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Evaluation of the presence and the pathogenicity of a novel HNF1A p.Lys120Gln mutation identified in a patient suspected of Maturity Onset Diabetes of the Young.

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Abstract

Maturity Onset Diabetes of the Young (MODY) is a form of monogenic diabetes characterized by autosomal dominant inheritance and early onset. At least 14 different subtypes of MODY have been characterized based on the gene involved, and the specific subtype determines the most effective treatment. Hepatocyte nuclear factor-1 alpha (HNF1A)-MODY, also called MODY3 is one of the most common MODY subtypes seen among 20-60% of MODY patients. Patients with HNF1A-MODY are highly sensitive to sulfonylureas, and do not require insulin treatment in early stages. Therefore, proper genetic characterization is important in the diagnosis of MODY. In prior research aimed at developing a Next Generation Sequencing (NGS) based test for the diagnosis of MODY, a novel HNF1A c.358 A>C (p.Lys120Gln) mutation has been identified, which has not previously been reported, and the pathogenicity unknown. The objective of the current research was to confirm the presence of this mutation by an alternative method, and to carry out *in silico* structural and functional characterization, as well as population studies in order to determine its pathogenicity. We have confirmed the presence of this mutation in the tested sample by Sanger sequencing, thereby verifying the accuracy of NGS results. *In silico* structural analysis predicts possible deleterious effects of this mutation. Population databases show extremely low incidence of this mutation in the general population. Together with interpretations from other relevant literature and databases, we have deduced the HNF1A c.358 A>C (p.Lys120Gln) mutation to be a likely pathogenic variant causative of MODY, which may be utilized for the diagnosis of HNF1A-MODY.

Keywords: MODY3, HNF1A, p.Lys120Gln, Sanger sequencing, Pathogenicity

1. Introduction

MODY is a subset of monogenic diabetes characterized by an autosomal dominant inheritance that can be transmitted by either parent or occur as a de-novo mutation.[1] It is classically characterized by a non-acute and non-ketotic presentation in lean subjects, typically before 25 years of age.[2] About 1-6% of patients with diabetes suffer from MODY, and distinguishing it from type 1 or type 2 diabetes is a diagnostic challenge.[2,3,4] MODY often faces misclassification as type 1 diabetes (T1D) due to its onset at a young age, or as early onset type 2 diabetes (T2D) due to relatively low risk of ketosis, and the low-dependence on insulin. MODY stands apart from T1D and T2D genetically, as it is primarily caused by a single gene mutation.

There are at least 14 different subtypes of MODY, categorized based on the specific gene that is mutated. The most common among these, MODY1, MODY2 and MODY3 are associated with HNF4α, GCK and HNF1α respectively. Together these account for up to 80% of MODY cases, while the rest have mutations in one of the other MODY genes, namely, PDX, HNF1β, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11, and

APPL1. The different genes varied in terms of the age at which symptoms appear, how well they respond to treatments, and if extra-pancreatic symptoms are present.[5] Epidemiologically, variants in MODY genes have been reported from every region of the world.[5] Very limited studies have been carried out thus far with Sri Lankan patients suspected of MODY, which have identified mutations in some of these genes including HNF1A.[6]

The most frequently mutated gene in MODY overall is GCK followed by HNF1A.[5] However, in some populations, mutations in HNF1A gene are the most common cause of MODY.[7] HNF1A is a transcription factor that is expressed in the gut, kidney, liver, and pancreas, among other organs, and the gene is located on chromosome 12 (NC_000012.12) in the region 12q24.2.[8]

HNF1A is a regulatory protein that controls the expression of many genes in pancreatic beta cells, liver, kidneys and intestines. HNF1A-MODY occurs due to inhibition of the key steps of glucose transport and metabolism as well as mitochondrial metabolism in pancreatic-β cells.[6] In a study involving human islets from a person with a missense variation in the HNF1A gene, researchers revealed that, even though having normal β cell mass and key β cell characteristics, the individual experienced difficulty releasing insulin in response to glucose. This issue was linked to changes in genes related to glucose metabolism and ATP production. Additionally, significant alterations were observed in various metabolic functions, including gene transcription, protein synthesis and degradation, and cellular communication. The study suggests that a mutation in HNF1A may contribute to diabetes not by reducing β cell mass, but by disrupting the normal function of β cells required for insulin release in response to glucose, impacting β cell transcriptional regulatory networks.[9]

Individuals with HNF1A-MODY are usually non-insulin dependent at diagnosis, but their beta-cell function declines over time, leading to worsening hyperglycaemia. They show increased sensitivity to sulfonylureas, which can restore insulin secretion by bypassing the defective pathways, often making them more effective than insulin in the early stages.

In a previous study aimed at developing a Next Generation Sequencing (NGS) based test for the diagnosis of MODY, we have identified a patient with a novel HNF1A c.358 A>C (p.Lys120Gln) variant (unpublished research). This variant has not been previously reported in any MODY patient but given the role of HNF1A gene in MODY and the general attributes of the specific mutation, we carried out further verification of this variant and its pathogenicity analysis. Our studies confirmed the presence of this variant by the alternative method of Sanger sequencing[10], and established the variant as likely pathogenic in MODY.

2. Methodology

This study was approved by the Ethics Committee of the National Hospital of Sri Lanka (approval AAJ/ETH/COM/2024/OCT).

2.1. Samples. The c.358A>C (p.Lys120Gln) variant in the HNF1A gene was initially identified in the SN10 sample by NGS method. To confirm the presence of the detected variant, PCR & Sanger sequencing was subsequently performed with SN10 sample, along with SN05 (sample negative by NGS) and no template (nuclease free water) negative control.

Specific primers targeting the HNF1A gene were designed using Primer 3 Plus tool as below: Forward primer 5'-TACCTCACCGTCCCTGAGTC-3' and reverse primer 5'-CTGGTTGAGGCCAGTGGTAT–3'. The specificity of the primers for the target region was confirmed using Primer BLAST.

2.2. Sanger sequencing process. Genomic DNA were extracted from peripheral blood samples using the QIAmp DNA Mini Kit (Qiagen, Germany) following the manufacturer's protocols. PCR amplification of the target region was performed in a 15µL reaction using 5X FIREPol® Master Mix (Solis Biodyne, Estonia), primers, nuclease-free water, and genomic DNA. PCR Thermocycling was conducted with an initial 10 minute denaturation at 95°C, followed by 35 cycles of 95°C for 30 seconds, 57°C for 30 seconds, 72°C for 2 minutes, and a final extension at 72°C for 10 minutes using a SimpliAmp™ Thermal Cycler.

Following amplification, PCR products were loaded on a 1% agarose gel and resolved at 70V for 15 minutes, with a 100bp DNA ladder used as a molecular size marker. Gel was visualized through the UV-transilluminator. PCR products were then purified using magnetic bead purification methods to eliminate unincorporated primers, nucleotides, and other impurities before downstream Sanger sequencing.

Chain termination PCR was performed using the bead purified PCR products as templates. The 10 µL reaction mixture contained 0.5µL of 2.5X Ready Reaction Mix, 1.75 µL of 5X Dilution Buffer, 2µL of 0.8 µM HNF1A forward primer, and 5.75µL of purified template. PCR thermal cycling was conducted with an initial denaturation at 96°C for 1 minute, followed by 25 cycles of denaturation at 96°C for 10 seconds, primer annealing at 50°C for 5 seconds, and extension at 60°C for 4 minutes, with a final hold at 4°C.

Post-PCR cleanup was performed using EDTA (0.125µM) and ethanol purification. DNA pellets were dissolved in 10µL of Hi-Di Formamide (Thermo Fisher Scientific). Purified products were then subjected to capillary electrophoresis using the SeqStudio Genetic Analyzer (Thermo Fisher Scientific). Sequence data were analyzed using the BioEdit sequence alignment editor.

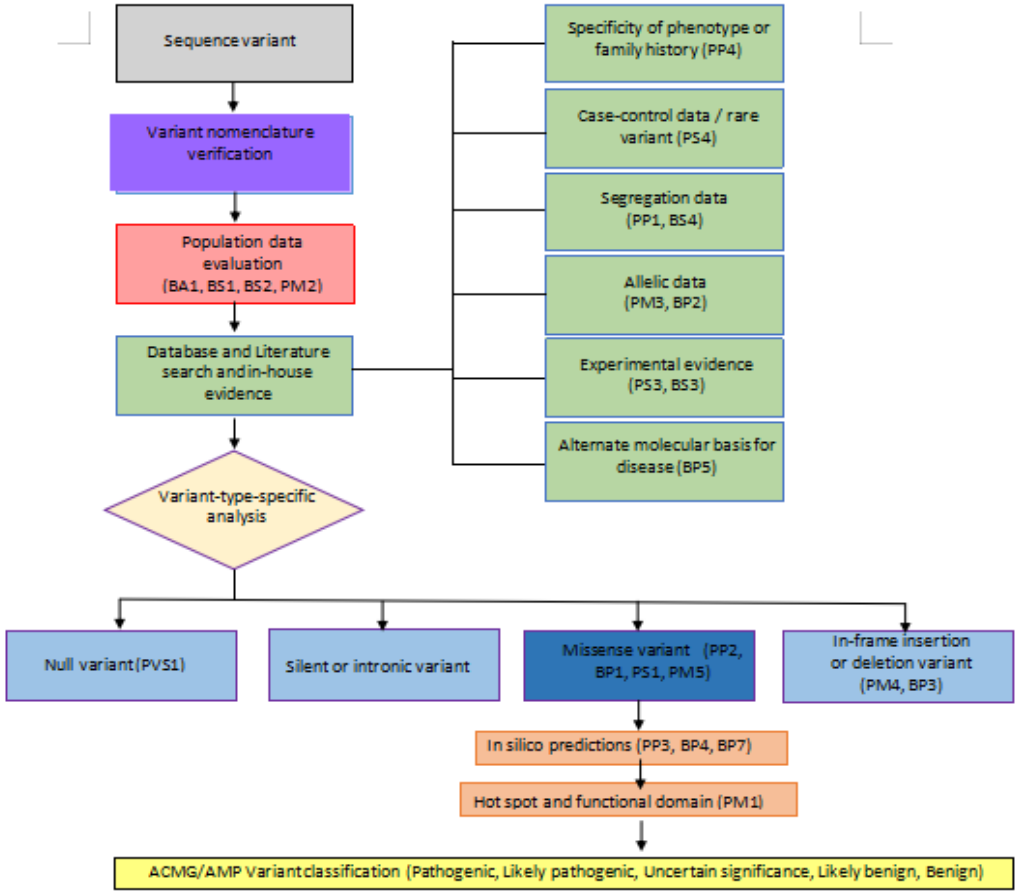

Figure 01: The variant classification and interpretation workflow as per ACMG/AMP guidelines (2015). [11]

2.3. Variant data pathogenicity analysis

2.3.1. Preliminary NGS analysis. Post NGS analysis was conducted using the Ion Reporter platform (ThermoFisher Scientific) with the in-built annotation workflow to identify and annotate potential variants.

The HNF1A c.358A>C (p.Lys120Gln) variant identified in the SN10 sample was further confirmed by aligning and evaluating the sequencing reads using the Integrative Genomics Viewer (IGV).

2.3.2. Assessment of the clinical significance. To assess the clinical significance of the identified HNF1A gene variant of SN10 patient, first followed the standardized variant interpretation workflow recommended by the American College of Medical Genetics and Genomics (ACMG). The analysis began with the identification and nomenclature verification of the variant according to the Human Genome Variation Society (HGVS) standards.[12] Population frequency was evaluated using public databases such as Genome Aggregation Database (gnomAD), Exome Aggregation Consortium (ExAC), 1000 Genomes and Bravo to determine the rarity or absence of the variant.[13] Next, a literature and clinical databases search was conducted, including resources like NCBI, Google scholar, Clinvar, ClinGen, Franklin and OMIM as well as internal data maintained by Genelabs Medical Pvt Ltd for any prior classifications of the variant, to check for any previously reported clinical significance. Variant interpretation also included evaluations based on its type among missense, silent, in-frame insertion/ deletion, intronic or null variants. [12]

In silico predictions using tools like SIFT, Phylop, Polyphen, Grantham, FATHMM, Mutation Taster and CADD scores were used to evaluate the potential impact on protein function/ amino acid substitution.[14] Additionally, the variant was examined based on whether it's occurred within a known mutational hotspot or functional domain. Supporting data such as segregation analysis, phenotype specificity, functional studies and de novo occurrence were analyzed. Based on the combination of these criteria, the variant was classified into one of five ACMG categories: pathogenic, likely pathogenic, uncertain significance, likely benign or benign.[12] The framework method outlined in Figure 01 ensures a comprehensive and evidence-based interpretation of the genetic variant's clinical relevance according to ACMG/ AMP standards and guidelines.

3. Results and Data Analysis and Findings

3.1. Patient Characteristics

The patient (SN10), a 33 years old female of Sinhala ethnicity, clinically diagnosed with diabetes at 19 years of age. At the time of diagnosis, her height was 154cm and weight 47kg, consistent with a normal body mass index (BMI). The clinical presentation was consistent with non-ketotic diabetes. Patient has a strong family history of diabetes mellitus, with the mother diagnosed with type 2 diabetes and her sister also developing diabetes in early adulthood (20s). Treatment data specific to sulfonylurea response in this patient were not available at the time of this analysis and familial segregation analysis was not performed as relatives were not available for genetic testing.

3.2. Variant Detection and confirmation

A heterozygous missense variant described as c.358A>C, leading to an amino acid substitution p.Lys120Gln was identified by NGS in SN10 patient sample. This variant is located on chromosome 12 at position Chr12:120988864 (hg38). This is a missense variant, resulting in a change from a positively charged lysine (Lys) to a polar, uncharged glutamine (Gln) at codon 120 within the highly conserved DNA binding domain of the HNF1A protein.

3.3. Sanger sequencing analysis

In gel electrophoresis of the PCR products of the region of interest, both SN05 and SN10 samples showed distinct bands at

approximately 389 base pairs, indicating the successful amplification of the targeted HNF1A gene region in both samples. Conversely, no band was present in the negative control (No template), indicating that there was no contamination in the PCR process. (Figure 02)

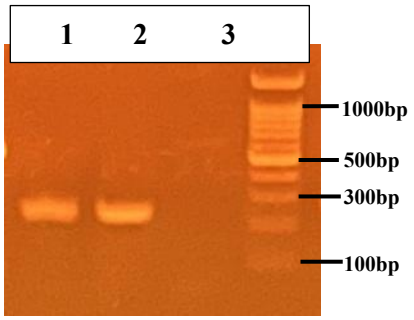

Figure 02: Gel electrophoresis of the PCR product. Lane 1-SN05 (negative sample), Lane 2-SN10 (positive sample), Lane 3: No template control, Lane 4: 100bp Ladder.

The PCR products of both SN05 and SN10 were sequenced by Sanger sequencing, and the electropherogram demonstrated a double peak at nucleotide position c.358 for sample SN10, confirming the presence of a heterozygous A>C (M: degenerative nucleotide) substitution (Figure 03). This mutation was not present in the SN05 sample, which was also negative by NGS.

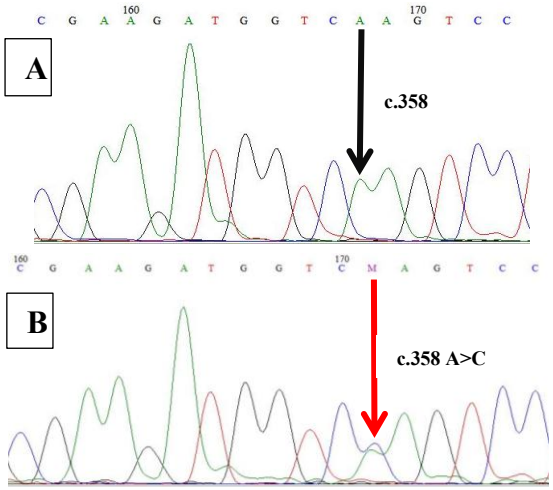

Figure 03: Electropherograms of the SN05 (A) and SN10 (B) samples. The arrows indicate the HNF1A c.358 position where an Adenine nucleotide is present in the wild type sequence of SN05 samples, and the A>C mutation is detected the SN10 mutated sample.

3.4. Population Frequency Analysis

Assessment of population frequency data was conducted using gnomAd, ExAC, 1000 Genomes and Bravo. The variant was not identified in any of these databases.[13] The absence of the variant in a large population database indicates that it is extremely rare or potentially novel in the general population and it supports the ACMG criterion PM2 (moderate evidence of pathogenic).[13]

3.5. Database and Literature Search

A search for clinical interpretations for this variant did not show any entries in available variant databases, including Clinvar, ClinGen and OMIM. Comprehensive literature searches using databases such as NCBI/PubMed, Google scholar, Google and Mastermind showed no prior studies or cases of the c.358A>C (p.Lys120Gln) variant on HNF1A gene. [12]

However, the variant analysis using Franklin by Genoox platform, showed the potential pathogenic significance by providing a computational prediction based on bioinformatics and other relevant data and giving a Likely Pathogenic outcome.

3.6. In silico (computational) Prediction Results

In silico analysis predictions collectively implied a probable damaging effect of the p.Lys120Gln mutation on protein (Table 01)

Table 01: *In silico* prediction scores

***In silico* tool**	**Result**	**Interpretation**
SIFT	0	Damaging
PolyPhen-2	0.927	Probably Damaging
Grantham	53	Moderately Radical Change
Phylop	8.87	Highly conserved
Other tools	Not available	No data

Several other tools such as FATHMM, Mutation Taster and CADD scores were used, but did not show interpretable scores for this specific variant.[14]

Although strong support was provided by *in silico* predictions and structural analysis, there was no available additional data for familial segregation analysis, phenotype specificity, de novo occurrence, or functional studies. Furthermore, there were no previous internal case records from our laboratory identifying this variant.[12]

Table 2. Variant classification and interpretation for HNF1A c.358A>C according to ACMG/AMP (2015) guidelines. [12]

ACMG Criteria	**Description**	**Results (Strength)**	**Evidence**
PM1	Located in a critical functional domain (DNA binding domain) with no benign variation	Moderate	DNA binding domain of HNF1A
PM2	Absent from population databases	Moderate	Not found in population databases (gnomAD v4.1.0, n=1614242)
PM5	Novel missense change at an amino acid residue where different pathogenic missense changes have been seen previously	Moderate	P.Lys120Glu previously reported as likely pathogenic
PP2	Missense variant in a gene where missense is a common mechanism of disease	Supporting	HNF1A intolerant to benign missense variation

PP3	Multiple *in silico* tools predict a deleterious effect. Aggregated score predicts a deleterious effect.	Supporting Aggregated prediction score ranges: Benign supporting 0-0.15, Pathogenic Supporting 0.7-0.8, Pathogenic Moderate 0.8-0.9, Pathogenic Strong 0.9-1.0 (Franklin Genoox)	SIFT, Polyphen, Phylop and Grantham

3.7. ACMG/AMP Classification

Based on three moderate (PM1, PM2 &PM5) and two supporting (PP2, PP3) ACMG criteria, this HNF1A c.358A>C (p.Lys120Gln) variant was classified as Likely Pathogenic.[12]

Table 2 summarizes the available interpretation of this variant classification according to the ACMG/ AMP guidelines.

4. Discussion and Conclusion

HNF1A-MODY (MODY3) is a monogenic disease with autosomal-dominant inheritance due to HNF1A haplo-insufficiency, where the loss of one functional copy of the HNF1A gene results in insufficient protein levels to sustain normal pancreatic beta cell function and glucose regulation, or dominant-negative effects.[15] The HNF1A gene has ten exons and a promoter that are prone to mutations, with exons 1–6 accounting for more than 80% of the total.[16] Specifically, most of the mutations have been found in HNF1A exons 2 and 4, while the lowest number have been found in exons 5 and 10.[15] Missense, frame shift, nonsense, splicing mutations, in-frame deletions, insertions and duplications of amino acids, and partial or whole-gene deletions are among the variations that have been reported to far. The dimerization domain, DNA-binding domain, and transactivation domain are among the 631 amino acids that make up the HNF1A protein.[17]

We have previously identified c.358A>C (p.Lys120Gln) mutation in Exon 2 of HNF1A in a suspected MODY patient (unpublished data), using an NGS-based method. In the current study we have verified this result by an alternative method of Sanger sequencing, to rule out sequencing artifacts in NGS. While the heterozygous substitution was clearly observed in SN10 through Sanger sequencing, its absence in SN05 confirms the variant is real, and is unlikely to be a sequencing artifact. Such use of multiple complementary approaches minimized the likelihood of false positive findings, which is particularly important in interpretation of rare variants.[11]

It was important to understand the significance of this variant in MODY, since it was not available in commonly used variant databases. Thus a systematic analysis was carried out to determine its pathogenicity in MODY, following the ACMG/AMP standards and guidelines.

The HNF1A gene encodes a transcription factor essential for pancreatic beta-cell development and glucose homeostasis, with pathogenic variants well established in association with Maturity-Onset Diabetes of the Young type 3 (MODY3).[18] The variant c.358A>C (p.Lys120Gln) identified in this study is located within the DNA-binding domain, a highly conserved and functionally critical region of the protein. Therefore, the variant supports the PM1 ACMG criteria.[12]

Pathogenicity in HNF1A is predominantly driven by missense mutations, with low rate of benign missense variation at the amino acid level. The p.Lys120Gln variant is consistent with this known disease mechanism, supporting the classification of PP2.[12]

Although the specific variant p.Lys120Gln has not been previously reported in major clinical variant databases, another substitution at the same codon p.Lys120Glu has been classified as likely pathogenic. This highlights the functional importance of lysine at position 120. The substitution to glutamine represents a potentially disruptive change in physicochemical properties and may impair protein-DNA interactions required for transcriptional regulation. The identification of another pathogenic variant at this residue supports the application of the PM5 criterion.[12]

The absence of this variant in large-scale population databases such as gnomAD, ExAc, 1000 Genomes and Bravo supports its rarity, a characteristic commonly associated with pathogenic variants in Mendelian conditions. The rarity of the variant supports its classification under the PM2 criterion (ACMG). [13]

The use of multiple computational prediction tools further strengthens this clinical result. SIFT and PolyPhen-2 both predicted the variant to be damaging, with SIFT producing a score of 0 and PolyPhen shows a score of 0.927, indicating a likelihood of functional impairment. The affected lysine residue at position 120 showed high evolutionary conservation, as demonstrated by a PhyloP score of 8.87. This suggests that alterations at this site are poorly tolerated and may have significant functional consequences. Furthermore, the Grantham score, which quantifies the physiochemical difference between amino acids to assess the effect of a substitution, gave a score of 53 indicating a moderately radical change, supporting a potential impact on protein structure, function and DNA binding capacity. While some predictive results were unavailable, the consistent deleterious findings among the available computational tools support the ACMG/AMP PP3 criterion, thereby supporting the likely pathogenic classification of the variant.[14]

This classification aligns with the interpretations carried out by Franklin database. Comprehensive literature searches using databases such as NCBI/PubMed, Google scholar, Google and Mastermind, showed no prior studies or cases on the c.358A>C (p.Lys120Gln) variant on HNF1A. The absence of published data further emphasizes the novelty of this variant and the importance of functional and clinical studies to clarify its clinical significance.[12]

However, this study has several important limitations. First, the study is limited by the availability of data from a single patient, the findings should be interpreted with caution and require further validation in larger cohorts. Second, no segregation analysis was performed due to the unavailability of genetic samples from family members, limiting the ability to confirm inheritance patterns or establish genotype/ phenotype correlations. Additionally, functional validation assays such as transcriptional activity or protein expression studies were not conducted, limiting the ability to assess the biological impact of the identified HNF1A c.358A>C (p.Lys120Gln) variant. Although multiple in-silico tools predicted deleterious effects and these predictions require experimental support.

Furthermore, treatment response data, particularly regarding sulfonylurea sensitivity, which is often informative in HNF1A MODY cases, were not available for the SN10 patient. Future studies including familial samples, longitudinal clinical data and functional assays are essential to confirm the clinical relevance of this novel HNF1A variant.[12]

Based on ACMG/AMP 2015 standards and guidelines, the combination of three moderate (PM1, PM2, PM5) and two supporting (PP2, PP3) criteria led to classification of the HNF1A c.358A>C (p.Lys120Gln) variant as Likely Pathogenic.[12]

In conclusion, this study verified and characterized a novel missense variant in the HNF1A gene, c.358A>C (p.Lys120Gln), using a combination of bioinformatic tools, database analysis, literature search and standardized ACMG/AMP classification criteria. Although the variant is absent from population and clinical databases and no experimental evidence is currently available, its location in a conserved functional domain, damaging *in silico* predictions, and structural context provide significant support for pathogenic potential. According to the applied ACMG criteria (PM1, PM2, PM5, PP2, PP3), the variant is classified as Likely Pathogenic. While no functional data are available at present, together with the clinical features suggestive of MODY, the findings support a significant role of the HNF1A c.358A>C (p.Lys120Gln) variant in monogenic diabetes and justify further investigation through familial segregation and functional assays.[12,13]

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