



PILOT STUDIES ON THE PREVALENCE AND INCIDENCE OF HNF-1 α EXON 2, EXON 4 SINGLE NUCLEOTIDE POLYMORPHISMS IN THE REGIONAL POPULATION OF SOUTH TAMILNADU, INDIA.

***Rohini Gomathinayagam, Kumaravel Velayutham, Balaji Ramanathan, Sivan Arul Selvan, Vishali Thavamani, Jeyasudha Murugan**

Alpha Health Foundation, Alpha Hospital and Research Center, Mela Anuppanady, Madurai-9, TN, India.

***Corresponding Author: Rohini Gomathinayagam**

Alpha Health Foundation, Alpha Hospital and Research Center, Mela Anuppanady, Madurai-9, TN, India.

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ABSTRACT

Maturity onset diabetes of the Young (MODY) is a monogenic form of diabetes that is caused by mutations/ single nucleotide polymorphisms (SNPs) in a spectrum of genes. Amidst the most prevalently incident forms of MODY, the HNF-1 α (Hepatocyte Nuclear factor 1 α) MODY is associated with the aggressive disease pathogenesis. While a few studies have reported that HNF-1 α MODY is incident in the south Indian population, population specific studies that enable evaluation and determination of the prevalence of HNF-1 α gene variants in south Tamil Nadu is still lacking. Hence, the present study aims to assess the incidence of single nucleotide polymorphisms in the coding regions of exon 2, exon 4 of HNF-1 α gene and the flanking intronic regions in clinically defined MODY patients. Whole genomic DNA from the patients and controls were utilized for obtaining PCR amplification of the genomic areas of interest and were assessed for the presence or absence of SNPs utilizing direct sequencing. The genotyping results reveal the presence of reported intron 1 variants and absence of MODY3 associated gene variants in exon 2, exon 4. About 78 % of the assessed study group exhibited the rs1169292 polymorphism, 100 % of the assessed participants presented the rs1169293 polymorphism and 78% of the population presented rs1169294 polymorphism. Cumulatively the results indicate that HNF-1 α exon 2, exon 4 SNPs associated with MODY3 may be less prevalent in the population and that with further large scale studies, the HNF-1 α intronic variants, haplotypes may also serve to predict population specific risk of incidence of MODY3.

INTRODUCTION

The autosomally dominant monogenic form of diabetes mellitus, MODY (Maturity onset diabetes of the young), is characterized by an early onset and absence of pancreatic autoimmunity markers.^[1] While European countries seem to exhibit a higher prevalence of heterozygous mutations of GCK (Glucokinase MODY/MODY2), HNF-1 α (Hepatocyte nuclear factor 1 α MODY/MODY3)^[2], MODY studies in the south Indian population indicate that HNF-1 α MODY/MODY3 is incident in the population and may be of relatively higher incidence.^[3] As a major transcription factor that is primarily associated with tissue specific regulation of genes, HNF-1 α MODY is hall marked by severe pancreatic insufficiency. While patients with MODY3 may present secondary microvascular complications involving the retinas and the kidneys^[2, 3, 4], the clinical spectrum is diverse. Further, due to the fact that HNF-1 α MODY exhibits a pathophysiology that is remarkably similar to Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus, these patients are often misdiagnosed and are subject to inappropriate therapy. Hence it becomes critically important to diagnose HNF-1 α MODY at an earlier time point using clinical and molecular diagnostic

methodologies. While recent years have brought in an improved awareness and possible management strategies for MODY3, a huge deficit with regard to population specific knowledge in the prevalence of MODY3 in India continues to exist due to the lack in the genetic literature.

The exon 2, exon 4 regions of HNF-1 α have been reported to harbor several mutations/SNPs that are associated with MODY3 and the flanking intronic regions of these exons are also reported to contain variants that influence the mRNA structure, function and stability.^[6] Hence, the present study focused in examining the prevalence of polymorphisms that are incident in exon 2, exon 4 regions of the HNF-1 α gene and their flanking intronic regions, in the regional population of south Tamil Nadu, India.

METHODS

The present pilot study was reviewed by the Institutional Ethical Committee (IEC, AHRC), and was permitted to be carried out in Alpha Health Foundation (AHF)/ Alpha Hospital and Research Center (AHRC), Madurai. Consenting patients who displayed an early onset,

sensitivity to sulphonyl urea, and negative for autoimmune markers were included in the study. Genomic DNA was extracted from 11 clinically diagnosed MODY3 patients and 3 age matched controls. PCR amplification for regions pertaining to exon 2, exon 4 and flanking intronic regions at the 3', 5' ends was carried out. The PCR conditions pertaining to the initial denaturation of 2 min at 96°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 55°C and 2 min at 72°C were utilized for the 593 bp product with the forward primer 5'-GTTGTGTGTCTGTGTATCCATG-3', and the reverse primer 5' CCAGCCCCACCTATGAGTTA-3' for exon 2 and the PCR conditions pertaining to the initial denaturation of 2 min at 96°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 55°C and 30 sec at

72°C were utilized for the 385 bp product with the forward primer 5'-ATTGGAACCCAGATCTGCCAGC-3', and the reverse primer 5'-CCACATACCACTTACCGTGGA-3' for exon 4. The products were gel extracted, purified and direct sequenced in order to determine the presence or absence of SNPs in exon 2 (rs774996577, rs758199396, rs1057520291), exon 4 (rs193922603, rs1057520504, rs886039386, rs762555237) and the corresponding flanking intronic regions.

RESULTS AND DISCUSSION

The study results evidence that none of the assessed participants presented prior reported MODY3 associated exon 2, exon 4 polymorphisms.

Table.1

	rs1169292	rs1169293	rs1169294
% Prevalence	78.57	100.00	78.57

Table 1. Percentage prevalence of HNF-1 α intron 1 SNPs in the assessed control, clinically diagnosed MODY patients of south Tamil Nadu, India. From Table 1 it can further be evidenced that the assessed population exhibited the intron 1 variants, rs1169292, rs1169293 and rs1169294. About 78.57% of the participants presented the intronic variants rs1169292, rs1169294 and 100% of the participants presented rs1169293.

Fig.1

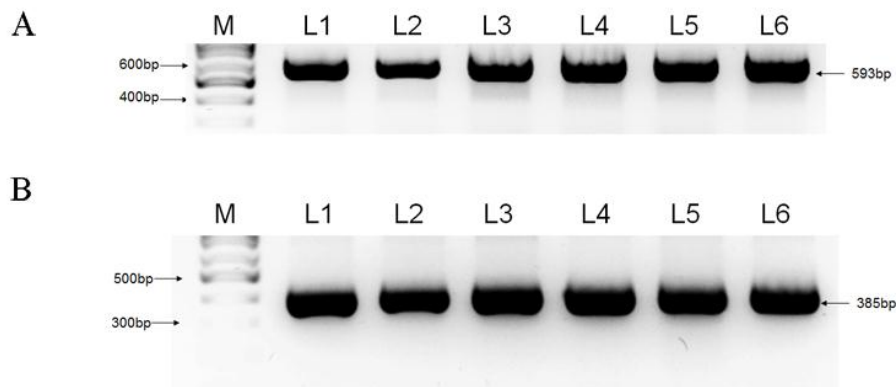


Fig.1. Determination of the incidence of exon 2, exon 4 and the flanking intronic region(s) HNF-1 α polymorphisms in the regional population. **A:** Representative image for the 593bp PCR product that encompasses the exon 2 (rs774996577, rs758199396, rs1057520291) and intron 1 regions of HNF-1 α . **B:** Representative image of a PCR amplified 385bp product that encompasses the exon 4 (rs193922603, rs1057520504, rs886039386, rs762555237) and its flanking intronic region (intron 3, 4).

Table.2

Patients Genotype	Wildtype		Heterozygous		Homozygous	
	Genotype	Allelic expression in %	Genotype	Allelic expression in %	Genotype	Allelic expression in %
rs1169292	CC	21.4	CT	42.9	TT	35.7
rs1169293	GG	0.0	GA	7.1	AA	92.9
rs1169294	GG	21.4	GA	42.9	AA	35.7

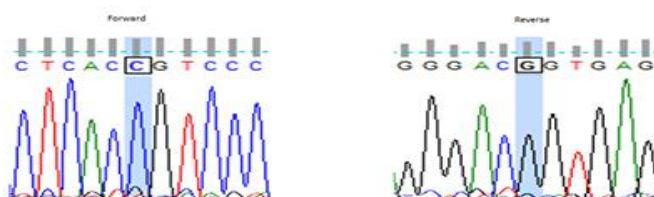
Table 2. Allelic distribution in HNF-1 α intron 1 SNP positive participants and the percentage of wild type, minor and major carriers of the SNPs. Genotyping results indicated that 42.9 % of the participants positive for rs1169292 and rs1169294 were heterozygous (CT, GA respectively) and 35.7% of the patients were homozygous positive (TT, AA respectively). 7.1 % of the participants positive for the rs1169293 were heterozygous (GA) and 92.9% of the participants positive for the rs1169293 exhibited a homozygous (AA) distribution.

Fig.2

A

Alignment of Sequence_1: [MODY SU EX2/IN1 F.xdna] with Sequence_2: [MODY SU EX2/IN1 R.xdna]

5' -GTCTGTGTCCCTGTGTCCTGGCATAAATGACCATACCTCACCGTCCCTGAGTCTATGTGT-3'
 3' -CAGACACAGGACACAGGACCGTATTTACTGGTATGGAGTGCAGGGACTCAGATACACA-5'



B

Alignment of Sequence_1: [MODY SK EX2/IN1 F.xdna] with Sequence_2: [MODY SK EX2/IN1 R.xdna]

5' -TCCCTGTGTCTGGCATAAATGACCATACCTCACATGTCCTGAGTCTATGTGTAGGCCCC-3'
 3' -AGGGACACAGGACCGTATTTACTGGTATGGAGTGCAGGGACTCAGATACACATCCGGGG-5'

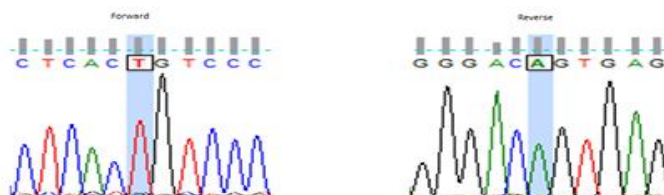


Fig.2 Representative Sequence chromatogram for rs1169292(C>T) in the intron 1 region of HNF-1 α . **A:** The forward and reverse sequences for a rs1169292 negative participant was aligned and the wild type alleles are indicated. **B:** The forward and reverse sequences for a rs1169292 homozygous positive participant was aligned and the corresponding T, A alleles are indicated.

Fig.1 represents the PCR amplified DNA fragments corresponding to exon 2 (593 bp product) and exon 4 (395bp product). Direct sequencing results as presented in Fig 2 (rs1169292), Fig 3 (rs1169293) and Fig 4 (rs1169294), table 2 depicts the distribution of the alleles pertaining to the intronic variants in the participants. It is observed that 21.4% of the study group participants were negative for the rs1169292 (presented CC) and rs1169294 (presented GG) SNPs while 100% of the population presented the rs1169293 (presented either

the heterozygous GA or homozygous AA distribution instead of the wild type GG) polymorphism. About 42.9% of the participants presented the heterozygous distribution, CT, GA for the SNPs 1169292, 1169294 respectively. About 35.7% of participants presented a TT or AA homozygous genotype for the rs1169292, rs1169294 respectively. While 100% of the assessed population was positive for rs1169293, 7.1% exhibited the heterozygous allele distribution (GA), and 92.9% presented the homozygous alleles (AA).

Fig.3

Alignment of Sequence_1: [MODY SK EX2/IN1 F.xdna] with Sequence_2: [MODY SK EX2/IN1 R.xdna]

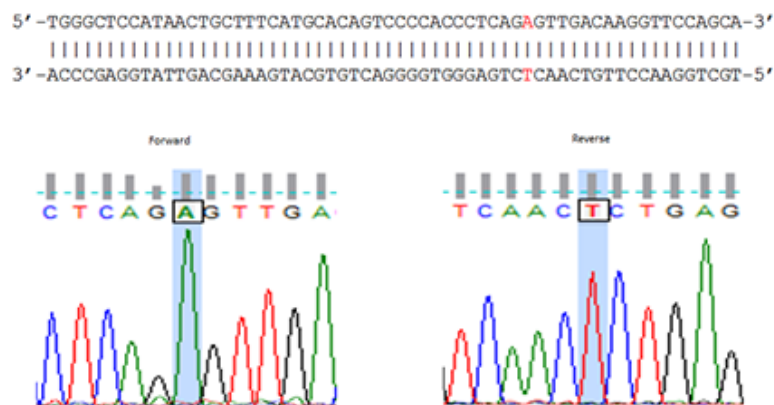
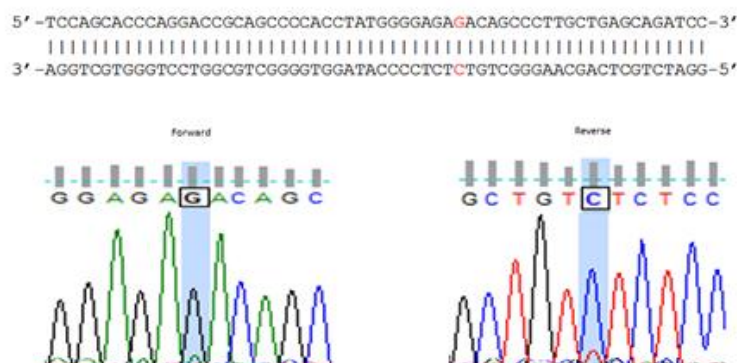


Fig.3. Representative Sequence chromatogram for rs1169293 (G>A) in intron 1 region of HNF-1 α . The forward and reverse sequences for a rs1169293 homozygous positive participant was aligned and the corresponding A, T changes are indicated.

Fig.4

A

Alignment of Sequence_1: [MODY SU EX2/IN1 F.xdna] with Sequence_2: [MODY SU EX2/IN1 R.xdna]



B

Alignment of Sequence_1: [MODY SK EX2/IN1 F.xdna] with Sequence_2: [MODY SK EX2/IN1 R.xdna]

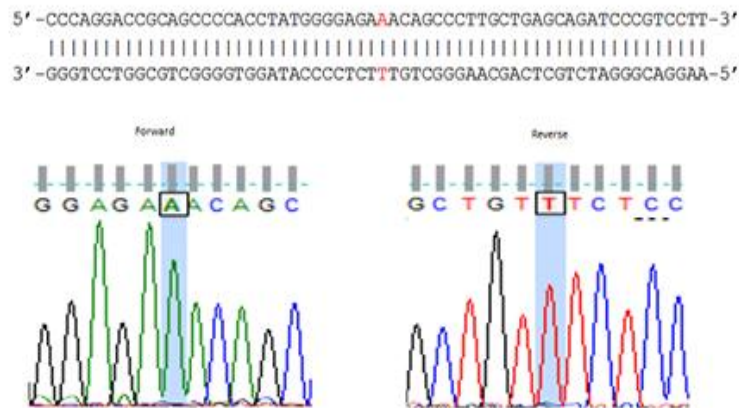


Fig.4. Representative Sequence chromatogram for rs1169294 (G>A) in intron 1 region of HNF-1 α . A: The forward and reverse sequences for a rs1169294 negative participant was aligned and the wild type alleles are indicated. B: The forward and reverse sequences for a rs1169294 homozygous positive participant was aligned and the corresponding A, T changes are indicated.

The genotyping results of the present study reveal that intron 1 HNF-1 α variants may commonly occur in the population and may tend to have a population specific distribution. Earlier studies have also indicated that HNF-1 α intronic variants can modulate gene expression, stability and splicing of the mRNA^[7] thereby playing a key role in post transcriptional regulatory mechanisms. Limited population specific studies also pin point that some HNF-1 α intronic variants are incident in MODY3 negative controls as well as in MODY3 patients.^[8, 9]

In concordance with these reports our pilot studies also bring forward that the identified intronic variants are dispersed in the control and patient samples. The genetic influence of HNF-1 α in disease pathogenesis has long been recognized and well established in diabetes and cardiovascular diseases.^[10] Additional evidences have also brought to light that the presence of the major/minor alleles of haplotype distributed intronic variants in HNF-1 α (rs1169293 and rs1169294) may influence/reflect high levels of C-reactive protein and cardiovascular risks.^[11] Similarly, the intronic variant rs1169294 is also associated with other disease conditions^[12] indicating that the intron 1 variants identified may have an implicative role in disease pathogenesis. Thus, based on the present pilot study data and the previous literature, it could be suggested that population specific HNF-1 α haplotypes/ the co-segregating alleles may determine the incidence, and enable the prediction of the risk of MODY3 incidence.

CONCLUSION

The results of the study bring forward that HNF-1 α gene variants pertaining to exon 2, exon 4 may be rarely

incident in the regional population and that intronic variants/intron 1 variants, haplotypes may play a participatory role in disease predisposition. Further large scale studies in the regional population may offer better insight on HNF-1 α gene variants and their potential role in the incidence of MODY3.

REFERENCES

1. Velho G, Robert JJ. Maturity-onset diabetes of the young (MODY): genetic and clinical characteristics *Horm Res*, 2002; 57(1): 29-33.
2. Balamurugan K, Bjørkhaug L, Mahajan S, Kanthimathi S, Njølstad PR, Srinivasan N, Mohan V, Radha V. Structure-function studies of HNF1A (MODY3) gene mutations in South Indian patients with monogenic diabetes. *Clin Genet*, 2016 Dec; 90(6): 486-495.
3. Radha V, Ek J, Anuradha S, Hansen T, Pedersen O, Mohan V. Identification of novel variants in the hepatocyte nuclear factor-1alpha gene in South Indian patients with maturity onset diabetes of young. *J Clin Endocrinol Metab*, 2009; 94: 1959-1965.
4. Vaxillaire M, Rouard M, Yamagata K, Oda N, Kaisaki PJ, Boriraj VV, Chevre JC, Boccio V, Cox RD, Lathrop GM, Dussoix P, Philippe J, Timsit J, Charpentier G, Velho G, Bell GI, Froguel P. Identification of nine novel mutations in the hepatocyte nuclear factor 1 alpha gene associated with maturity-onset diabetes of the young (MODY3). *Hum Mol Genet*, 1997 Apr; 6(4): 583-6.
5. Nyunt O, Wu JY, McGown IN, Harris M, Huynh T, Leong GM, Cowley DM, Cotterill AM.

- Investigating maturity onset diabetes of the young
Clin Biochem Rev, 2009 May; 30(2): 67-74.
6. Mohan V, Radha V, Nguyen TT, Stawiski EW, Pahuja KB, Goldstein LD, Tom J, Anjana RM, Kong-Beltran M, Bhangale T, Jahnvi S, Chandni R, Gayathri V, George P, Zhang N, Murugan S, Phalke S, Chaudhuri S, Gupta R, Zhang J, Santhosh S, Stinson J, Modrusan Z, Ramprasad VL, Seshagiri S, Peterson AS. Comprehensive genomic analysis identifies pathogenic variants in maturity-onset diabetes of the young (MODY) patients in South India *BMC Med Genet*, 2018 Feb 13; 19(1): 22.
 7. Kaisaki PJ, Menzel S, Lindner T, Oda N, Rjasanowski I, Sahm J, Meincke G, Schulze J, Schmechel H, Petzold C, Ledermann HM, Sachse G, Boriraj VV, Menzel R, Kerner W, Turner RC, Yamagata K, Bell GI. Mutations in the hepatocyte nuclear factor-1alpha gene in MODY and early-onset NIDDM: evidence for a mutational hotspot in exon 4. *Diabetes*, 1997 Mar; 46(3): 528-35.
 8. Labriet A, De Mattia E, Cecchin E, Lévesque É, Jonker D, Couture F, Buonadonna A, D'Andrea M, Villeneuve L, Toffoli G, Guillemette C. Improved Progression-Free Survival in Irinotecan-Treated Metastatic Colorectal Cancer Patients Carrying the HNF1A Coding Variant p.I27L. *Front Pharmacol*, 2017 Oct 10; 8: 712.
 9. Ellard S, Colclough K. Mutations in the genes encoding the transcription factor-1 alpha (HNF1A) and 4 alpha (HNF4A) in maturity – onset diabetes of the young. *Hum Mutat*, 2006 Sep; 27(9): 854-69.
 10. Johansen A, Ek J, Mortensen HB, Pedersen O, Hansen T. Half of clinically defined maturity-onset diabetes of the young patients in Denmark do not have mutations in HNF4A, GCK, and TCF1. *J Clin Endocrinol Metab*, 2005 Aug; 90(8): 4607-14.
 11. Yang Z, Wu SH, Zheng TS, Wang SJ, Lu HJ, Xiang KS. The genetic and clinical characteristics of transcription factor 1 gene mutations in Chinese diabetes. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, 2007 Apr; 24(2): 157-61.
 12. Reiner AP, Barber MJ, Guan Y, Ridker PM, Lange LA, Chasman DI, Walston JD, Cooper GM, Jenny NS, Rieder MJ, Durda JP, Smith JD, Novembre J, Tracy RP, Rotter JI, Stephens M, Nickerson DA, Krauss RM. Polymorphisms of the HNF1A Gene Encoding Hepatocyte Nuclear Factor-1a are Associated with C-Reactive Protein *Am J Hum Genet*, 2008 May; 82(5): 1193-201.
 13. Richter D, Harsch S, Strohmeyer A, Hirobe-Jahn S, Schimmel S, Renner O, Müller O, Schäffeler E, Kratzer W, Schwab M, Stange EF. MALDI-ToF mass spectrophotometry screening of cholelithiasis risk markers in the gene HNF1 alpha. *J Proteomics*, 2012 Jun 27; 75(12): 3386-99.