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Research Article

GLIMPSES OF GENETIC TESTING FOR HNF4A AND HNF1A FORM OF MODY GENES

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Abstract

This paper presents glimpses of genetic testing for HNF4A and HNF1A form of MODY genes. Literature review has been carried out on the topic of genetic testing for HNF4A and HNF1A form of MODY genes. It is observed from the literature that (i) Mutations in HNF1A are the most common cause of MODY, responsible for 52% of monogenic diabetes, (ii) HNF4A mutations can paradoxically cause foetal macrosomia and transient neonatal hypoglycaemia and (iii) with a clinical phenotype of *HNF1A* diabetes but negative genetic testing for *HNF1A* mutations, 29% were found to have mutations in the *HNF4A* gene. It is anticipated that PCR hybridization capture for selected genes of interest and very high-coverage sequencing of specific gene panels will replace Sanger sequencing. Ongoing refinements in the design of capture reagents, sequencing technologies and bioinformatics will, however, most likely ultimately lead to exome and possibly whole-genome sequencing as state-of-the art in molecular diagnostics of MODY.

Keywords: HNF4A, HNF1A and MODY genes.

Introduction

Monogenic forms of diabetes mellitus cover a heterogeneous group of diabetes which are uniformly caused by a single gene mutation and are characterised by impaired insulin secretion of the pancreatic beta cell. It is estimated that they account for up to 5-10% of all cases of diabetes mellitus, which are often not diagnosed or are misclassified as T1D or T2D. However, accurate diagnosis is important because of the special implications for treatment, prognosis and family risk. The knowledge of typical clinical features such as mode of inheritance, age at diagnosis and impaired insulin secretion, as well as genetic testing establishes the diagnosis of MODY. MODY (Maturity-onset diabetes of the young) was described by Tattersall in 1974–1975, is a heterogeneous group of diabetes caused by single gene defects in at least ten genes affecting pancreas development and beta-cell function. The most common MODY forms are caused by mutations in the hepatocyte transcription factor genes HNF1A and HNF4A (MODY3 and MODY1,

respectively) typically lead to progressive beta-cell dysfunction and high risk for late complications and patients often untreated with leads to early death. Until recently the outlook for a youth or young adult diagnosed with diabetes, which was almost universally type 1, was bleak. Indeed, using data from the National Health Interview Survey as recent as from 1984 to 2000, it was estimated that U.S. children diagnosed with diabetes at 10 years of age had a life expectancy approximately 19 years less than seen in the general population [1]. However, more recent data from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study suggest those diagnosed with childhood-onset diabetes between 1965 and 1980 have a life expectancy of almost 69 years, which is less than 4 years lower than the comparable U.S. population [2]. This good news has been accompanied by the observation from the Finnish Diabetic Nephropathy (FinnDiane) study that virtually all of the excess mortality seen in type 1 diabetes is related to the development of micro- or macroalbuminuria [3]. This seminal observation has been confirmed and extended for up to a 20-year period in the EDC population [4]. Although systematic studies are lacking, it is noted that the molecular genetic testing reveals a mutation in one of the common MODY genes in about 50% of probands referred to our laboratory testing. The remaining cases would also benefit from a genetic diagnosis, but the cost of sequencing other candidate genes often precludes further testing. A standard, complete investigation of HNF4A and HNF1A includes sequencing of exons, where each sequencing reaction must be evaluated separately. Hence, the current approach is expensive and time-consuming, and establishes a molecular diagnosis only among a limited number of genes. Whole-exome capture and high-throughput sequencing has a great potential to detect causal gene variants in dominant and recessive disorders as well as in diseases due to *de novo* mutations.

HNF1A and HNF4A encode transcription factors important to pancreatic development and beta cell differentiation and function. Diabetes caused by mutations in these genes follows a similar clinical picture with progressive beta cell failure. Microvascular complications are common and are related to overall glycaemic control. *HNF1A* and *HNF4A* mutations cause MODY that is often particularly sensitive to low-dose sulphonylurea therapy. Sensitivity to sulphonylureas has been demonstrated in *HNF1A*-MODY and reported in *HNF4A*-MODY, but not established by randomized trials [5]. Mutations in *HNF1A* are the most common cause of MODY, responsible for 52% of monogenic diabetes in the large UK series. Mutations in *HNF1A* are highly penetrant. Diabetes develops by age 25 years in 63% of mutation carriers and by age 50 years in 94%. *HNF1A* mutations also result in a low renal threshold for glucose; thus, glycosuria is commonly found in mutation carriers even at relatively normal blood glucose levels. Sulphonylureas are the treatment of choice in *HNF1A* diabetes.[14] In one study, 70% of individuals with a genetic

diagnosis of *HNF1A* diabetes successfully switched from insulin to sulphonylurea treatment and remained off insulin at a median of 39 months with good glycaemic control. Individuals with diabetes caused by *HNF1A* mutations have also been shown to have a 5·2-fold greater response to sulphonylureas than to metformin for reduction in fasting plasma glucose [5].

Diabetes caused by HNF4A mutations accounts for ~10% of MODY. In a study of subjects with a clinical phenotype of HNF1A diabetes but negative genetic testing for HNF1A mutations, 29% were found to have mutations in the HNF4A gene. HNF4A mutations caused a similar clinical phenotype as HNF1A diabetes characterized by progressive insulin secretory defects, diabetes onset before 25 years and a sensitivity to sulphonylureas. HNF4A mutations can paradoxically cause foetal macrosomia and transient neonatal hypoglycaemia. A personal or family history of these diagnoses should raise suspicion of HNF4A-MODY. This paper glimpses of genetic testing for HNF4A and HNF1A form of MODY genes.

2.0 Literature Review

Genetic studies have defined a number of subtypes of MODY. Mutations in the genes encoding hepatic nuclear factor 4 (HNF4), glucokinase (GCK), hepatic nuclear factor 1 alpha and 1 beta (commonly known as HNF1A and HNF1B), insulin promoter factor 1 (IPF-1), and NEUROD1 are the cause of the six known forms of MODY (MODY1-6). Table 1 presents comparison of the MODY-related genes. MODY1 and MODY3 are the most common causes of MODY but remain relatively uncommon causes of diabetes.

Table-1: Comparison of MODY-related genes.

Type of MODY	Gene	Molecular basis
MODY1	HNF4A	Abnormal regulation of gene transcription in beta cells causes a defect in the metabolic signaling of insulin secretion, beta cell mass, or both.
MODY2	Glucokinase	Reduced phosphorylation of glucose results in a defect in sensitivity of beta cells to glucose and a defect in the storage of glucose as glycogen in the liver.
MODY3	TCF1 (HNF1A)	Abnormal regulation of gene transcription in beta cells causes a defect in the metabolic signaling of insulin secretion, beta cell mass, or both.
MODY4	IPF1	Abnormal transcriptional regulation of beta cell development and function.

MODY5	TCF2 (HNF1B)	Abnormal regulation of gene transcription in beta cells causes a defect in the metabolic signaling of insulin secretion, beta cell mass, or both.
MODY6	NeuroD1 (BETA2)	Abnormal transcriptional regulation of beta cell development and function.

Note: *MODY, maturity onset diabetes in the young; HNF4A, hepatocyte nuclear factor 4α; TCF1, Transcription Factor 1; HNF1A, Hepatocyte Nuclear Factor 1α; IPF1, Insulin Promoter Factor; TCF2, Transcription Factor 2; HNF1B, Hepatocyte Nuclear Factor 1β; NeuroD1, Neurogenic differentiation factor; BETA2, Beta cell E-box transactivator* [2].

Misdiagnosis of maturity-onset diabetes of the young (MODY) remains widespread, despite the benefits of optimized management [6-14]. Fig. 1 shows the typical Fig. 1 Typical Genetic testing for monogenic diabetes [5].

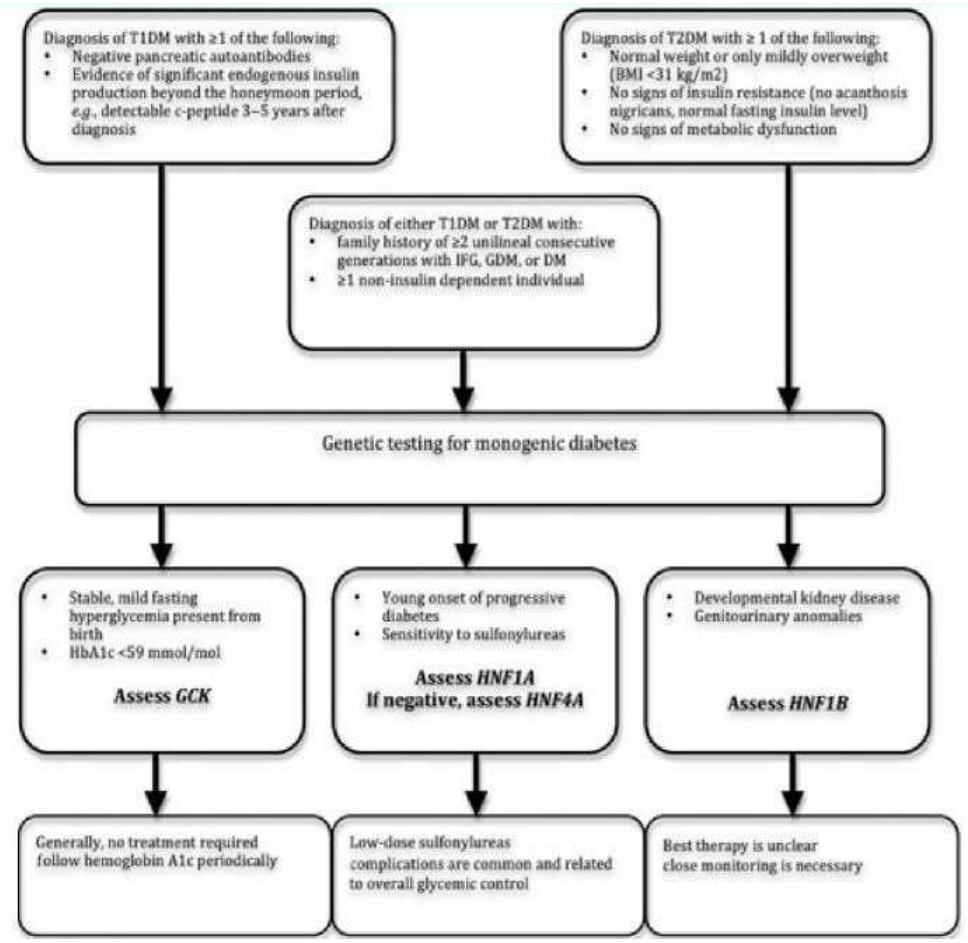

Fig. 1 Typical Genetic testing for monogenic diabetes

Gaya Thanabalasingham et al. [15] examined the cross-sectional study of diagnostic misclassification of MODY in subjects with clinically labeled young adult-onset type 1 and type 2 diabetes by extending genetic testing beyond current guidelines. Their study confirms that MODY is misdiagnosed as both type 1 and type 2 diabetes and that an accurate molecular diagnosis is often delayed for many years. Until falling costs for diagnostic resequencing allow more comprehensive investigation of MODY genes in all patients with young-onset diabetes, using much wider

selection criteria than present, based on simple clinical features, can be used to identify individuals at high risk of having MODY. This is the widest and most extensive study of its kind to date and led to high positive rates of HNF1A/HNF4A-MODY (10–25% of those tested), particularly within the young adult-onset type 2 diabetic subjects. These results of course require validation in other populations, particularly non-European. Figures 2 and 3 show the typical flow chart for investigation of individuals with clinically labeled type 1 diabetes and type 2 diabetes [15].

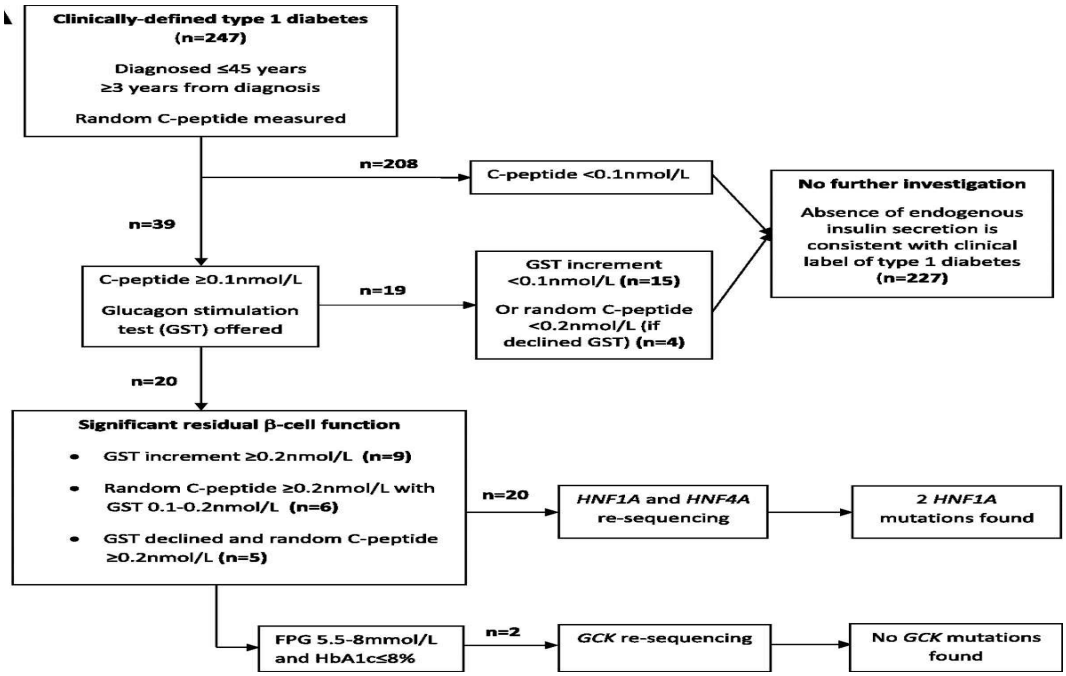

Fig. 2 Flowchart for investigation of individuals with clinically labeled type 1 diabetes

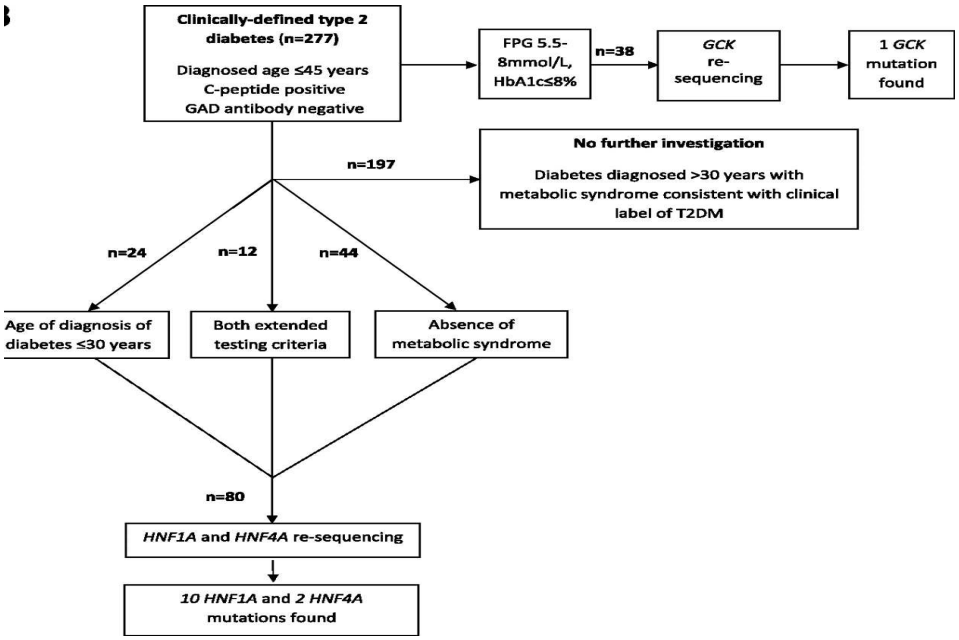

Fig. 3 Flowchart for investigation of individuals with clinically labeled type 2 diabetes

Fajans et al. [16] mentioned that, not unexpectedly, the pathophysiologic mechanisms of MODY due to mutations in the HNF4A gene (MODY1) and MODY due to mutations in the HNF1A (MODY3) are very similar since HNF4-

alpha regulates the expression of HNF1-alpha. Patients with mutations in these genes may present with a mild form of diabetes. Despite similarly mild elevations in fasting plasma glucose concentrations, patients with mutations in HNF4A or HNF1A have significantly higher plasma glucose concentrations 2 hours after glucose administration than do persons with glucokinase mutations. The hyperglycemia in patients with MODY1 and MODY3 tends to increase over time, resulting in the need for treatment with oral hypoglycemic drugs or insulin in may of these patients (30 to 40% require insulin). These forms of MODY are associated with a progressive decrease in insulin secretion. In most populations, mutations in the HNF1A gene are the most common cause of MODY. Patients with MODY1 or MODY3 may have the full spectrum of complications of diabetes.

Microvascular complications, particularly those involving the retina or kidneys, are as common in these patients as in patients with type I or type II diabetes (matched according to the duration of diabetes and the degree of glycemic control) and are probably determined by the degree of glycemic control. Patients with MODY1 lose the glucose priming effect of mild hyperglycemia on insulin secretion. Both prediabetic and diabetic persons with mutations in the HNF4A gene secrete decreased amounts of insulin in response to glucose and in response to arginine and also have an impairment of glucagon secretion in response to arginine. Furthermore, a defect in the hypoglycemia-induced secretion of pancreatic polypeptide has been found in prediabetic and diabetic persons who have mutations in the gene for HNF4A. These findings suggested that a deficiency of HNF4A resulting from mutations in this gene may affect the function of the beta, alpha, and pancreatic polypeptide cells within pancreatic islets. Patients with mutations in HNF1A have decreased renal absorption of glucose (i.e., a low renal threshold for glucose) and glycosuria. It is mentioned that a deficiency of HNF4A affects triglyceride and apolipoprotein biosynthesis and is associated with a 50% reduction in serum triglyceride concentrations and a 25% reduction in serum concentrations of apolipoproteins AII and CIII and Lp(a).

Fajans et al. [16] further reported that mutations in the HNF1A gene have been identified in all racial and ethnic backgrounds, including European, Chinese, Japanese, African, and American Indian. Mutations in the HNF1A gene appear to be the most common cause of MODY among adults seen in diabetic clinics.

Ellard [17] described that 65 different mutations in the TCF1 gene had been found to cause MODY3 in a total of 116 families worldwide. They noted that diagnostic and predictive genetic testing is possible for the majority of patients with MODY, opening new avenues for the classification, prediction, and perhaps eventually the prevention of diabetes in these families.

Vaxillaire et al. [18] examined linkage in 12 French MODY families in which diabetes was not genetically linked to previously identified MODY loci. By a genomewide segregation analysis of highly informative microsatellite markers, they localized the gene for a MODY susceptibility locus (MODY3) to 12q in 6 families. The locus in question was thought to lie within a 7-cM interval bracketed by D12S86 and D12S342 (in 12q22-qter). The patients exhibited major hyperglycemia with a severe insulin (176730) secretory defect, suggesting that the causal gene is implicated in pancreatic beta-cell function.

Lesage et al. [19] performed the possible implication of the MODY3 locus in late-onset NIDDM. In 600 affected sib pairs from 172 French families, linkage was rejected by all methods of analysis, implying that the MODY gene on 12q is not a major gene in late-onset NIDDM in this population.

Menzel et al. [20] observed evidence of linkage to chromosome 12 in 3 families with MODY from Denmark, Germany, and the U.S. (Michigan) and suggestive evidence of linkage in a family from Japan. They placed the locus in a 5-cM interval between markers D12S86 and D12S807/D12S820. It is observed that the age of onset of NIDDM was less than 25 years of age in the youngest generation in each pedigree and the segregation was consistent with autosomal dominant inheritance. In 1 pedigree, the body weight of 18 of 22 diabetic subjects is known and only 1 was obese. Diabetes was diagnosed in all but 1 of the subjects before 20 years of age. From the location of the linked markers the MODY3 locus was thought to be in the region 12q24.1-q24.32.

Mahtani et al. [21] screened over 4,000 individuals from a Swedish-speaking population isolate in western Finland and identified 26 families enriched for NIDDM. Families with the lowest insulin levels showed linkage to 12q24 near D12S1349. Unlike MODY3 families, the Finnish families with low insulin had an age of onset typical for NIDDM (mean = 58 years). Mahtani et al. [21] inferred the existence of a gene, NIDDM2 (601407), causing noninsulin-dependent diabetes mellitus associated with low insulin secretion and suggested that NIDDM2 and MODY3 may represent different alleles of the same gene. Lehto et al. [22] investigated the phenotype of affected members in 4 large Finnish MODY3 kindreds showing linkage to 12q with a maximum lod score of 15. They found evidence of severe impairment in insulin secretion, which was present also in those normal glycemic family members who had inherited the MODY3 gene. In contrast to patients with NIDDM, MODY3 patients did not show any features of the insulin resistance syndrome. They could be discriminated from patients with insulin-dependent diabetes mellitus by lack of glutamic acid decarboxylase antibodies. Taken together with the finding of linkage between this region on chromosome 12 and an insulin-deficient form of NIDDM, designated NIDDM2, as demonstrated by Mahtani et al.

[21], the data suggested to Lehto et al. [22] that mutations at the MODY3/NIDDM2 gene(s) result in a reduced insulin secretory response that subsequently progresses to diabetes, and underlines the importance of subphenotypic classification in studies of diabetes. MODY3 and NIDDM2 may be different alleles of the same gene; NIDDM2 has an average age of onset of 58 years.

Aguilar-Salinas et al. [23] examined possible defects in the insulin sensitivity and the acute insulin response in a group of Mexican patients displaying early-onset NIDDM and evaluated the contribution of mutations in 3 of the genes linked to MODY. Authors studied 40 Mexican patients diagnosed between 20 and 40 years of age, in which the insulin sensitivity as well as the insulin secretory response were measured using the minimal model approach. A partial screening for possible mutations in 3 of the 5 genes linked to MODY was carried out by PCR-SSCP. It was noted that among this group they found 2 individuals carrying missense mutations in exon 4 of the HNF4A gene and 1 carrying a nonsense mutation in exon 7 of the HNF1A gene; 7.5% had positive titers for glutamic acid decarboxylase antibodies. Thirty-five percent of cases had insulin resistance; these subjects had the lipid abnormalities seen in the metabolic syndrome. The authors concluded that a defect in insulin secretion is the hallmark in Mexican diabetic patients diagnosed between 20 and 40 years of age. Mutations in either the HNF1A or the HNF4A genes were present among the individuals who developed early-onset diabetes in their population.

Barrio et al. [24] estimated the prevalence of major MODY subtypes in Spanish MODY families and analyzed genotype-phenotype correlations. Twenty-two unrelated pediatric MODY patients and 97 relatives were screened for mutations in the coding region of the GCK (138079), HNF1A, and HNF4A genes using PCR-SSCP and/or direct sequencing. Mutations in MODY genes were identified in 64% of the families. Four pedigrees (18%) harbored mutations in the HNF1A/MODY3 gene, including a previously unreported change. The age at diagnosis was prepubertal in MODY2 index patients and pubertal in MODY3 patients. Overt diabetes was rare in MODY2 and was invariably present in MODY3 index patients. Chronic complications of diabetes were absent in the MODY2 population and were present in more than 40% of all relatives of MODY3 patients. Clinical expression of MODY3 and MODY1 mutations was more severe, including the frequent development of chronic complications.

3. Summary and concluding Remarks

Brief literature review has been carried out on Genetic testing for HNF4A and HNF1A form of MODY genes. From the literature, it is observed that a molecular diagnosis of monogenic diabetes alters management and identifies affected and at-risk family members. Thus, genetic testing should be pursued in all patients meeting a clinical

diagnosis of maturity-onset diabetes of the young. Moreover, such patients should be followed longitudinally through registries to facilitate our understanding of the unique features and best treatment of each genetic cause of maturity-onset diabetes of the young. Sequencing is currently an important complement when Sanger sequencing is negative, or in patients with atypical clinical presentation. In the near future, it is believed that PCR hybridization capture for selected genes of interest and very high-coverage sequencing of specific gene panels will replace Sanger sequencing. Ongoing refinements in the design of capture reagents, sequencing technologies and bioinformatics will, however, most likely ultimately lead to exome and possibly whole-genome sequencing as state-of-the art in molecular diagnostics of MODY.

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