor-1, PTF-1A: Pancreatic transcription factor 1 alpha subunit, PNDM: Permanent neonatal diabetes mellitus, TNDM: Transient neonatal diabetes mellitus

MATURITY ONSET DIABETES OF THE YOUNG

The early 1970's witnessed identification of a novel form of diabetes wherein younger people were affected with a familial clustering.^[33] These patients had mild or no disease progression and did not require insulin. Subsequently this group came to be called as maturity onset diabetes of the young and was shown to have an autosomal dominant mode of inheritance. Tattersall and Fajans in 1975, proposed the clinical criteria for diagnosing MODY in a diabetic patient which were as follows:^[34]

1. Early onset of Diabetes (<25 years)
2. Early onset Diabetes in at least 2 or ideally 3 family members
3. Autosomal dominant mode of inheritance
4. Non-insulin dependence (not requiring insulin even after 5 years of diagnosis)

Based on these criteria, the reported prevalence of MODY was 1-2% in Europe.^[35] A high prevalence of MODY was reported from India also as early as 1985.^[36] However this was based on clinical presumption and not substantiated by genetic testing. The molecular genetic basis of this condition was unravelled in the 1990's when 5 of the genetic loci were identified and characterised by various investigators. Thereafter, these conditions were referred to as MODY 1-5 based on the sequence of genes identified. Subsequent years have witnessed an explosion of knowledge in this field with 6 more gene loci harbouring mutations resulting in MODY being identified. It is now clear that the proverbial iceberg effect exists in MODY as well. This was due to a vast majority of diabetic patients being misclassified stereotypically as either type 1 diabetes or type 2 diabetes in the presence of overlapping or atypical features.

Genetic basis of maturity onset diabetes of the young

The MODY group of monogenic diabetes results from

heterozygous mutations in the genes coding for various transcription factors involved in the foetal development of the pancreas and the β -cells as well as those regulating the maturation and maintenance of β -cell function after birth. In addition, mutations in enzymes directly or indirectly involved in glucose sensing of the β -cell have also been shown to result in early onset diabetes. There is wide clinical as well as genetic heterogeneity within and among the different MODY subtypes. The following section gives a brief account of the molecular mechanism and clinical peculiarities of each MODY subtype. Table 2 displays a summary of the different MODY mutations described so far.

MODY due to mutations in transcription factor genes

The different transcription factors involved in foetal pancreatogenesis include hepatocyte nuclear factor 1- α (HNF-1 α), HNF-4 α , HNF-1 β , IPF-1 (Insulin Promoter Factor-1), Neuro-D1 (Neurogenic Differentiation Factor-1) and Pax 4 and 6 (Paired Homeobox).^[37]

HNF-1 α mutations (MODY3)

HNF-1 α or TCF-1 (Transcription Factor 1) is a homeo-domain containing transcription factor developmentally expressed in the liver, kidney, pancreas and gut. It has a crucial role in regulation of transcription of insulin gene in the mature β -cell as well as the glucose transporter GLUT2.^[37,38] The 631 amino acid protein is encoded by the HNF-1 α gene located on chromosome 12q. Mutations in the HNF-1 α gene leads to progressive insulin secretory defect resulting in a severe form of inherited diabetes that is classically termed MODY3.^[39] Recently, it has been established that in HNF-1 α mutation carriers β -cell apoptosis is increased. The apoptotic cells induce production of pancreatic stone protein (PSP)/reg1 A from the surviving cells, which serves as a marker of the pathologic process.^[40] The reduced β -cell mass results in

Table 2: MODY subtypes described so far with clinical characteristics^[78-80]

MODY type	Gene locus	Chromosome location	Presentation age	Phenotypic features
1	HNF-4A	20q13	Adolescent/early adult	Progressive insulin secretory defect. Fetal macrosomia, transient neonatal hypoglycemia
2	GCK	7p13	Since birth	Stable mild fasting hyperglycemia. Asymptomatic/abnormal OGTT/GDM/LBW. Microvascular complications rare
3	HNF-1A	12q24	Adolescent/early adult	Progressive insulin secretory defect renal glycosuria
4	IPF-1	13q12	Early adult	Very rare, mild diabetes (in heterozygous form)
5	HNF-1B	17q12	Adolescent	Progressive diabetes, cystic renal disease (RCAD), genitourinary anomalies, pancreatic atrophy, exocrine dysfunction, hyperuricemia
6	Neuro-D1	2q31	Early adult	Rare
7	KLF-11	2p25	Early adult	Pancreatic atrophy, exocrine pancreatic dysfunction
8	CEL	9q34	Early adult	Rare
9	Pax-4	7q32	Early adult	Rare
10	INS	11p15.5		Rare
11	BLK	8p23		Rare
X	Unknown	-		-

MODY: Molecular diagnosis of maturity onset diabetes of the young, GCK: Glucokinase, CEL: Carboxy ester lipase, RCAD: Renal cyst and diabetes

defective glucose handling which is evidenced by large increments in postprandial 2 hour blood sugar values during a 75 g OGTT. HNF-1 α is also functional in the kidneys in the regulation of proximal renal tubular reabsorption of glucose mediated through sodium glucose transporter-2.^[41] Hence patients with MODY3 also exhibit low renal threshold for glucose resulting in renal glycosuria. MODY3 patients are sensitive to sulfonylureas, though many patients finally require insulin on a long term basis. Long term microvascular complications occur with the same frequency as in type 2 and type 1 diabetes.^[42] Liver adenomas have also been reported to occur in MODY3 subjects, though the association is rare.^[43]

The HNF-1 α mutation is the most common form of MODY reported in studies from UK and from many European countries.^[44-47] A recent study from Argentina reported a prevalence of 10% in a group of clinically characterised MODY patients of Caucasian ethnicity.^[48] However studies in Japanese and Brazilian populations showed a very low prevalence of the HNF-1 α gene defects in early onset diabetes.^[49,50] In a recent publication, the French Monogenic Diabetes Study group reported a prevalence of 39% of HNF-1 α gene mutation in their cohort. 40% of the mutation proven MODY 3 subjects had their age at diagnosis above twenty five years and only about half of these subjects had 3 generations of family history of diabetes. Nearly one third (28%) of the subjects had a BMI of >25kg/m².^[51] Similar figures had been reported from an Irish MODY cohort.^[52] This points towards the fact that many cases of MODY3 may be missed if strict criteria are used for patient selection. During a median follow up of twenty years in the French cohort, 45% of the patients required initiation of insulin therapy.

HNF-4 α mutation (MODY1)

HNF-4 α and HNF-1 α transcription factors have been found to exhibit a mutual regulatory function in the mature β -cell.^[53] Heterozygous mutations in HNF4 α results in a phenotype that resembles MODY3.^[54] In addition, it has been observed that such patients are more sensitive to the hypoglycaemic effect of sulfonylureas.^[55,56] Mutations in HNF-4 α have been reported to result in macrosomia and transient hyperinsulinemic hypoglycaemia in the neonatal period and these subjects subsequently develop an early onset diabetes mellitus (MODY1).^[57] The reported prevalence of HNF-4 α mutations is less than 5%.^[58] However, up to a 30% mutation detection rate for HNF-4 α has been reported in those screened negative for HNF-1 α mutations in a clinically suggestive MODY cohort.^[59]

Insulin Promoter Factor-1/Pancreatoduodenal homeobox-1 (Pdx-1) mutations

IPF-1 is a transcription factor which is important in early pancreatic development, differentiation, maturation and maintenance of β -cells as well as transcriptional regulation of insulin, GLUT2, Glucokinase and Glucagon genes in the mature β -cell. Homozygous mutations in IPF-1 leads to pancreatic agenesis resulting in permanent neonatal diabetes and exocrine pancreatic insufficiency.^[60] Heterozygous mutations lead to a variable defect in insulin secretion and diabetes (MODY4).^[61]

HNF-1 β mutations (MODY5)

HNF-1 β , also known as transcription factor-2 (TCF-2), is a member of basic helix-loop-helix proteins. In the embryonic period HNF-1 β is involved in the differentiation of the visceral endoderm. Apart from its role in pancreatic development, HNF-1 β is required for development of the kidneys and the genital tract. Hence mutations in

HNF-1 β results in pancreatic insufficiency that includes both exocrine and endocrine dysfunction, cystic renal disease (RCAD-Renal cyst and diabetes syndrome) and anomalies of the vagina and the mullerian structures ranging from uterine aplasia to a bi-cornuate uterus. Early onset slowly progressive non diabetic renal failure may occur in some affected patients.^[62,63] A wide spectrum of renal and genitourinary manifestations has been reported in HNF-1 β mutation carriers.^[64] The renal defect can manifest well before the onset of diabetes.

Neurogenic differentiation factor 1 (MODY6)

Neurogenic differentiation factor 1 (Neuro-D1) is an important transcription factor required for pancreatic development as well as β -cell differentiation. It has also been expressed in the enterocytes and the neurons. Mutations in Neuro-D1 results in an autosomal dominant disorder which resembles type 2 diabetes (MODY6).^[65,66]

MODY due to enzymatic mutations

Glucokinase gene mutation (MODY2)

The prime role of Glucokinase (GCK) enzyme in the glucose sensing mechanism of the β -cell is already explained in the section of neonatal diabetes. Heterozygous mutations affecting the GCK gene results in mild fasting hyperglycemia that has been demonstrable from birth but is non progressive.^[67] The Glucokinase enzyme is also functional in the liver. As a result of the molecular defect, there is reduced hepatic glucose sensing especially after a meal resulting in reduced glycogen synthesis and stimulation of hepatic gluconeogenesis manifesting as postprandial hyperglycemia. However due to a milder degree of hyperglycemia, long term microvascular complications rarely occur in MODY2 compared to other forms of diabetes. GCK mutations may remain asymptomatic in nearly half of the carriers. Pregnancy unmasks the defect in some patients. GCK mutations have been shown to be responsible for gestational diabetes mellitus in 80% of Caucasian pregnant women when strict clinical criteria were applied for patient selection.^[68] An affected mother can transmit the genetic defect to the offspring in which case the foetus is born with low birth weight owing to reduced insulin availability in the intrauterine period. However an unaffected foetus can develop macrosomia under the effect of maternal hyperglycemia.^[69] Till date more than one hundred twenty different GCK mutations have been identified, each of which causes a variable degree of functional impairment.

Carboxy ester lipase enzyme-CEL (MODY8)

CEL, also known as Bile salt activated lipase, is secreted by the pancreatic acinar cells and is involved in hydrolysis of cholesteryl esters. Genome wide screen detected mutations in the carboxy ester lipase (CEL) gene to be a rare cause

for diabetes and exocrine pancreatic insufficiency.^[70] Subsequent research has suggested that the underlying functional defect could be due the accumulation of the misfolded protein product of the mutant gene in the pancreas.^[71]

Other MODY mutations described

Several other gene mutations have been discovered in recent years as rare causes for early onset diabetes.

The Kruppel like factor 11 is a transcription factor which regulates the transcription of IPF-1 (Pdx-1), one of the crucial transcription factors involved in early pancreatic development.^[72] Recently mutations in this gene are identified to be linked with MODY7. Pax 4 and Pax 6 are transcription factors involved in islet cell differentiation. In 2007 two novel mutations in the Pax 4 gene were identified in 2 patients from a Thai population as the cause for diabetes (now termed MODY9).^[73]

Homozygous mutations in insulin gene resulting in PNDM have been discussed under the section of neonatal diabetes. The search for a genetic cause for diabetes in children with type 1 diabetes with negative autoantibodies identified mutations in the insulin gene as a rare cause for MODY (MODY10).^[74]

The BLK gene codes for a non receptor tyrosine kinase of the *src* family of proto oncogenes which are involved in cell proliferation and differentiation. It is also found to exert a modulatory role in β -cell development and function. Mutations in BLK gene result in β -cell dysfunction and have been shown to be associated with early onset diabetes (MODY11).^[75] Lately, there are reports suggesting mutations in ABCC8 and KCNJ11 genes as causes for MODY (MODY12 and 13 respectively).^[76,77]

WHY IS MOLECULAR DIAGNOSIS OF MODY IMPORTANT?

There is extensive genetic heterogeneity with a wide phenotypic spectrum within and among the MODY groups. Table 2 displays the known MODY mutations and the clinical characteristics.^[78-80] It is therefore important to characterize the different types of MODY and to distinguish it from other types of diabetes, because the treatment protocols for achieving optimal glycaemic control are specific for each type of MODY. Hyperglycemia due to mutations in HNF-1 α has been found to be well controlled with Sulfonylureas for a number of years.^[81] On the other hand, heterozygous mutations in the GCK gene (MODY2) do not require any pharmacological treatment except in certain physiologically demanding situations like pregnancy.

The clinical spectrum of MODY overlap with the features of both type 1 and type 2 diabetes, thus presenting a diagnostic challenge. Especially in a developing country like India, where there is lack of awareness for a genetic diagnosis of MODY, the probability of MODY being falsely diagnosed as either type 1 or type 2 diabetes is rather high. This may result in patients receiving inappropriate therapy with suboptimal glycaemic control. Identifying specific genes involved in MODY will have potential therapeutic benefit by streamlining the therapy for the particular genotype, better prognostication of the clinical course of the disease and widening our knowledge to give appropriate genetic counselling to the affected individuals.

On a wider perspective, molecular diagnosis of monogenic diabetes is necessary for:

1. Characterising the frequency of disease in the population

Shields *et al.* has reported that 80% of MODY cases in UK are misdiagnosed as either type 1 or type 2 diabetes.^[82] While considering the high prevalence of diabetes in India, these figures are more likely to be higher. An alarming proportion (28%) of adolescents and young adults from Southern India were detected to have impaired fasting glucose during a search for metabolic abnormalities and their anthropometric correlates.^[83] Some of these subjects could be harbouring asymptomatic MODY mutations particularly involving the GCK gene.

2. Establishing a direction for applied pharmacogenomics

Patients with mutations in HNF-4 α (MODY1) and HNF-1 α (MODY3) respond well to sulphonylureas alone, while mutations in GCK (MODY2) are managed with diet alone, and rarely require pharmacotherapy. Mutations in the HNF-1 β gene results in severe β -cell dysfunction and these patients require insulin right from a very early stage of the disease. Even though one can diagnostically suspect MODY based on the phenotypical features we have mentioned earlier, one cannot treat the patient without knowing the specific genotype. The specific management protocols for each genetic subtype mandates genetic analysis in patients with clinical suspicion of MODY.

3. Avoiding insulin therapy

Given the fact that MODY is misdiagnosed as either type 1 or type 2 diabetes, many patients end up receiving insulin, along with the complications of insulin therapy and manage to achieve only suboptimal glycaemic control.^[84] This can be avoided, and treatment can be streamlined if the genetic subtype is known via molecular genetic methods.

4. Understanding the relationship between MODY mutations and birth weight

It has been proven beyond doubt that a strong

relationship exists between maternal malnutrition, low-birth weight and the predisposition to metabolic and cardiovascular disease in later life.^[85,86] Studies have demonstrated that exposure to sub-optimal diet during pregnancy leads to gene expression changes resulting in 'intra uterine programming', thus helping the foetus in adapting to a suboptimal environment. Overfeeding of these infants in the postnatal period results in the onset of metabolic disease in the early years of life.^[87] Epigenetic studies have shown reduced transcription of the HNF-4 α gene (HNF-4 α is a key transcription factor required for β -cell differentiation and glucose homeostasis) in rat models exposed to low-protein maternal diet during pregnancy and lactation. Such rats also developed diabetes at seventeen months of age showing continued repression of the gene throughout the ageing process.^[88] Given the status of maternal malnutrition in our country, molecular studies might help establish the possible association between epigenetic regulation of HNF-4A, low birth weight and early onset diabetes. Genome-wide association scans have further proven the genetic association between low birth weight and type 2 diabetes in early adulthood. Genetic variants in the ADCY5 (Adenylate Cyclase 5) and CCNL1 (Cyclin L1) gene loci have been shown to be associated with low birth weight in Europeans.^[89] However, the birth weight lowering effect of the ADCY5 polymorphism could not be replicated in a large Indian birth cohort, though its effect on glucose-insulin homeostasis and the risk of type 2 diabetes was well documented.^[90]

5. Identifying the effect of consanguinity on the frequency of MODY

The 1992-1993 National Family Health Survey had shown high frequency of consanguinity and co-efficient of inbreeding in Southern Indian states of Tamil Nadu, Karnataka and Andhra Pradesh.^[91] In this context, a higher rate of genetic disorders as MODY may be expected in the population. It is likely that MODY is an under diagnosed subset of diabetes in our country than is thought of.

6. Unravelling the impact of variations in the susceptibility genes on MODY

Genome-wide association studies have identified several susceptibility genes for developing type 2 diabetes.^[92] Polymorphisms in the transcription factor 7-like 2 (TCF-7L2) gene have been shown to be strongly associated with increasing risk of developing type 2 diabetes in populations of various ethnicities including Asians.^[93,94] Hypothetically speaking, the TCF-7L2 polymorphisms can augment the onset of MODY at an earlier age or modify its clinical expression. It would

be interesting to investigate this possible interaction through genetic studies.

What is the status of monogenic diabetes worldwide?

Significant progress in molecular research is taking place worldwide. At the time of writing this paper eleven genes have been identified to harbour MODY related mutations. Epidemiological studies from different ethnic groups have shown varying frequencies of prevalence of these mutations.^[95-97] Many countries are now offering molecular diagnosis to young people with diabetes before a therapeutic decision can be made. Additionally some of them have established monogenic diabetes registries.

Molecular diagnostic platform used in MODY

Sanger sequencing is a widely used genetic diagnostic platform for identifying MODY and is the gold standard sequencing platform to test single-gene genetic disorders. This sequencing technique has been reported to be >99% sensitive to detect a heterozygous base substitution in MODY genes. Only 3 genes i.e. HNF-1 α , HNF-4 α and GCK genes are sequenced and are available under as a diagnostic modality in European countries. The turn around time for the genetic diagnosis of MODY for sequencing these 3 genes is around forty working days for unknown mutations and twenty working days for known mutations.

Based on the 2008 best practice guidelines for genetic diagnosis of MODY, it has been recommended that genetic testing should be performed only for subjects who fit into the clinical criteria of MODY.^[80] However it has been reported that >50% of patients with MODY do not present with a classical picture of MODY. The genetic diagnosis of MODY has been recommended by a clinician only when he considers that a genetic diagnosis may make a difference in the clinical management of the patient. Furthermore, owing to issues of affordability, there is a delay from the time of diagnosis of diabetes to the definitive genetic diagnosis of MODY. At present due to limitations in throughput and scalability of the present Sanger sequencing, there are limitations to genetically characterizing patients with MODY, in a fast, comprehensive and cost-efficient manner.

Status of MODY genetics in India

India is making rapid advances in the field of molecular genetics and its application in the field of health and disease. However, few groups of investigators have embarked on the molecular genetic studies in diabetes particularly MODY. The performed studies have been centred on mutations on very few genes which were reported to be relatively common in the western population. Hence, data about MODY in India is basic and preliminary. In 2005, Mohan *et al.* reported the first single nucleotide polymorphism in HNF-1 α gene associated with MODY and early type 2

diabetes from India.^[98] They identified that frequency of Ala98Val polymorphism in the HNF-1 α gene is significantly higher in MODY patients. The same polymorphism was associated with earlier age of diabetes onset in type 2 diabetes. However, since this study specifically looked at only 1 polymorphism in HNF-1 α gene among the several genes involved and there could be several polymorphisms associated with each MODY gene, it cannot be representative of the entire genetic subtypes of MODY.

Another study from Northern India by Sahu *et al.* showed that A3243G mitochondrial mutation and HNF-1 α gene mutations were infrequently associated with early onset type 2 diabetes.^[99] However, this study attempted to look at the specific aetiologies of early onset type diabetes and was not uniquely focused on the genetic basis of MODY. In 2009 Radha *et al.* demonstrated that mutations in the HNF-1 α gene comprise about 9% of clinically diagnosed MODY subjects in southern India.^[100] They sequenced the coding and promoter regions of HNF-1 α gene for mutations in unrelated South Indian subjects, in whom a clinical diagnosis of MODY was made. Nine novel variants comprising 7 mutations (1 novel mutation-538G > C at promoter region and 6 novel coding region mutations) and 2 polymorphisms in the HNF-1 α gene were identified. They also observed co-segregation with diabetes of the Arg263His coding region mutation in eight members of one MODY family. In this study, mutations in only 1 gene that was implicated in MODY was looked for, while there are more than ten genes on the list currently. In 2011, the first report on HNF-4 α mutation (MODY1) was published by the same group constituting 3.4% of the clinically diagnosed MODY.^[101] They identified three novel mutations in the P2 promoter region of HNF-4 α . In addition, they also reported single nucleotide polymorphisms of HNF-4 α that are both susceptible to and protective against MODY and early onset T2DM.

CONCLUSION

MODY is a clinically and genetically heterogenous group of monogenic disorder causing diabetes in the younger population. Genetic diagnosis of this disorder has huge therapeutic and prognostic benefits. A comprehensive genetic service for the diabetic population particularly for the younger age group may benefit in the long run in terms of treatment as well as genetic counselling. The complexities of the molecular genetic techniques as well the financial requirements for the sophisticated instruments and reagents and the need for expert manpower limits the widespread availability of such a facility in our country at present, however steps in this direction are in the phase of evolution.

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