

Role of Urinary C Peptide and Targeted NGS to Unveil Hidden MODY in Type 1 Diabetes

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Commentary

Classification of diabetes has always been a challenge for endocrinologists, especially in young patients [1]. Monogenic diabetes results from inheritance of one or more variants in a single gene, accounting for 1–3% of diabetes cases diagnosed within an age of 30 years. Inheritance of variants can be in a dominant or recessive fashion. The majority (90%) of monogenic diabetes cases were initially misdiagnosed as type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM). Hence a correct genetic diagnosis plays an important role to predict the clinical course, to look for other associated clinical features, provide genetic counselling, diagnose family members, and most importantly to provide appropriate treatment.

Maturity-onset diabetes of the young (MODY) and T1DM are easily confused with each other because of the early onset of diabetes and lack of obesity. Also, a simple family history and insulin resistance markers did not reliably distinguish between MODY and T2DM in adults [2-4]. In a study by Farmer and Fox, it was reported that only 15% of young diabetes patients had been correctly diagnosed [5]. A misdiagnosis can lead to improper insulin prescription, and it may significantly impact the quality of life and long-term health outcomes [3].

Prevalence estimates of MODY from large systematic surveys are between 1% and 3% of young-onset diabetes. In the UK Using Pharmacogenetics to Improve Treatment in Early-onset Diabetes (UNITED) study, 3.6% of all diabetes cases were diagnosed under 30 years of age [6] and 2.5% of all diabetes cases diagnosed under 20 years of age were MODY [7], while the SEARCH for Diabetes in Youth study undertaken in the USA showed that 1.2% of all cases diagnosed under 20 years of age were MODY [8].

The key approach to diagnosing MODY is to initially look for clinical features that are unusual for T1DM and T2DM and to undertake genetic testing in these individuals to confirm monogenic diabetes.

Since molecular testing is relatively expensive, it is recommended that testing be restricted to those individuals with a moderate to high clinical possibility of a positive result. Since traditional clinical features, such as age of onset, a parental history of diabetes, and non-insulin treatment, overlap considerably between MODY and other types of diabetes [9] and hence independently have poor discriminatory value, relying on these criteria solely would lead to inappropriate screening for MODY.

A MODY probability calculator provides an excellent way to establish whether a diagnosis of MODY is likely. This combines clinical information to predict the probability of testing positive for MODY [10]. The calculator has markedly enhanced the sensitivity and specificity of identifying MODY compared with standard criteria of diagnosis by an age under 25 years with an affected parent.

Presence of pancreatic autoantibodies is another effective way to distinguish between type 1 diabetes and MODY.

Pancreatic autoantibodies are positive when measured near to diagnosis in approximately 90% of people with T1DM if glutamic acid decarboxylase (GAD), islet antigen 2 (IA2), and zinc transporter 8 (ZnT8) antibodies are measured, compared to only 1% of patients with MODY [11]. A negative test does not necessarily exclude T1DM, especially as with increasing duration of disease, the antibody positivity is lost. Testing pancreatic autoantibodies in patients treated with insulin is therefore of value, if positive, in excluding MODY.

The presence of preserved C-peptide secretion in cases of long-term T1DM can also help to stratify those in whom MODY testing must be considered, however previous studies have found that around 8% of people with long-term T1DM have stimulated C-peptide levels of more than 200 pmol/l [12].

Serum C-peptide is widely used for assessment of islet beta cell function, and hence helpful for individualizing treatment in diabetes. The half-life of C-peptide being 20–30 min is much longer than that of insulin (half-life, 3–5 min).

Also, other methods of C peptide estimation like urinary C-peptide (UCP) to creatinine ratios was found to be useful in differentiating MODY from T1DM and avoids the need for blood samples [13].

UCP is a non-invasive test and can be performed in an outpatient setting [14]. When collected in boric acid UCP remains stable at room temperature for up to three days. UCP quantity is reflective of 5–10% of the total C-peptide secreted by the pancreas provided patients have a normal renal function [15].

The 24 h UCP sample collection (24 h UCP) is a more time-consuming and inconvenient method for the patient, making it a less attractive option than spot UCP.

In subjects with normal glucose tolerance, UCP to creatinine ratio (UCPCR) was found to correlate well with 24 h UCP [16]. Moreover, in the past decade, UCPCR was reported to differentiate between T1DM and non-T1DM (i.e., T2DM and MODY) with a high sensitivity and specificity [17-19]. This suggests that UCPCR is a simple, reliable, and convenient method of estimating C-peptide.

UCPCR has been used as a test to discriminate between two of the most common types of MODY: HNF1A and HNF4A heterozygous mutations, and long-term T1DM [20]. UCPCR was found to be significantly lower in patients with T1DM of greater than 5 years duration, compared to subjects with HNF1A/4A MODY.

C-peptide is a useful tool in the classification of diabetes to differentiate T1DM from T2DM or MODY. Level of C-peptide is associated with the duration of disease as well as the age at diagnosis.

UCPCR cut-off of ≤ 0.20 nmol/mmol for a differential diagnosis of diabetes is also useful for identifying patients who need insulin or secretagogue therapy added to their treatment regimen to achieve their goal of glucose control.

Both UCPCR and serum C-peptide can be used for assessment of pancreatic beta cell function [21]. Compared with serum C-peptide, which has to be separated from the serum via centrifugation and subsequently frozen to avoid protease

hydrolysis, UCPCR was found to be more convenient [22]. UCP is stable even if it stays in room temperature for 3 days in boric acid [23] or if it is frozen at -80°C for 4 months. These advantages of UCPCR including stability and non invasiveness can potentially facilitate further experiments.

UCPCR has been used widely for measurement of endogenous insulin secretion and to differentiate between diabetes subtypes [24-27]. Sebahat *et al.* reported that postprandial UCPCR ≥ 0.22 nmol/mmol could differentiate MODY from T1DM in children with 96.3% sensitivity and 85.7% specificity [26]. Besser *et al.* performed multiple studies on UCPCR. They also demonstrated that UCPCR ≥ 0.20 nmol/mmol distinguished between MODY1 or MODY3 and T1DM with a sensitivity of 97% and a specificity of 96% in long term adult diabetes patients with duration more than 5 years [24]. An absolute insulin deficiency was seen in most T1DM patients with a disease duration of greater than 5 years, and therefore, the above-mentioned studies, which included mainly long-term T1DM patients (5.8 ± 3.3 years in Sebahat *et al.*'s study, >5 years in Besser *et al.*'s study), had similar optimal UCPCR cut-off levels.

However, it was found that UCPCR did not reliably distinguish between MODY and T2DM as noted in study by Besser *et al.* [25]. This may be due to the fact that a rapid decline in pancreas beta cell function is infrequent in both T2DM and monogenic diabetes.

In this hospital-based cross-sectional study from single centre in South India, we included 100 clinically diagnosed T1DM patients (according to ADA criteria) including both autoantibody positive and negative cases, with disease duration of more than 3 years.

In most cases, the presence of autoantibodies for T1DM precludes further testing for monogenic diabetes, but the presence of autoantibodies in people with monogenic diabetes has been reported [28]. In a large Australian pediatric diabetes cohort, the prevalence of one or more antibodies among MODY cases was 18% [29]. Hence, we have included even antibody positive cases in our study.

Also, since 5–10% of people with T1DM do not have antibodies, we have included the antibody negative cases of clinically diagnosed T1DM individuals in our study and further aimed to detect MODY through NGS in patients with a preserved C-peptide.

Since the autoantibody level of anti-islet cell autoantibodies decreases with disease duration and can become negative, it is essential to measure them early in the onset of T1DM for accurate diagnosis. Seventy-six percent of subjects had one or more anti-islet auto antibodies in our study, but the limitation was that only IA 2 and GAD autoantibodies were considered.

Although duration of 3 years was considered in our study, preserved β -cell function with low insulin requirements and detectable C-peptide (either in blood or urine) over an extended partial remission phase (at least 5 years after diagnosis) may be seen.

The percentage of T1DM patients who attain remission ranges from 18% to 72% whereas complete remission is extremely rare occurring in less than 3% [30]. The partial remission phase (honeymoon phase) occurs around 3 months from the initiation of treatment, previous studies show that it may range from 3 to 12 months [30-32]. Studies quote that 35%-70% of remission occurs within the initial 3 months of diagnosis and the rate of remission was seen to progressively decline with increasing duration of the disease [33]. The duration of honeymoon phase or partial remission ranges from 1 month to 13 years with a mean of 7 months [32]. Similar to factors described in the diabetes risk [34], the duration of partial remission is also influenced by multiple factors. Remission was observed to be longer in patients with one antibody positivity, older age of onset of diabetes, higher blood pH at the time of diagnosis, and in boys [35,32,30]. In addition to the factors described above, others like inflammatory markers, diet, and nicotinamide could also have a role in predicting the occurrence of remission [36].

In our study, the patients who met the above inclusion criteria were subjected to further screening using MODY probability score and UCPCR to assess the endogenous insulin reserve and clinical probability of MODY. Targeted next-generation sequencing (NGS) for MODY-related genes (13 genes) was performed for individuals with UCPCR>0.2 which was observed in eight out of hundred individuals.

DNA samples of these eight patients were taken for targeted NGS in 13 MODY related genes (HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, and KCNJ11). Among them, one (12.5%) had a non-pathogenic gene variant in KLF11.

Our study also found that the mean HbA1c values were lower, and the MODY probability score was higher in individuals with preserved endogenous insulin.

More than two-thirds of the population was found to be positive for GAD autoantibodies in our study. Out of hundred clinically diagnosed T1DM patients for more than 3 years, eight individuals had a preserved endogenous insulin reserve. Among them, four had a history of DKA episodes, and three had a family history of T1DM.

One study individual was found to have a gene variant for KLF11. She was diagnosed with T1DM at the age of 21 years with an episode of DKA. She had a significant family history of diabetes with both father and paternal grandmother

being diagnosed with diabetes at about 35 years of age and were well-controlled with oral anti-diabetic drugs. She was overweight (BMI 23.5 kg/m²) with a low insulin requirement. Anti-islet antibodies were absent, and the MODY probability score was lower (12.6%).

Considering a strong family history of young onset diabetes with absent auto antibodies and low insulin requirement should prompt a consideration for MODY although the MODY probability score was low. In our case this patient also had a UCPCR>0.2 further prompting a need to rule out monogenic diabetes.

NGS revealed a heterozygous variant at Exon 3, c.1120G>A (p.Val374Met) position of the *KLF11* gene which was described as VUS (variant of uncertain significance) in the ACMG classification system. Sanger sequencing for this gene variant, in the father and paternal grandmother, also revealed the same variant in *KLF11* indicating the inheritance of this variant which likely would have caused the disease. Since our patient had poor response to sulfonylurea, she was continued on insulin.

A total of 14 subtypes of MODY have been described, the most common among which are *HNF1A*-MODY (MODY3), *GCK*-MODY (MODY2), *HNF4A*-MODY (MODY1), *HNF1B*-MODY (MODY5), and *ABCC8*-MODY (MODY12). The rarer subtypes include *NEUROD1*-MODY (MODY6), *IPF1/PDX1*-MODY (MODY4), and *INS*-MODY (MODY10). Other purported forms of MODY such as *BLK*-(MODY 11), *PAX4*-(MODY9), *KLF11*-(MODY7), and *APPL1*-(MODY14) have been subsequently unclassified as true MODY.

In studies by Mohan *et al.*, it was found that HNF1 alpha was the most common MODY variant (7.6%), followed by ABCC8 (3.3%). *KLF11* mutation is considered an infrequent cause of MODY in South India [37].

Neve *et al.* studied the role of transcription factor, KLF11 on pancreatic beta cell function and was considered as a cause of MODY 7 prior to 2022 (during the conduct of the study).

However, according to Laver *et al.*, the lack of co segregation of published MODY-causing variants, presence in the population at a high frequency, and lack of enrichment of rare variants in a MODY cohort was found to be consistent with the observation that these genes do not cause MODY [38]. They recommended that these genes should not be included for MODY genetic testing.

The KLF11 mutation in our patient although reported as VUS, in the presence of Sanger sequencing showing same variant in two other generations in the background of preserved C peptide indicates the possibility of monogenic diabetes which substantiates the need for further study of rarer genes like MODY7 (KLF11) in the future.

The limitations of our study were that the sample size of our study was small. Also as mentioned, we estimated only two auto-antibodies, i.e., GAD-65 and IA-2.

Whole exome sequencing should have been done given the expanding number of genes responsible for MODY, which would have probably detected more genetic variants.

Hence our study emphasizes the need to precisely differentiate T1DM from monogenic diabetes in the background of multiple overlapping features between them.

Since precise diagnosis of monogenic diabetes requires a genetic analysis which might not be always economically feasible, screening at a preliminary level using cost effective and non invasive investigations like UCPCR can be done. Patients with preserved UCPCR which correlates well with 24 hour urinary C peptide can be subjected to a genetic study, ideally a whole exome sequencing.

Implementing such a screening followed by genetic diagnosis would help diagnose more cases of monogenic diabetes and hence enable healthcare providers to provide appropriate care and also screen them for associated conditions.

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