Original Article

Our Experiences and Learnings in Diagnosing MODY from Non-Institutional-Based Diabetes Care Clinics

Arunkumar R. Pande, Santosh Chaubey[1], Dinesh Kumar[2], Kumar P. Chandra[3], Thenral Geetha[4], Akshita Sharma[4]

Department of Endocrinology, Lucknow Endocrine Diabetes and Thyroid Clinic, Uttar Pradesh, India, [1]Department of Endocrinology, Cairns and Hinterland Hospital and Health Service, 165 The Esplanade Cairns North QLD, Queensland, Australia, [2]Department of Internal Medicine, Harsh Clinic and Diabetes Care Centre, Singar Nagar Alambagh, Lucknow, Uttar Pradesh, [3]Department of Diabetology, Chandra Diabetes Clinic, Vijayant Khand, Gomti Nagar, Lucknow, Uttar Pradesh, [4]Department of Genetics, MedGenome Labs, Tamil Nadu, India

Abstract

Introduction: Maturity-onset diabetes of the young (MODY) is a rare group of disorders characterised by impaired functions or development of pancreatic islets and monogenic diabetes at a young age. Diagnosing MODY can be rewarding for both clinicians and patients as it can change the management from generic to targeted therapy. **Methods:** This study reports the retrospective analysis of data collected from four clinics between March 2016 and February 2023 from Lucknow, a city in northern India. Fifty-three individuals are suspected to be affected by MODY based on ISPAD guidelines. Following a detailed clinical evaluation, they were referred for genetic diagnostic testing. **Results:** The cohort consists of 19 females and 34 males with a mean age of diagnosis of 25.3 years and a body mass index of 22.3 Kg/m^2. Genetic testing detected variants in 13/53 (~24.5%) individuals. Five cases had significant pathogenic/likely pathogenic variants, *HNF1A* gene in two [(p.Phe268LeufsTer74) (p.Arg200Gln)], one each in *HNF4A* (Arg311His), *PDX1*(p.Ala228GlyfsTer33), and a case with suggestive digenic variants in *HNF1A* gene (p.Arg200Gln) and *HNF1B* [(p.Leu13Met)]. Variants of uncertain significance (VUSs) with inconclusive evidence of pathogenicity were reported in eight patients, and five were considered to be clinically significant as they are lean young onset, sulfonylurea-responsive, and presented with diabetes without acanthosis nigricans and with high pretest probability. These individuals harboured variants in *HNF1A* (p.Thr425_Thr429delinsPro), *HNF1B* (p.Ser19Phe), *CEL* (p.Val681ArgfsTer6), *ABCC8* (p.Ile872Met), and *KCNJ11* (p.Arg221Cys) genes. **Conclusion:** We found a diagnostic yield of around 10% of pathogenic or likely pathogenic variants in individuals who were suspected to have MODY. As it is a field that is still evolving, we might consider starting with oral agents under close supervision in those individuals who have VUS; there are some proportions of individuals who might not have classical sulfonylurea-responsive genetic variants, but they might respond to it.

Keywords: HNF1A MODY, MODY, monogenic diabetes, pediatric diabetes, young diabetes

Introduction

Maturity-onset diabetes of the young (MODY) is a clinically heterogeneous group of disorders characterised by non-ketotic diabetes mellitus, an autosomal dominant mode of inheritance, an onset usually before the age of 25 years and frequently in childhood or adolescence, and a primary defect in the function of the pancreatic beta cells. This was a definition given by Fajans *et al*.[1] A lot has changed over the years; new genes have been identified, and interestingly, many exceptions to the above definition have been well documented in the scientific literature.[2]

These are the first few cases of MODY reported from India for various genetic forms of diabetes *HNF1A* MODY in 2009, *GCK* MODY in 2014, and *HNF1B* MODY in 2015.[3-5] However, there is a dearth of data on MODY in India.[6-9] This study is an effort to bring detailed case studies from our privately run diabetes care clinics (four clinicians), collected over 7 years on MODY patients and our learnings.

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Address for correspondence: Dr. Arunkumar R. Pande, Department of Endocrinology, Lucknow Endocrine Diabetes and Thyroid Clinic, Uttar Pradesh, India.
E-mail: drarunendocrine@gmail.com

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As we delved into the field of genetic diabetes research, we encountered several terminologies that initially posed challenges. Terms such as MODY X, why there are so many cases of variants of unknown significance (VUS), and digenic mutation were unfamiliar to us, prompting further exploration and understanding. In this paper, we also aim to shed light on these concepts, discussing their significance in the context of genetic diabetes research. By elucidating these terminologies and with the help of this work to simplify and explain them, we hope to contribute to a better understanding of the complexities inherent in the genetic basis of diabetes and its implications for diagnosis, treatment, and research. Additionally, by addressing these terminologies, we aim to provide valuable insights for individuals who are new to this area of work, equipping them with foundational knowledge to navigate the complexities of genetic diabetes research effectively.

Materials and Methods

This study reports data collected in Lucknow, a city in the northern part of India, from four clinics between March 2016 and February 2023. Approximately 18,000 unique patients have been treated from LEDTC clinics. The clinics witness an annual average of approximately 6000 diabetes-related visits. The individuals who were suspected to be affected with MODY based on detailed evaluation and with either positive family history with no signs of insulin resistance or negative antibodies (GAD Antibody, CLIA, Snibe diagnostic, Maglumi series, Shenzhen, China And IA2 antibody CLIA, Snibe diagnostic, Maglumi, Shenzhen, China) and no history of diabetic ketoacidosis (wherever applicable). Fifty-three individuals underwent genetic testing: one from Chandra Diabetes Clinic, two from Harsh Clinic and Diabetes Care Centre, three from Dr SK Chaubey clinic in India, and 47 from Lucknow Endocrine Diabetes and Thyroid Clinic from Lucknow, India.

MODY was suspected if (1) the patient was <35 years of age at the time of diabetes diagnosis, (2) had a family history suggestive of autosomal dominant inheritance, (3) lacked evidence suggesting a diagnosis of T1D [evidence of endogenous insulin production outside the honeymoon period with detectable C peptide level (>200 nmol/L) when glucose is >140 mg/dL and absence of autoantibodies], and/or (4) lacked evidence supporting a diagnosis of T2D (normal body weight, absence of acanthosis nigricans, and no evidence of insulin resistance with normal fasting C peptide level). There were also slight deviations in a few cases based on the clinical judgment of the clinicians.

Fifty-three individuals from out-patient clinics were tested for MODY by four different genetic lab services. The details of the genes and samples in these four labs are given in the supplementary Table [Tables S1 and S2]. Variants in disease-relevant genes were detected in 13 individuals tested for MODY and were classified as pathogenic/likely pathogenic or uncertain significance [Table S3]. Following analysis, the prioritised variants were classified into five tiers based on American College of Medical Genetics (ACMG) recommendation: Pathogenic (P), Likely pathogenic (LP), Uncertain significance (VUS), Likely benign (LB), and Benign (B), depending on the applied criteria according to the parameters suggested by the ACMG guidelines.[10] Some of the primary criteria are a) reported in the literature/disease databases (HGMD, Clinvar, etc.), b) variant functional evidence, and c) segregation of the variant in affected/unaffected individuals with similar phenotypes from the literature along with d) allele frequencies and e) *in silico* prediction tools as supporting values. Based on the criteria, significant variants were classified as pathogenic or likely pathogenic. Benign variants were excluded, and variants whose role was inconclusive were classified as of uncertain significance. The databases and tools used have been listed above. The details of the applied criteria for the ACMG parameters are added to Table 1. A few of the cases were presented in various conferences (Case Summaries – Supplementary Material [Figures S6 and S10]).[11,12]

Ethical aspect

The ethical clearance was taken from the institutional review board (Sanjivini Lung Centre-ECR/963InstUP/2017) ethics committee. The index patient and/or their parents were informed about the benefits and expected outcomes of genetic testing. Based on their consent, the samples were sent to the four genetic testing centers. The de-identified retrospective data have been presented here.

Results

The cohort consisting of 53 individuals who underwent genetic testing and had a mean age of 25.3 years at the time of clinical diagnosis. Thirteen had one or more variants in one of the MODY genes, eight males and five females [Tables S4 and S6]. The mean age of diagnosis of diabetes was 18.1 years, so there was a lag of more than 7 years from the diagnosis of diabetes to the genetic diagnosis of MODY. As per Indian body mass index (BMI) cut-offs of less than 23 kg/m^2, two of the individuals had obesity, two were overweight, and one was found to be underweight. The mean BMI (for the calculation of the mean, individuals below 18 years of age were excluded) was 22.3 Kg/m^2. Five out of 13 individuals were labeled as type 2 based on clinical diagnosis, three were diagnosed as type 1, and five individuals who visited us first at the onset of diabetes were diagnosed as MODY. Genetic testing detected variants in 13/53 (24.5%), of whom five had significant pathogenic/likely pathogenic variants and eight had rare variants with inadequate literature evidence.

HNF4A (p.Arg311His), *HNF1A* (p.Phe268LeufsTer74), and *PDX1* (p.Ala228GlyfsTer33) were found to be likely pathogenic, and *HNF1A* (p.Arg200Gln – two cases) were pathogenic according to ACMG classification [Table 1]. The frameshift variant observed in *HNF1A* and *PDX1* has not been reported in the literature and is novel to this study cohort.[13-18]

Table 1: Details of all the cases with likely pathogenic and pathogenic variants

Case ID	Sex	Age of diagnosis	Age of MODY diagnosis	Current Age	BMI	HbA1c at diagnosis	Baseline treatment	Current Treatment	Current HbA1c	Complication	Additional Features	Gene	Variant	MODY type	Pathogenicity	Previous reports
PP1	M	20	52	56	23.12	8.6	Regular Insulin (10-0-8), Glargine (16-0-10), Vildagliptin 50 (1-0-0),	Glimepiride 3 mg (OD), Metformin SR 500 (BD), Empagliflozin 25 + Linagliptin 5 (OD)	7.27	Neuropathy, Proteinuria, Hypertension	-	*HNF4A*	(Arg311His)	MODY type I	Likely Pathogenic (PS1, PM2, PP3)	[13]
PP3	M	17	26	26	25.04	8.1	Gliclazide 60 (1 1/2-0-0)	Gliclazide XR 90 (OD)	6.4	Hypertension	Renal glycosuria	*HNF1A*	(p.Phe268 LeufsTer74)	MODY type III	Likely Pathogenic (PVS1, PM2)	-
PP4	M	22	37	44	26.42	9.4	Glimepiride 3 mg (1-0-0), Empagliflozin 25 (1-0-0)	Glimepiride 3 (OD), Dapagliflozin 10 + Metformin XR 100 (OD), Metformin SR 1000 (OD)	8.7	-	-	*HNF1A*	(p.Arg200Gln)	MODY type III	Pathogenic (PS3, PM1, PM2, PP3, PP5, PM5)	[15]
PP7	F	9	12	12	21.65	8.8	-	Gliclazide 40 mg Metformin 500 (1-0-1)	8.2	-	-	*HNF1A* + *HNF1B*	(p.Arg200Gln) (p.Leu13Met)	MODY type III + MODY type V	Pathogenic (PS3, PM1, PM2, PP3, PP5, PM5)/ Uncertain Significance (PM2, PP3)	-
PP8	M	13	13	13	18.99	13.6	Glargine (0-0-8), Metformin 500 (1-0-1)	Gliclazide 30 modified release (1-0-0), Metformin 500 (1-0-1)	6.4	-	-	*PDX1*	(p.Ala228Glyfs Ter33)	MODY type IV	Likely pathogenic (PVS1, PM2)	-

Individual PP8 (*PDX1*; p.Ala228GlyfsTer33) inherited the frameshift variant from a similarly affected father. He responded to gliclazide and metformin. His HbA1c dropped from 13.6% to 6.4%, challenging the usual belief that they respond only to insulin therapy. When last visited the clinic, which was 2 years and 7 months after the onset of diabetes, he was still responding to it.

Variants of uncertain significance (VUSs) were detected in eight individuals [Tables 2 and S5]. We know if the pre-test probability of a test is high, then there is less likelihood of false positivity.[19] We had five individuals who were detected as VUSs – *HNF1A* [(p.Thr425_Thr429delinsPro)], *HNF1B* (p.Ser19Phe), *CEL* [(p.Val681ArgfsTer6)], *ABCC8* [(p.Ile872Met)], and *KCNJ11*[(p.Arg221Cys)]. These individuals had a high pre-test probability ranging from 45.5 to 75.5%. These individuals were lean, young-onset diabetes, sulfonylurea-responsive for various durations [Figure 1], without acanthosis nigricans.

Additionally, the variants of uncertain significance in genes *RFX6*, *LIPC*, *KLF11*, *PAX4*, and *ABCC8* in this study were excluded based on the current population frequency of >1% or homozygous variants in the population database. The details have been added in the supplementary material [Table S3].

Discussion

We found five individuals who had pathogenic or likely pathogenic variants [Table S1]. Similar to the case series from South India by Mohan *et al*., *HNF1A* was found to be the most common in both case series [Table 1].[8,20] Interestingly, *NEUROD1* variants were common in the southern MODY case series, but we did not find any case of this variant in North India.[6,8] There was a recent publication from northern India where single-gene variants were found, but there is a lack of case series involving a detailed evaluation of multiple genes.[9]

There are many well-characterised genes for MODY. Clinically, it may be difficult to differentiate between MODY and the genetic form of type 2 diabetes. Individual PP1 on the initial visit to the clinic stated the duration of diabetes was 15 years. His genetic test came positive for MODY [*HNF4A*; p.Arg311His].

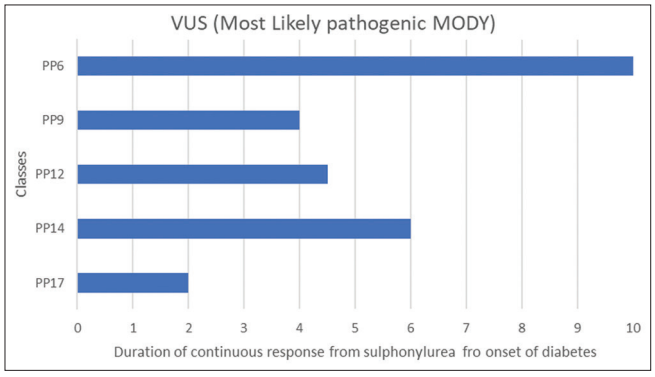

Figure 1: Duration of response to sulfonylurea in a few cases who were detected as VUSs

Of note, he was actually diagnosed with diabetes at the age of 23 years, making the duration of diabetes almost 28 years before a gene test was considered. So, the duration of diabetes and the age of onset of diabetes, which is a very important history in the case of MODY, can be a little tricky to elicit; however, the details can help to clinch the diagnosis of MODY. In this case of PP1, probably, he was considered as type 2 diabetes; that is why he was on insulin. A diagnosis of MODY led us to a trial of sulphonylurea to which he responded very well. His HbA1c reduced from 8.6 to 7.3%. A detailed description of the cases is given in the supplementary information (PP1, PP4, PP9, PP3, PP10, PN9, PN11, PN20, PN29).

There were two cases (PP8 and PP9) presented in their teens to us with diabetes. Although we had a suspicion of MODY, they were treated as type 1 diabetes as it is the most common cause of diabetes in this age group and also to be on the safer side. Later on, after the diagnosis of MODY, they were shifted to sulphonylurea and they responded very well to it.

PP8 was a jeweler who presented to us with a typical history of MODY that is the early onset of diabetes and family history of diabetes in three generations. He was on four oral agents except for sulphonylurea. He was an exercise enthusiast running on the treadmill for 1-1 and a half hours; still, HbA1c was above 9%. The typical history prompted us to shift him to sulphonylurea, to which he responded very well, and his HbA1c came to 7%. PP7 had a similar surname to one of our previous cases and had a strong family history of diabetes suggested by MODY. This led us to ask a question as to whether she is related to him and the response was positive for that. So, this led us to have a suspicion of MODY type 3 and as her glucose was not on the higher side, she was started on oral agents right at the diagnosis of diabetes.

MODY X

In the southern part of India, it was previously thought to have a large number of MODY cases,[21] but subsequently, an actual evaluation of the prevalence of MODY by the same group found it less than that estimated before.[22,23]

In this study, GCK MODY, commonly observed in Western populations, was notably absent in our Indian cohort. Furthermore, only a limited number of GCK MODY cases have been documented in India to date. These observations suggest a lower prevalence of GCK MODY in India compared to Western countries, as noted in the findings of Sujeet Jha *et al*.[4,24]

The recognition of the role of non-coding and regulatory regions in the pathogenesis of MODY underscores the need for a comprehensive genetic evaluation. Current screening methods primarily focus on coding exons; however, expanding our analysis to include promoter and intronic regions could potentially identify causative variants not detected by traditional methods. Coupling these comprehensive genetic screenings with functional diagnostic tests such as enzyme kinetics, enzyme stability, and binding assays may enhance our diagnostic capabilities, particularly for variants where literature evidence is conflicting or insufficient.[25]

Table 2: Details of all the cases with VUSs

Case ID	Sex	Age of diagnosis	Age of MODY diagnosis	Current Age	BMI	HbA1c at diagnosis	Baseline treatment	Current Treatment	Current HbA1c	Complication	Additional Features	Gene	Variant	MODY type	Pathogenicity	Previous reports	MODY probability (%)
PP6	F	28	37	38	21.58	9.8	-	Gliclazide 90 (1-0-0), Metformin 500 + Sitagliptin 50 (1-0-0)	7.9	-	-	*HNF1A*	(p.Thr425_ Thr429 delinsPro)	MODY type III	Uncertain Significance (PM1, PM2, PM4)	-	62.4
PP9	M	18	18	22	20	>10	Glimepiride 2 mg (1-0-0)	Gliclazide XR 90 (OD)	9.2	-	-	*HNF1B*	(p.Ser19Phe)	MODY type V	Uncertain Significance (PM2, PP3)	-	75.5
PP12	M	26	31	31	21.71	-	-	Gliclazide 60 (1-0-0) Sitagliptin 100 + Metformin 500 (1-0-1)	9.5	Neuropathy	-	*CEL*	(p.Val681 ArgfsTer6)	MODY type VIII	Uncertain Significance (PM1, PM2)	-	45.5
PP14	F	18	19	24	24.78	-	-	Glimepiride 4 mg (OD), Linagliptin 2.5 + Metformin 1000 (OD)	6.7	-	-	*ABCC8*	(p.Ile872Met)	MODY type XII	Uncertain Significance (PM2, PP3)	-	75.5
PP17	M	7	8	9	-	-	-	Gimlipride 2mg (OD)	-	-	-	*KCNJ11*	(p.Arg221Cys)	MODY type XIII	Uncertain Significance (PM2, PP3)	-	75.5

PP: Proband Positive; PN: Proband Negative; FP: Family Positive, FN: Family Negative; M: Male; F: Female; BMI: Body Mass Index; FBS: Fasting Blood glucose; PPBS: Post Prandial Blood glucose; HbA1c: Glycosylated Haemoglobin

Refinement of bioinformatics methods aimed at improving the prediction accuracy of non-synonymous amino acid substitutions in glucokinase has not yet met satisfactory standards. Despite efforts to enhance these methodologies through evidence-based adjustments, the specificity of predictions remains at 75% or lower, indicating significant room for improvement.[26]

It is intriguing to consider future investigations that may elucidate the underlying biochemical abnormalities exhibiting familial traits in our cases. Given the prevalent understanding that fasting hyperglycaemia can result from anomalies in the glucose-sensing pathway, including the glucokinase regulation mechanism, we hypothesise that the cases of fasting hyperglycaemia we observed, which did not test positive for GCK MODY, may involve defects in other yet-unidentified genes within this pathway. This hypothesis underscores the necessity of exploring beyond the known MODY genes, potentially involving a broader spectrum of genetic defects affecting glucose regulation.[27]

Numerous mutations in HNF1A and HNF4A have been identified as causes of MODY, highlighting the genetic complexity of this disease.[28] In a recent study by Malikova *et al*. (2020),[29] functional analyses were performed on 17 previously unreported HNF1A variants. These studies revealed inconsistencies between in silico predictions and functional outcomes, allowing for the re-classification of 10 out of 17 variants as significant or benign. This underscores the limitations of current predictive models and the essential role of functional validation in the diagnostic process. Addressing these challenges in a clinical setting may require enhanced academic collaboration and better access to comprehensive genetic databases.

Furthermore, prediction methods are vital in both biomedical and biotechnological research; however, their application in clinical settings has often been critiqued for a lack of specificity, primarily when relying solely on biochemical or molecular data. Recent efforts to refine these methods through evidence-based adjustments have shown promise. For example, modifications to the default settings of tools like EVmutation, SNAP2, and PoPMuSiC 2.1 have led to marked improvements in the accuracy of clinical effect predictions, demonstrating significant advances in predictive genomics.[30]

In our cohort, a few cases, notably PN9 and PN29, exhibited clinical characteristics typical of MODY, such as lean body type, young-onset diabetes, a strong family history, and responsiveness to sulfonylurea, yet genetic testing did not confirm these as MODY through the known genes. Despite extensive screening, including for a newly reported gene in 60.4% of cases, results remained negative (Supplementary Material: SU Responsive MODY Case summary: PN9, PN29). This observation underscores the potential existence of yet unidentified genetic factors contributing to the MODY phenotype. The consistent absence of known MODY gene mutations in such phenotypically suggestive cases points towards the necessity for further genetic exploration, possibly revealing novel MODY-related genes in future studies.

Variants of unknown significance (VUS) menace

Eight individuals were detected with VUSs despite significant clinical suspicion. Interpreting the VUS can be challenging to decide on the treatment program. This is because their variants did not receive the required ACMG score to be classified as pathogenic or likely pathogenic,[10] especially when the clinical history and family history strongly suggest a clinical diagnosis of MODY. Case PP10, the *HNF1B* variant, was detected at the transactivation domain of the gene (p. Pro335Arg: classified as a VUS); the clinical diagnosis was made based on the symptoms of young-onset diabetes, renal cyst, and family history of diabetes in mother, and her death was due to renal disease at the age of 37 years and requiring insulin in order to have good glycemic control (case PP10). It would be interesting to see why some *HNF1B* MODY respond to oral agents and others do not. Segregation of the variants in the mother could not be possible, which may have given insight into the significance of the observation. The classification of the variants can change over time based on new literature evidence, and thus, it is imperative to see if the databases that update them have changed their classification of the pathogenicity status.

Digenic mutation

We have one case with the combination of a pathogenic variant and a variant of uncertain significance (PP7) of MODY with heterozygous variants, suggestive of digenic inheritance (*HNF1A* + *HNF1B*). There is a library for this type of variant which was previously known as DIDA (DIgenic diseases DAtabase), but now, DIDA has been overhauled to improve the data quality, content, and accessibility in the new database OLIDA (OLIgogenic diseases DAtabase).

Strength

Identifying MODY cases following systematic screening for all the individuals with a pre-defined process is one of the strategies to find large numbers of cases. Our case series is unique as it reports cases that were diagnosed over 7 years in out-patient settings of private clinics in North India. Our case series substantiates that MODY can be diagnosed in individual care clinics. A diagnosis of MODY can lead to better management in the form of treatment and follow-up as well as the screening of the other family members.

Limitations of the study

It is a retrospective chart audit that has been done at four different clinics. Hence, individual biases in advising the screening tests are expected. Not all individuals who were advised of genetic evaluation underwent the testing because of financial and other reasons. Also, detailed family screening for MODY could not be done because of the prohibitory price of genetic testing and other reasons.

Conclusion

This is one of the few handful of case series from northern India that feature the diagnostic yield based on genetic testing for MODY. Our case series highlights when there is limited awareness of genetic testing, we may get people for consultation with non-insulin anti-diabetic agents responsive MODY in their fourth or fifth decade of age.

We found a diagnostic yield of around 10% of pathogenic or likely pathogenic variants in individuals who were suspected to have MODY. As it's a field that is still evolving, we might consider starting with oral agents under close supervision in those individuals who have VUS; there are some proportions of individuals who might not have classical sulfonylurea-responsive genetic variants, but they might respond to it.

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Authors' contribution

Arunkumar R Pande: Conceived the idea for the study, led the project direction and coordination, wrote the initial draft, and reviewed subsequent edits. Santosh Chaubey, Dinesh Kumar, Kumar Prafull Chandra: Assisted with case studies, providing critical data that shaped the research. Thenral Geetha, Akshita Sharma: Contributed to genetic interpretation, editing of the manuscript, and addressing reviewer comments, ensuring the accuracy and integrity of the final submission.

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Conflicts of interest

There are no conflicts of interest.

Data availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on a reasonable request.

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Table S1 - Number of cases positive for Maturity Onset of Diabetes of Young (MODY) from various labs

Labs	Total eligible cases for score	Number of samples +ve rate in cases Qualified for score (%) including VUS	Number of samples +ve rate in cases Qualified for score (%)
Lab Med Genome	30	8(26.7)	3(10)
Lab Geneplore	1	0	-
Lab Eurofins	1	-	-
Lab Christian Medical College Vellore	21	5(23.8)	2(9.5)
Total	53	13(24.5)	5(9.4)

Table S2- Genes screened by various labs when sent for Maturity Onset of Diabetes of Young (MODY) screening

Laboratory Name	Gene Name
Christian Medical College Vellore	*HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, KCNJ11, ABCC8*
Medgenome	*ABCC8, APPL1, CAV1, EIF2AK3, GATA6, GLUD1, HNF1B, INS, KLF11, NEUROD1, NKX6-1, PPARG, SLC19A2, AGPAT2, BLK, CEL, FOXP3, GCK, HADH, HNF4A, INSR, LMNA, NEUROG3, PAX4, PTF1A, SLC2A2, AKT2, BSCL2, CISD2, GATA4, GLIS3, HNF1A, IER3IP1, KCNJ11, MNX1, NKX2-2, PDX1, RFX6, WFS1, ZFP57*
Geneplore (clinical exome sequencing gene testing was done for 5200+ genes including MODY genes)	*ABCC8, APPL1, CAV1, EIF2AK3, GATA6, GLUD1, HNF1B, INS, KLF11, NEUROD1, NKX6-1, PPARG, SLC19A2, AGPAT2, BLK, CEL, FOXP3, GCK, HADH, HNF4A, INSR, LMNA, NEUROG3, PAX4, PTF1A, SLC2A2, AKT2, BSCL2, CISD2, GATA4, GLIS3, HNF1A, IER3IP1, KCNJ11, MNX1, NKX2-2, PDX1, RFX6, WFS1, ZFP57*
Eurofins (clinical exome sequencing gene testing was done for 5200+ genes including MODY genes)	*ABCC8, APPL1, CAV1, EIF2AK3, GATA6, GLUD1, HNF1B, INS, KLF11, NEUROD1, NKX6-1, PPARG, SLC19A2, AGPAT2, BLK, CEL, FOXP3, GCK, HADH, HNF4A, INSR, LMNA, NEUROG3, PAX4, PTF1A, SLC2A2, AKT2, BSCL2, CISD2, GATA4, GLIS3, HNF1A, IER3IP1, KCNJ11, MNX1, NKX2-2, PDX1, RFX6, WFS1, ZFP57*

Table S3: Details of all cases

Sr No	Case ID	Gene	Positive/ Negative	Proband/ Family	Age at diagnosis of Diabetes	Age of MODY diagnosis	Current Age	M/F	Current Rx details	BMI	FBS	PPBS	HbA1c
1	PP1	*HNF4A*	Positive	Proband	20	52	56	M	Glimepiride 3 mg (OD), Metformin SR 500 (BD), Empagliflozin 25 + Linagliptin 5 (OD)	23.12	72	177	6.9
2	PP3	*HNF1A*	Positive	Proband	17	26	26	M	Gliclazide XR 90 (OD)	25.43	86	160	6.4
3	PP4	*HNF1A*	Positive	Proband	22	37	44	M	Glimepiride 3 mg (1-0-0), Dapagliflozin 10 + Metformin 500 (1-0-0), Metformin 1000 (0-0-1)	26.42	-	-	-
4	FP1	*HNF1A*	Positive	Family	-	14	-	F	-	-	-	-	-
5	FN1	-	Negative	Family	-	-	36	F	-	-	-	-	-
6	FN2	-	Negative	Family	-	-	-	-	-	-	-	-	-
7	PP5	*HNF1A*	Positive	Proband	-	25	25	F	-	-	-	-	-
8	PP6	*HNF1A*	Positive	Proband	28	37	38	F	Gliclazide 90 (1-0-0), Metformin 500 + Sitagliptin 50 (1-0-0)	21.58	300	350	9.8
9	PP7	*HNF1A+HNF1B*	Positive	Proband	9	12	12	F	Metformin 500 (1-0-1)	21.65	121.4	294.8	8.8
10	PP8	*PDX1*	Positive	Proband	13	13	13	M	Glargine (0-0-21), Gliclazide 40 (1-0-0), Metformin 500 (1-0-1)	18.99	99		8.5
11	FP2	*PDX1*	Positive	Family	-	45	-	M	-	-	-	-	-
12	FN3	-	Negative	Family	-	-	-		-	-	-	-	-
13	PP9	*HNF1B*	Positive	Proband	21	18	21	M	Gliclazide XR 90 (OD)	-	-	-	-

14	FN4	-	Negative	Family	32	-	56	M	Lispro (10-19-22), Glargine (0-0-53)	-	-	-	-
15	PP10	*HNF1B*	Positive	Proband	18	23	23	F	Aspart (12-10-8), Glargine (0-0-30)	17.7	194	-	9.1
16	FP3	*CEL*	Positive	Family	-	57	-	M	-	-	-	-	-
17	FN5	-	Negative	Family	35	-	56	F	Glimepiride 3 (1-0-1), Vildagliptin 50 + Metformin 1000 (1-0-1)	23.27	79	149	6.3
19	PP12	*CEL*	Positive	Proband	26	31	31	M	Gliclazide 60 (1-0-0), Metformin 500 + Sitagliptin 50 (1-0-1)	21.71	222	293	9.5
20	PP14	*ABCC8*	Positive	Proband	18	19	24	F	Glimepiride 4 mg (OD), Linagliptin 2.5 + Metformin 1000 (OD)	24.78	-	-	-
21	PP15	*ABCC8*	Positive	Proband	-	27	27	M	Metformin 500 + Teneligliptin 20 (1-0-0), Metformin 500 (0-0-1), Glargine (0-0-22)	22.02	-	-	-
22	PP17	*KCNJ11*	Positive	Proband	7	8	9	M	-	-	-	-	-
23	PN1	-	Negative	Proband	18	-	29	M	Glimepiride 2 mg +Metformin 500 (1-0-1)	20.84	112	-	6.5
24	PN2	-	Negative	Proband	10	-	14	F	Metformin 500 (1-0-1), Glargine (0-0-11)	15.16	92	374	9.3
25	PN3	-	Negative	Proband	23	-	38	M	Aspart (8-8-6), Glargine (0-0-16)	24.48	246		11
26	PN4	-	Negative	Proband	24	-	25	M	Gliclazide 60 (1-0-0), Metformin 500 + Teneligliptin 20 (1-0-0), Metformin 500 (0-0-1), Glargine (0-0-10)	24.46	115	257.4	6.1
27	PN5	-	Negative	Proband	50	-	71	M	Gliclazide 60 (1-0-0), Dapaglifliozin 10 mg + Metformin 1000 (1-0-0), Metformin 1000 mg + Teneligliptin 20 (0-0-1), Saroglitazar 4 (0-01)	35	110	170	7.7
28	PN6	-	Negative	Proband	26	-	29	F	Regular (10-10-0), Glargine (0-0-15)	18.98	312	104	7.33

29	PN7	-	Negative	Proband	17	-	28	F	Gliclazide 60 + Metformin 500 (1-0-0), Metformin 1000 + Teneligliptin (0-0-1)	33.33	145	224	10.2
30	PN8	-	Negative	Proband	51	-	54	F	Metformin 500 (1-0-1)	29.86	180	177	7.8
31	PN9	-	Negative	Proband	17	-	22	F	Metformin 500 + Teneligliptin 20 (1-0-0)	26.97	148		6.2
32	PN10	-	Negative	Proband	-	-	-	M	Vildagliptin 500 + Metformin 1000 (1-0-1), Pioglitazone 30 (1-0-0)	-	-	-	5.5
33	PN11	-	Negative	Proband	34	-	67	M	Metformin 500 (1-0-0), Metformin 1000 + Teneligliptin 20 (0-0-1), Saroglitazar 4 (0-0-1), Glargine (0-0-14)	25.84	152	154	6.8
34	PN12	-	Negative	Proband	-	-	-	M	-	-	-	-	-
35	PN13	-	Negative	Proband	-	-	33	M	Metformin 500 (1-0-1)	30.5	122	188	6.82
36	PN14	-	Negative	Proband		-	43	M	Glargine (0-0-8), Linagliptin 2.5 + Metformin 1000 (1-0-1)	25.8	-	-	-
37	PN15	-	Negative	Proband	35	-	39	F	Glimepiride 4 (1-0-1), Metformin SR 500 (0-0-1), Glargine (0-0-20), Teneligliptin 20 + Metformin 500 (1-0-0)	23.18	196.9	-	6.6
38	PN16	-	Negative	Proband	-	-	38	M	Glimepiride 4 (1-0-0), Metformin 1000 + Sitagliptin 50 (1-0-1), Glargine (0-0-50)	31.61	187	204	8.8
39	PN17	-	Negative	Proband	30	-	45	M	Lispro (5-0-5), Glargine (13-0-11), Linagliptin 2.5 + Metformin 850 (1-0-1)	23.62	90	138	7.1
40	PN18	-	Negative	Proband	-	-	25	M	Lispro (8-8-6), Glargine (0-0-24)	17.26	240	198	11.06
41	PN19	-	Negative	Proband	18	-	26	M	Gliclazide 60 (1-0-0), Linagliptin 2.5 + Metformin 850 (1-0-1)	19.76	225	180	9.1
42	PN20	-	Negative	Proband	57	-	74	M	Glimepiride 2 + Metformin 500 (1-0-0), Metformin 500 + Teneligliptin (0-0-1)	23.28	108	162	6.7

43	PN21	-	Negative	Proband	-	-	-	M	-	-	-	-	-
44	PN22	-	Negative	Proband	Unknown	-	39	F	Regular (6-6-4), Glargine (0-0-10)	17.91	164	-	9.8
45	PN23	-	Negative	Proband	Unknown	-	14	F	Glulisine (7-9-7), Glargine (0-0-11)	15.33	157	-	15.7
46	PN24	-	Negative	Proband	Unknown	-	47	M	Glimepiride 3 (1-0-1), Metformin 500 (1-0-1)	30.41	-	-	-
47	PN25	-	Negative	Proband	-	-	-	F	-	-	-	-	-
48	PN26	-	Negative	Proband	41	-	49	M	Dapagliflozin 10 + Metformin 500 (1-0-0), Sitagliptin 100 + Metformin 500 (0-0-1)	32.31	139.97	-	7.2
49	PN27	-	Negative	Proband	20	-	29	M	Metformin 500 (1-0-1), Glargine (0-0-11)	19.48	178.4	241.2	11.8
50	PN28	-	Negative	Proband	-	-	32	M	Metformin 500 (1-0-1), Glargine (0-0-10)	-	-	-	-
51	PN29	-	Negative	Proband	20	-	30	M	Glipizide 5 (1-0-1/2), Metformin 500 + Sitagliptin 50 (1-0-1), Glargine (0-0-8)	19.06	111	176	7.5
52	PN30	-	Negative	Proband	57	-	66	M	Gliclazide 40 (1/2-0-0)	23.51	128	88	6.4
53	PN31	-	Negative	Proband	39	-	50	M	Gliclazide 120 (1/2-0-0), Metformin 500 + Sitagliptin 50 (1-0-1)	28.12	140	-	9.2
54	PN32	*CEL*	Negative	Proband	24	26	27	F	Aspart + Degludec (8-0-8)	18.46	115	135	12.1
55	PN33	*CEL*	Negative	Proband	24	30	34	F	Metformin 1000 (1-0-1), Aspart (10-10-8), Detemir (25-0-25)	27.12	217.7	232.5	-
56	PN34	*ABCC8*	Negative	Proband	28	41	41	M	Glimepiride 4 (1-0-0), Dapagliflozin 10 + Metformin 500 (1-0-0), Metformin 1000 + Sitagliptin 100 (0-0-1), Glargine (0-0-100	28.67	296.14	-	7.8

57	PP2	*HNF4A+RFX6*	Negative (*HNF4A* detected in asymptomatic father also; Single heterozygous *RFX6* variant was observed)	Proband	-	16	16	-	-	-	-	-	-
58	PP11	*KLF11*	Negative (1 homozygote observed in gnomAD database)	Proband	16	24	24	F	Gliclazide 60 (1-0-0), Metformin 500 + Sitagliptin 50 (1-0-1), Glargine (0-0-10)	22.11	183	425.4	5.2
59	PP13	*PAX4*	Negative (7 homozygotes observed in gnomAD database)	Proband	42	45	46	F	Lispro (8-12-7), Degludec (0-0-28)	25.7	153	-	9.2
60	PP16	*ABCC8*	Negative (1	Proband	19	19	19	M	Glipizide 5 (1-0-1), Metformin SR 500 (1-0-1), Aspart + Degludec (18-0-0)	26.2	-	-	8

			homozygote observed in gnomAD database										
61	PP18	*LIPC*	Negative (1 homozygote observed in gnomAD database)	Proband	24	25	32	F	Aspart (13-13-12), Glargine (0-0-18)	24.95	145	94	6.3
62	PP19	*PPARG*	Negative (Unrelated to the Clinical phenotype)	Proband	-	33	33	M	-	-	-	-	-

PP: Proband Positive; PN: Proband Negative; FP: Family Positive, FN: Family Negative; M: Male; F: Female; BMI: Body Mass Index; FBS: Fasting Blood Sugar; PPBS: Post Prandial Blood Sugar; HbA1c: Glycosylated Haemoglobin

Table S4- Prioritised Variants with Significance based Classification

Case ID	Gene	Variant c. position	Amino acid change	Literature	ACMG classification	ACMG criteria
PP1	*HNF4A*	c.932G>A	p. Arg311His	PMID: 24947580[1], 26059258[2], 25414397[3]	Likely Pathogenic	PS1, PM2, PP3
PP3	*HNF1A*	c.804del	p. Phe268LeufsTer74	Not reported	Likely Pathogenic	PVS1, PM2
PP4	*HNF1A*	c.599G>A	p. Arg200Gln	PMID: 35918471[4],37396188[5]	Pathogenic	PS3, PM1, PM2, PP3, PP5, PM5
PP5	*HNF1A*	c.403G>A	p. Asp135Asn	PMID: 18003757[6]	Uncertain Significance	PM1, PM2 ,PP3
PP6	*HNF1A*	c.1273_1285delinsC	p. Thr425_Thr429delinsPro	Not Reported	Uncertain Significance	PM1, PM2, PM4
PP7	*HNF1A*	c.599G>A	p. Arg200Gln	Reported (Gu et al. 2004) ; PMID: 35918471[4],37396188[5]	Pathogenic	PS3, PM1, PM2, PP3, PP5, PM5
	HNF1B	c.37C>A	p. Leu13Met	Not reported	Uncertain Significance	PM2, PP3
PP8	*PDX1*	c.683_699del	p. Ala228GlyfsTer33	Not reported	Likely Pathogenic	PVS1, PM2
PP9	*HNF1B*	c.56C>T	p. Ser19Phe	Not reported	Uncertain Significance	PM2, PP3
PP10	*HNF1B*	c.1004C>G	p. Pro335Arg	Not reported	Uncertain Significance	PM2, PP3
PP12	*CEL*	c.2040dup	p. Val681ArgfsTer6	Not reported	Uncertain Significance	PM1, PM2
PP14	*ABCC8*	c.2616C>G	p. Ile872Met	Not reported	Uncertain Significance	PM2, PP3
PP15	*ABCC8*	c.361G>A	p. Val121Met	Not reported	Uncertain Significance	PM2, PP3
PP17	*KCNJ11*	c.661C>T	p. Arg221Cys	Not reported	Uncertain Significance	PM2, PP3

ACMG: American College of Medical Genetics and Genomics

VUS: Variant of Unknown Significance

Table S5-significant VUS TABLE

Sr No	Case ID	Sex	Age of diagnosis	Age of MODY diagnosis	Current Age	BMI	HbA1c at diagnosis	Baseline treatment	Current Treatment	Current Hb A1c	Complication	Additional Features	Gene	Variant	MODY type	Pathogenicity	Previous reports	MODY Probability (%)
1	**PP5**	M		27	27	-	-	-	-	-	-	-	*HNF1A*	(p.Asp135Asn)	MODY type III	Uncertain Significance	-	
2	**PP6**	F	28	37	38	21.58	9.8	-	Gliclazide 90 (1-0-0), Metformin 500 + Sitagliptin 50 (1-0-0)	7.9	-	-	*HNF1A*	(p.Thr425_Thr429delinsPro)	MODY type III	Uncertain Significance	-	62.4
3	**PP9**	M	18	18	22	20	>10	Glimepiride 2 mg (1-0-0)	Gliclazide XR 90 (OD)	9.2	-	-	*HNF1B*	(p.Ser19Phe)	MODY type V	Uncertain Significance	-	75.5
4	**PP10**	F	18	23	23	17.7	-	Aspart Glargine	Aspart (12-10-8) Glargine (0-0-30)	8.1	-	Renal cyst	*HNF1B*	*(p.Pro335Arg)*	MODY type V	Uncertain Significance	-	

5	**PP12**	M	26	31	31	21.71	-	-	Gliclazide 60 (1-0-0) Sitagliptin 100 + Metformin 500 (1-0-1)	9.5	Neuropathy	-	*CEL*	(p.Val681ArgfsTer6)	MODY type VIII	Uncertain Significance	-	45.5
6	**PP14**	F	18	19	24	24.78	-	-	Glimepiride 4 mg (OD), Linagliptin 2.5 + Metformin 1000 (OD)	6.7	-	-	*ABCC8*	(p.Ile872Met)	MODY type XII	Uncertain Significance	-	75.5
7	**PP15**	M	-	27	27	22.02	-	-	Metformin 500 + Teneligliptin 20 (1-0-0), Metformin 500 (0-0-1), Glargine (0-0-22)	-	-	-	*ABCC8*	(p.Val121Met)	MODY type XII	Uncertain Significance	-	
8	**PP17**	M	7	8	9	-	-	-	Glimepride 2mg (OD)	-	-	-	*KCNJ11*	(p.Arg221Cys)	MODY type XIII	Uncertain Significance	-	75.5

Table S6- Comparison of our case series with the previous case series presented from India

MODY type	CHAPLA et al 2014	Mruthyunjaya et al 2017[5]	Mohan et al 2018	Sampathkumar et al 2022	Bhat et al 2022	Arthy et al 2023	Menon et al. 2023	Our data
Total cases	11	9	21	4	20	34	12	13
Setting	Getting Lab for MODY	Pregnancy	To discover new genes	Predefined Criteria		Records retrieved from EMR		Outpatient Department
HNF4A (MODY 1)	2 (p.Val169Ile) (p.Glu271Lys)	-		1		10	1	(p.Arg311His)
GCK (MODY 2)	(p.Glu440X)	Ile348Phe	1					-
HNF1A (MODY 3)	(p.Ala501Ser)	Ser3Cys	9	2	20	21		(p.Phe268LeufsTer74) (p.Arg200Gln) (2) d (p.Thr425_Thr429delinsPro) (p.Asp135Asn)
PDX1 (MODY 4)	(p.Glu224Lys) a (p.Glu224Lys) b	Glu224Lys His94Gln*	1					(p.Ala228GlyfsTer33)
HNF1B (MODY 5)	p.Leu92Phe	-	1					(p.Ser19Phe) (p.Leu13Met) d p.Pro335Arg
NEUROD1 (MODY 6)	(p.His241Gln) a (p.His241Gln) (p.Glu59Gln) b	Glu59Gln Phe318Ser						-
KLF11 (MODY 7)	-	-	1					

CEL (MODY 8)	-	-	1					(p.Val681ArgfsTer6)
PAX4 (MODY 9)	p.Arg31Leu	-	-					
INS (MODY 10)	-	Gly44Arg						
ABCC8 (MODY 12)	-	Arg620Cys	5	1		3	1	(p.Ile872Met) (p.Val121Met)
KCNJ11 (MODY 13)	-		1					(p.Arg221Cys)
APPL1 (MODY 14)	-							-
Others			*RFX6, WFS1, AKT2, NKX6−1, EIF2AK3, GLIS3, HADH, MNX1, NKX2−2, PTF1A*				9+1 (WFS1 + PTF1A) = 10	

Case Summaries:

Case PP1:

A 52-year-old man who was diagnosed with diabetes when he was 23 years old and has had diabetes for around 29 years came to us. His BMI was 25.43 kg/ m 2. When seen first, he was on Premix (30/70) (regular and NPH) insulin, and vildagliptin 50 mg once a day. His fasting blood glucose was 158 mg/dl and postprandial blood glucose was 301 mg/dl and HbA1c was 8.6%. On carefully reviewing the family history we found that he had an autosomal dominant pattern and young age of onset (< 35 years) in the family history of diabetes. He was shifted on twice a day regular and bedtime glargine insulin whereas Vildagliptin 50 mg was continued. Genetic analysis was done by Sanger Sequencing. Utilizing the next generation sequencing we have identified a novel *HNF4A* gene variant c.932G>A p. Arg311His The identified novel *HNF4A* gene variant was absent in the 1000 genome database and also in the Exome Aggregation Consortium database. Subsequently, his insulin was stopped and he was shifted to oral anti-diabetic medicines i.e. Gliclazide extended release 60 mg 1½ tablet before breakfast, Metformin 500 mg, and Sitagliptin 50 mg twice a day. He had a good response with the same and his fasting blood glucose came down to 124 mg/dl, and postprandial blood glucose came to 145 mg/dl with HbA1c was 7.3 %. [7] Oral glucose tolerance was normal for the daughters (table S5)

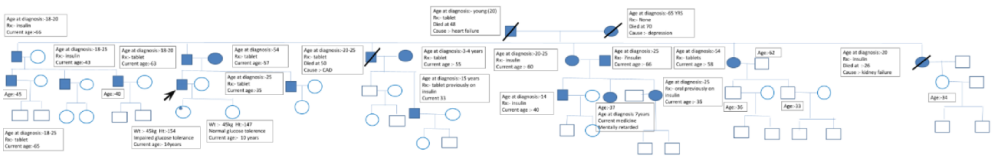
Table S5: Oral Glucose Tolerance Test of the three daughters of Case PP1

Glucose (mg/dL)	Fasting	Post 1 Hour	Post 2 Hours
25 yrs/daughter	82	99	87
23 yrs/daughter	90	97	95
13 yrs/daughter	79	88	86

Case PP4:

A lean 37-year male who came to us with a history of diabetes for 19 years was diagnosed at the age of 18 years. When seen first he was on 4 oral antidiabetic agents (OADA), metformin 1 gm, canagliflozin 100 mg, sitagliptin 100 mg, and pioglitazone 15 mg. His fasting blood glucose was 160 mg/dl and postprandial blood glucose pp 280 mg/dl and HbA1c of 9.4 %. On carefully reviewing the history we found that he had a family history of diabetes involving three generations at a young age. (figure S1) The patient was shifted to glimepiride 3 mg and all the other OADA was stopped. He had a good response to the same and his fasting blood glucose came to 110, postprandial blood sugar 160 and his HbA1c after three months was 6.7%. Genetic analysis was done by Sanger sequencing. His report came out to be positive for the novel Pathogenic variant c.599G>A p.Arg200Gln in the *HNF1A* gene. This variation is located in a conserved region and the bioinformatic analysis using PolyPhen_2, Sorting Intolerant from Tolerant (SIFT), and MutationTaster2 predicts this variant as probably damaging. This variant has not been described by an exome sequencing project or exome aggregation consortium. Therefore, this novel variation with previous reports of variant at this codon and damaging in silico predictions suggest that this variant is clinically relevant and is likely pathogenic based on American College of Medical *Genetics* and Genomics (ACMG) 2015 guidelines.[8,9]

Fig S1: Family tree of case PP4



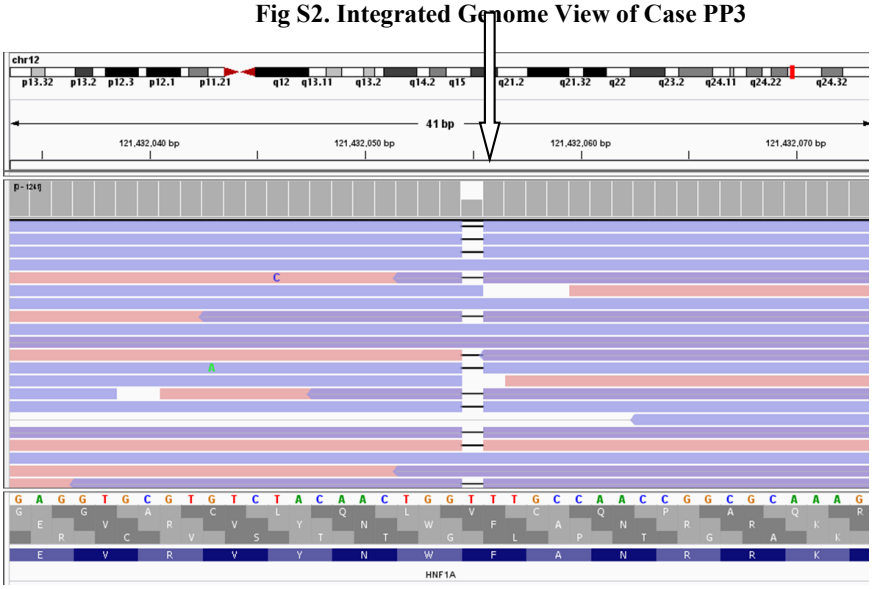
Case PP9:

A 21-year-old shopkeeper presented to us at the age of 18 years with a blood sugar of 550 mg/dL, an HbA1c of 12%, a lean body habitus, and osmotic symptoms. His mother and siblings also had diabetes. Given the very high blood sugar levels and osmotic symptoms, he was advised to take insulin, but he declined despite repeated persuasion. His ketones were negative, and with the patient's assurance of a regular follow-up, Glimepiride 3 mg was initiated. He responded quite well to this medication, and his blood sugars were reduced to 110 mg/dL fasting and 180 mg/dL postprandial. This led to suspicion of MODY, hence a genetic investigation was performed, which revealed an *HNF1B* variant. His elder brother who had been diagnosed with infertility and azoospermia and had been on insulin for the previous five years was switched to sulfonylurea medication with which he responded well. Based on ACMG 2015 guidelines this variation can be categorized as likely pathogenic.

Case PP3:

A 26-year-old man with a strong family history of diabetes spanning 3 generations and a family history to suggest premature coronary artery disease (CAD) was diagnosed with diabetes. He had a good response to sulfonylurea. Suspecting sulfonylurea-responsive MODY a genetic evaluation was performed which revealed an *HNF1A* variant. (fig S2) This case was the novel variant of the gene *HNF1A* and the MutationTaster2 of this variant came out to be damaging.[12]

Fig S2. Integrated Genome View of Case PP3



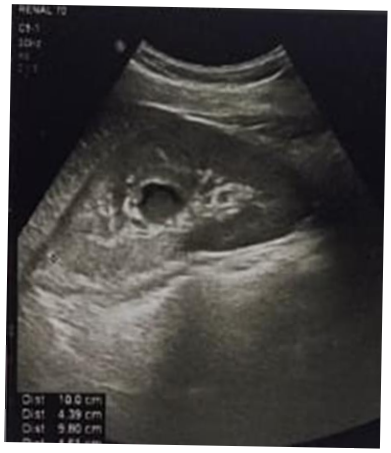
Note: The variant chr12:120994254delT was detected in the *HNF1A* gene. On IGV the positive strand corresponds to red colour reads and the negative strand corresponds to blue colour reads.

PP10: Integrated Genome View of *HNF1B*

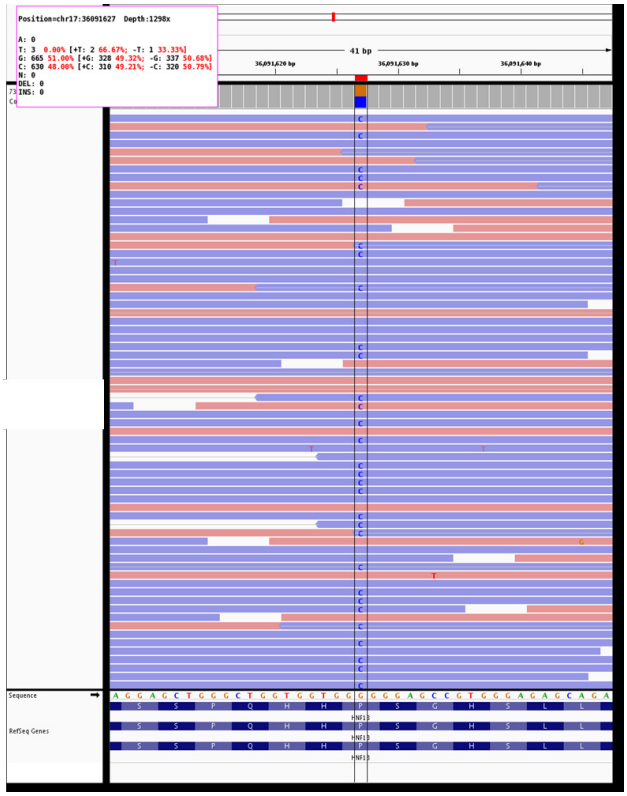
A 23-year-old female with a history of diabetes at an early age. Her mother had diabetes at a young age and died of renal complications of diabetes in her late 30s. Her USG revealed a renal cyst and an atrophic pancreas. (figure S3) We thus suspected it to be a

case of MODY type 5 and a genetic evaluation was advised. There was a novel genetic variant at *HNF1B*..[10] (figure S4)

Fig S3. Ultrasound of PP10 showing the presence of renal cyst



S4. Integrated Genome View of Case PP10 (*HNF1B*)



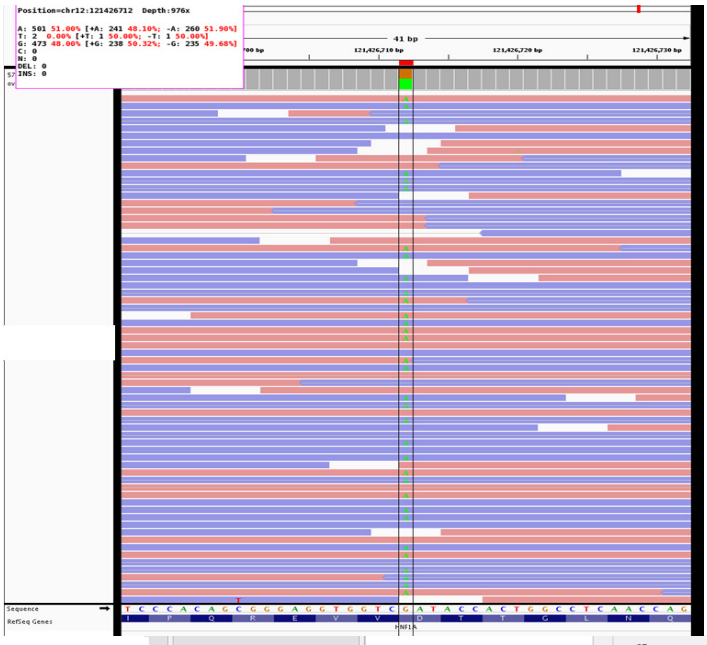
Note: The variant chr17:37731636G>C detected in the *HNF1B* gene has 1298x depth with 48% of the reads with alternate alleles. On IGV the positive strand corresponds to red colour reads and the negative strand corresponds to blue colour reads.

Case PP13:

A 45-year-old professional who was diagnosed with diabetes in her early 20s', was detected as a part of an evaluation of her underweight status. There was no history of diabetic ketoacidosis; her antibodies for type 1 diabetes including anti-IA2 and anti-GAD antibodies were negative. Considering the fact that she never had diabetic ketoacidosis and antibodies were negative MODY was suspected and a gene evaluation was done which turned out to be a *PAX4 variant*. (figure S5) We wanted to perform a genetic evaluation of the parents to evaluate the pathogenic status of the variant but unfortunately, they refused.

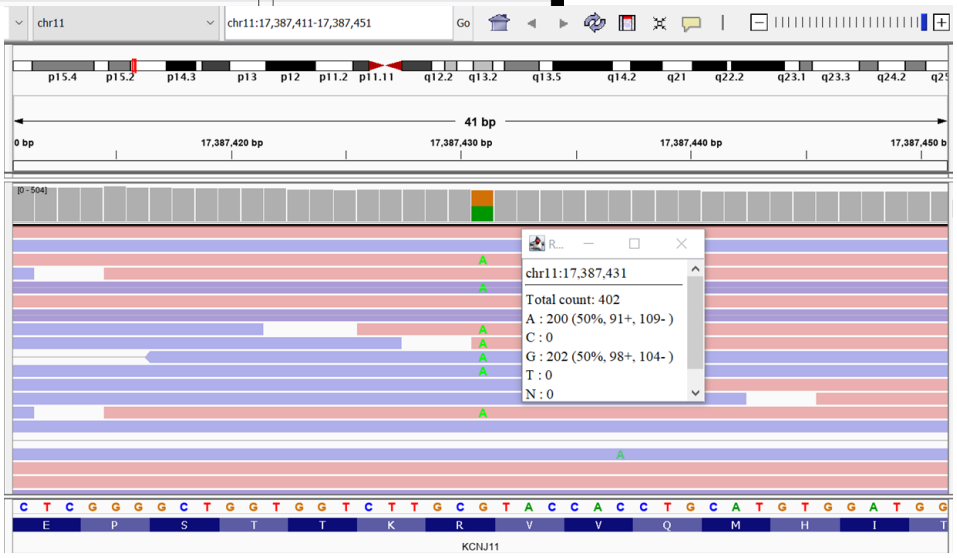
As the case history is becoming repetitive, we have just included the Integrated Genome View for reference.

Fig S5. Integrated Genome View of Case PP5



Note: The variant chr12:120988909G>A detected in the *HNF1A* gene has 976x depth with 51% of the reads with alternate alleles. On IGV the positive strand corresponds to red colour reads and the negative strand corresponds to blue colour reads.

Fig S6. Integrated Genome View of Case PP17



Note: The variant chr11:17387431G>A detected in the *KCNJ11* gene has 402x depth with 50% of the reads with alternate allele. On IGV the positive strand corresponds to red colour reads and negative strand corresponds to blue colour reads.

***GCK* MODY Case Summary**

Case PN11:

A 67-year-old man was diagnosed with diabetes at the age of 34 years and was treated with oral hypoglycemic agents for more than 3 decades. In these 3 decades, his HbA1c has ranged from 5.6 to 6.8. At the onset, his prescribed treatments were oral antidiabetic agents but he was on Metformin 1 Teneligliptin 20 mg daily and 14 units of Insulin glargine 100 IU /ml daily - as his fasting glucose values were consistently above 200 mg/dl. Considering the above scenario gene testing for *GCK* MODY was sent but surprisingly it came out to be negative.

Case PN20:

A 69-year-old male was diagnosed at the age of 57 years with diabetes for the very first time when blood glucose levels were tested as a part of routine investigation. He was treated with oral hypoglycemic agents (Glimepiride 2 mg and Metformin 500 mg once a day) and had no micro or macrovascular complications. At the time his BMI was 23.82 kg/m^2 and his HbA1c was 7.2%. His fasting blood glucose was 140 mg/dl and postprandial blood glucose was 142 mg/dl. He had a meticulous record-keeping habit and kept all the records of blood glucose testing safely. On reviewing his records (fig S7, S8), It was noticed that his fasting blood glucose was higher in comparison to postprandial blood glucose levels at majority of the times. Suspecting *GCK* MODY, he was advised to get the blood glucose levels of family members checked (fig S9). Interestingly his elder son was found to have similar blood glucose profiles i.e. the fasting blood glucose levels were higher than postprandial blood glucose levels, which were normal. Awaiting genetic report and as he agreed to a close follow up the treatment was ceased. On follow-up after stopping the treatment his fasting blood sugar was 145 mg/dl and postprandial blood sugar came to 127 mg/dl. There was no significant change seen in HbA1c results with or without treatment. This indicated the presence of fasting hyperglycemia.

Fig S7: Graphical representation of Blood Glucose (Fasting and Postprandial) of case PN20 from the initial diagnosis.

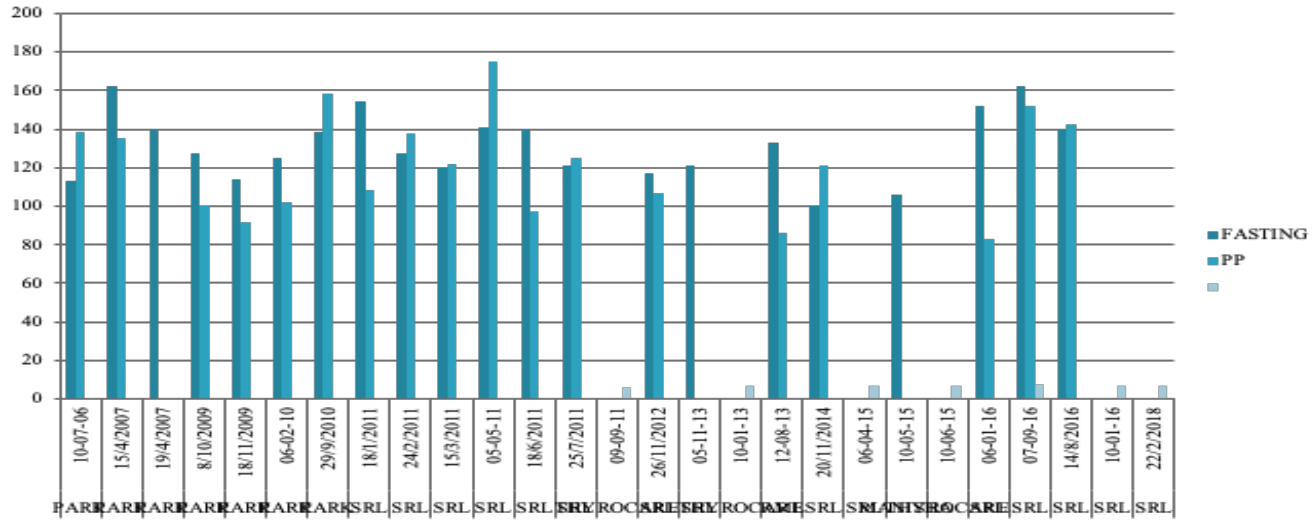
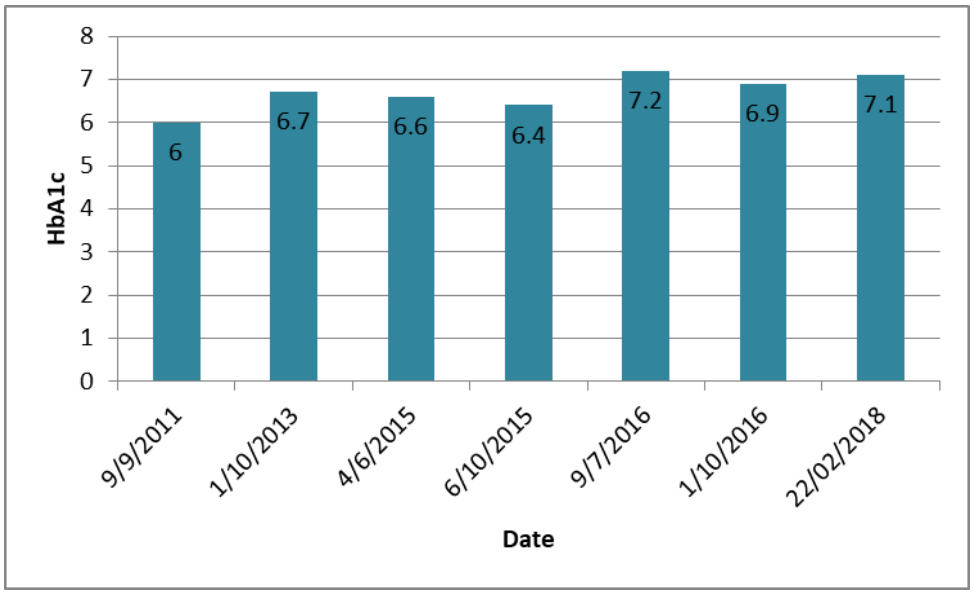
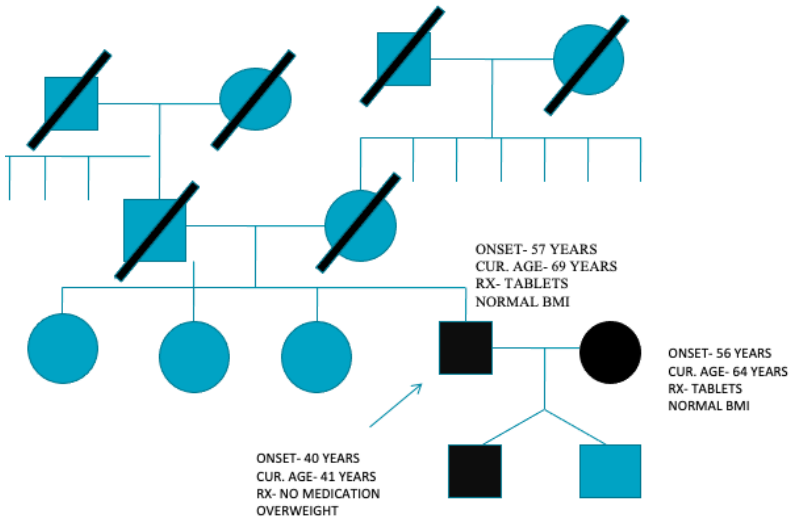


Fig S8: HbA1c of case PN20 over the course of 8 years.



HbA1c in the past 8 years has been fairly stable

Fig S9: Family tree of case PN20



Sulphonylurea: Responsive MODY Case Summary

<u>Case PN9:</u>

A 19-year-old female with a BMI of 26.97 kg/m² was found to have an HbA1c of 10.6%, a strong family history of diabetes, and a MODY score of >75.5%. On genetic testing, the results came out to be negative however she was responding to OADA (Metformin + Teneligliptin) and did not require insulin for her blood sugar management. Her HbA1c dropped from 10.6% to 6.2% on only OHA treatment signifying the likelihood of a certain form of MODY that might have not been discovered yet.

Case PN29:

A 30-year-old man came to us who was diagnosed with diabetes at the age of 20 years, his BMI was 19.06 Kg/m2 and he was labelled as type 1 diabetes, and has been on insulin for almost half a decade. After a thorough evaluation, a MODY score was found to be 8.2% when insulin use was taken into account but as soon as we changed it to non-insulin use, the score came out to >75.5%. He was tested for MODY as there was a strong family history of diabetes and despite the negative outcome on MODY gene testing and also the fact that family members were responding to sulphonylurea-based oral agents, under our close supervision the transition was made from a basal-bolus insulin regimen to an OADA (Glipizide (1-0-½), Metformin + Sitagliptin (1-0-1)). His HbA1c test 3 months after stopping insulin came down to 6.9%.

Brother of Case PN9:

The brother of the above patient, a 31-year-old doctor with a BMI of 26.06 kg/m^2 diagnosed with diabetes in his late teens. He was diagnosed with Type 1 diabetes and was on a basal-bolus regimen. Seeing the fact, that the sister was responding to oral agents, he was shifted onto oral agents (gliclazide 60 mg extended release, linagliptin 5 mg with metformin 1000 mg). His HbA1c before starting oral agents was 6.9 % and after being on OADA for 3 months it came to 7.5%. He was lost to follow-up for 10 months and came with an HbA1c of 11.4%. He started on basal insulin. Currently, the patient is being treated with gliclazide 30 mg (modified release), metformin 2000 mg + sitagliptin 100 mg, and a basal insulin glargine 30 units daily with his latest HbA1c being 7.5 %.

Fig S10: Integrated Genome View of Case PP8

Note: The variant chr13:g.27924532_27924548CCGTGACCTCCGGCGAGdel detected in the *PDX1* gene. On IGV the positive strand corresponds to red colour reads and the negative strand corresponds to blue colour reads.

Overview of NGS Methodology*

DNA isolation, Exome library preparation, and sequencing:

Genomic DNA isolated from whole blood using QIAamp DNA Blood Mini Kit (Qiagen, Germany) is quantified using Qubit fluorometer (Thermo Fisher Scientific, USA). For library preparation, 200ng of the Qubit quantified DNA is fragmented to ~250bp inserts. The library is hybridised and enriched using whole exome probes. The fragments are end-repaired, 3′ adenylated and ligated with the indexed adapters. The adapter-ligated fragments are then amplified with adapter-specific primers followed by size selection and purification to generate the gDNA library. The generated library is assessed for fragment size distribution using Tape Station (Agilent, USA) and quantified using Qubit (Thermo Fisher Scientifc, USA). The library is sequenced as 2 × 150 bp paired-end reads on an Illumina HiseqX / Novaseq (Illumina, CA) machine according to the manufacturer's protocol to an average sequencing depth of ≥80-100x.

Data processing, variant calling and annotation:

Following quality check and adapter trimming using fastq-mcf (version 1.04.676), the sequencing reads obtained are aligned to human reference genome (GRCh38.p13). The aligned reads are sorted, and duplicate reads removed. Single nucleotide variants (SNVs) and small Indels variants are called using GATK best practices pipeline using Sentieon (v201808.07)[11]. Gene annotation of the variants is performed using the VEP program against the Ensembl release 99 human gene model[12,13]. The variants are annotated for allele frequency [population databases GnomAD (v2.10), GnomAD (v3.0), 1000 genome, MedGenome population specific database], *in silico* prediction tools [PolyPhen-2, SIFT, Mutation Taster2, and LRT] and disease databases [OMIM, ClinVar and HGMD]. The clinically significant variants are sequentially prioritised based on a) minor allele frequency < 0.01; b) previously reported with disease literature; c) supporting damaging effect by ≥ 2 *in silico* prediction tools; d) clinical features of the proband and analysed using Varminer (MedGenome proprietary variant interpretation tool).

Copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth (v1.1.10) method[14]. Based on the comparison of read-depths of the test data with the matched aggregate reference dataset, the algorithm detects CNVs (≥ 400bp deletions and duplications).

Notes: The *in-silico* predictions are based on Variant Effect Predictor (v104), [SIFT version - 5.2.2; PolyPhen - 2.2.2; LRT version (November, 2009); CADD (v1.6); Splice AI; dbNSFPv4.2] and MutationTaster2 predictions are based on NCBI/Ensembl 66 build (GRCh38 genomic coordinates are converted to hg19 using UCSC LiftOver and mapped to MT2). Diseases databases used for annotation includes ClinVar (updated on 5082021), OMIM (updated on 5082021), HGMD (v2021.3), DECIPHER (population CNV) and SwissVar. *Based on the samples processed in MedGenome

Classification of the prioritised variant based on ACMG:

The prioritised variants were classified into five tiers: Pathogenic (P), Likely pathogenic (LP), Uncertain significance (VUS), Likely benign (LB), and Benign (B), depending on the applied criteria according to the parameters suggested by the ACMG guidelines10. Some of the primary criteria are a) reported in literature/ disease databases (HGMD, Clinvar etc) b) variant functional evidence, c) segregation of the variant in affected/ unaffected individuals with similar phenotypes from literature along with d) allele frequencies and e) in-silico prediction tools as supporting values. The classification is based on the weighted average of the available evidence. During variant interpretation, significant variants were classified as Pathogenic or likely pathogenic. Benign variants were excluded and variants whose role was inconclusive were classified of uncertain significance. The databases and tools used have been listed above. Additionally, the details of the applied criteria for the ACMG parameters have been added in the table.

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