Al Ameen J Med Sci 2013; 6(1):58-64 ● US National Library of Medicine enlisted journal ● ISSN 0974-1143

ORIGINAL ARTICLE CODEN: AAJMBG

Identification of novel variants in HNF1-alpha gene in maturity onset diabetes in young adults (MODY) subjects of Eastern India

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Abstract: *Background*: The disorder, Maturity Onset of Diabetes of the young (MODY) is a monogenic form of Non-Insulin dependent Diabetes Mellitus (NIDDM), characterized by autosomal dominant mode of inheritance and onset is usually before 25 years of age. Clinical studies of the subjects with the different forms of MODY indicate that each is associated with a different defect in the normal pattern of glucose stimulated insulin secretion. MODY can result from mutations in any one of the six different genes as of now, one of which encodes the glycolytic enzyme Glucokinase, associated with MODY2 and the other five encode transcription factors HNF4alpha associated with MODY1,HNF1alpha associated with MODY3, IPF with MODY4, HNF1Beta with MODY5 and NeuroD1 with MODY6.Studies related to mutations in the MODY genes have led to a better understanding of the genetic causes of the Beta cell dysfunction as genetic factors play a great role in this disorder. *Objective:* To investigate the mutation pattern/patterns in the different transcription factor genes with special reference to HNF1alpha gene which are highly penetrant with 63% mutation carriers manifesting clinical diabetes by the age of 25 years. Hence study of mutation pattern in this gene is essential in our population i.e. Eastern Indian population. Our study is focused on HNF1alpha related to MODY3, which is the most common one. *Methods:* In our study, the enzyme amplification (PCR) of the10 target exons of the said gene with simultaneous mutation detection in them by PCR-SSCP (Polymerase chain reaction followed by single strand conformational polymorphism) reaction analysis method was attempted by screening of exon1-10 with respect to normal healthy controls without Diabetes Mellitus. The nature of the specific mutations was also determined by sequencing. *Result:* It was observed in our study that there were sequence variants existing in exon7 and exon 8 of HNF1-alpha gene, revealed by PCR-SSCP study in our population which goes in agreement with the sequencing study. The age at diagnosis of the subjects with novel single base substitution polymorphisms in exon 7 seem to be little less than those in subjects with polymorphisms in exon 8 of the HNF1-alpha gene. *Conclusions:* The age at diagnosis of the subjects with novel single base substitution polymorphisms in exon 7 seem to be little less than that in subjects with polymorphisms in exon 8 of the HNF1-alpha gene, emphasizing that age at diagnosis is influenced by variations in mutational positions.

Keywords: Maturity onset diabetes of Young, PCR-SSCP, HNF1-Alpha, Non insulin dependant diabetes mellitus

Introduction

Heterozygous mutations in the hepatocyte nuclear factor1-alpha (HNF1-α) gene is the most common cause for Maturity-onset diabetes of the young or MODY3,which is characterized by reduced glucose stimulated insulin secretion, sensitivity towards sulphonylureas and a decreased renal thresholds for glucose reabsorption. The incidence of expression of MODY varies from one family to another extensively. The incidence of MODY significantly varies in different racial and ethnic groups extensively [1-2]. Since mild hyperglycemia may not cause the classic symptoms of diabetes mellitus, the diagnosis may not be made until adulthood [3]. Symptoms at diagnosis may also vary extensively. The severity and extent of insulin secretion defect may also vary since approximately one third of the patients are treated with insulin after 15 years of diabetes

duration, while in others diabetes was controlled by diet or oral hypoglycemic agents [1,3].Age at onset of diabetes is partially inheritable within MODY3 families[4].In an earlier study, it was shown that the age of diagnosis of patients with MODY was modulated by type and position of HNF-1α Mutations [4].Clinical manifestations of MODY 1 and MODY 3 diabetes are more or less identical. About 70% of people develop this clinical condition by age of 25 years, but it occurs at much later stage of life in a few cases. This type of diabetes mellitus can often be treated with sulfonylurea with excellent results for decades. However, the loss of insulin secretary capacity is slowly progressive and most of the cases eventually need insulin therapy [5]. With the identification of genes responsible for MODY, it is possible to identify members of pedigrees who have inherited, the specific mutations affecting their family, even before carbohydrate intolerance develops. Genetic screening for and identification of a specific MODY related mutation in children may have important prognostic and therapeutic implications [6-7].

Persons who are genetically susceptible to diabetes due to mutations in the genes for HNF1-alpha and HNF4–alpha, should be monitored frequently so that appropriate therapy can be instituted early in the course of their uncontrolled hyperglycemic state, because of impending risk of progression to severe hyperglycemia with both micro and macro vascular complications such as diabetic associated retinopathy, cardiac pathology and renal failure and insulin requirement [7-8]. In our earlier study, evidence of presence of novel variants were confirmed in exon 5 of HNF1-α gene. Three new polymorphisms were identified in exon 5 in our Eastern Indian population, which is already communicated in the journal entitled, "Novel Variants in the Hepatocyte Nuclear factor-1-alpha Gene in MODY and early onset NIDDM: evidence for a mutational hotspot in Exon 5", in Open journal of preventive medicine. In our present study we aimed at screening more mutations, in HNF-α gene, in study subjects of our eastern Indian population. Our objective in the present study was also to observe whether mutations in the said gene influences the age of onset of the disease or not.

Material and Methods

Study Subjects: Patients with diabetes with onset before the age of 25 years were selected in the study as probands. After receiving the detailed history (including family history) and duly signed informed consent forms, clinical examinations were done. The study protocols complied with the Declaration of Helsinki and was approved by the institution's ethics committee. Informed consent was obtained from each subject after explaining the details of purposes.

Control: Non-diabetic subjects who did not have any history of Diabetes Mellitus were considered as the Control subjects. The family history of first and second degree relative of diabetes mellitus were obtained for all the subjects**.**

Exclusion criteria: All the subjects were carefully examined and an investigation has been carried out to rule out Type 1 diabetes using GAD and IA2 Antibodies. Fibro calcific Pancreatic Diabetes, Pancreatic duct dilation and calcification were ruled out after Ultrasound examination of Pancreas.

Sample processing and sample collection: Both patient and control samples were being collected from Department of Endocrinology and Metabolism, Central laboratory of Institute of Post Graduate Medical Education and Research, and other Endocrinology clinics of the region.

Genomic DNA isolation: Genomic DNA from human whole blood was isolated using standard phenol-chloroform method of isolation and the quality of the DNA was checked by spectrophotometric analysis [9].

Polymerase chain reaction (PCR) amplification of the different exons of the HNF1-alpha gene was carried out with 120 ng of extracted DNA.The reaction Mixture in 50 µl consisted of 10mMTris-HCL (pH 8.3), 50mMKCL, 2.5mM $MgCl_2$, 2.5 mM deoxyribonucleoside triphosphates, 1 U Taq DNA polymerase (MBI Fermentas) and 20 µmol primers specific for each exon. The reactions were performed at various annealing temperatures specific for each exon in a thermo cycler (Biometra,T-personel 48) [Table1].

Table-1: Representing primer sequences and annealing temperatures of the respective Exons			
Name of the primer	**Sequence of the primer(5'-3')**	**Size of the amplicons**	**Annealing temperature**
EXON1 FP	GAG CAA AGA GGC ACT GAT CC	349 bp	60°C
EXON1 RP	CTC CAG CTC TTT GAG GAT GG		
EXON2 FP	GGA GGT GGT CGA TAC CAC TG	200 bp	58°C
EXON2 RP	ACG TAC CAG GTG TAC AGG GC		
EXON3 FP	AAG AGC CCA CAG GTG ATG AG	187 bp	61°C
EXON3 RP	CAC TAG CGT CTC TCG CTC CT		
EXON4 FP	GCA AAG AAG AAG CCT TCC G	242 bp	60°C
EXON4 RP	GTG GAC CTT ACT GGG GGA GA		
EXON5 FP	CGA CCA GTG AGA CTG CAG AA	152 bp	62°C
EXON5 RP	GTA CTC AGC AGG CTG TGG CT		
EXON6 FP	ACA GCT TGG AGC AGA CAT CC	202 bp	63°C
EXON6 RP	TGT TGG TGA ACG TAG GAC CC		
EXON7 FP	GAG TGT GCC GGT CAT CAA C	192 bp	61°C
EXON7 RP	TCT GGG TCA CAT GGC TCT G		
EXON8 FP	TCC CGT TCC CTT TCA TAC CT	122 bp	59°C
EXON8 RP	GAT CCA GGG CTG ATT TTC AA		
EXON9 FP	CCC AGG TCT TCA CCT CAG AC	145 bp	62°C
EXON9 RP	ACA GTG ACG GAC AGC AAC AG		
EXON10 FP	TGG AGA GCT AGG AGC AAA GC	445 bp	63°C
EXON10 RP	TCT CAG AGC TCA GCA GGT CA		

Mutation Detection: Molecular techniques of mutation detection has become more and more a focus of interest in clinical medicine because of the responsible genes are now known for an increasing number of diseases and the detection is essential to enable reliable predictions and to design individual therapies. Therefore the SSCP (Single-Stranded Conformation Polymorphism) analysis is one of the most commonly applied methods in detection of point mutations.

SSCP Analysis: The use of SSCP for detection of the pathogenic mutations of MODY (Maturity Onset Diabetes in the Young) is described as an example in the study [9].The polymorphisms were confirmed by sequencing.

Statistical Analysis: Differences between the median age of diabetes diagnosis between the described subsets of HNF1 alpha mutation in exon 7 and 8 carriers were examined for statistical analysis by using Mann – Whitney U test. All statistical analysis was carried out In Graph pad statistical soft ware (Graph pad, version 5.00, 2007, USA). $p < 0.05$ was considered as minimum level of significance.

Result and Analysis

We considered 98 subjects fulfilling stringent MODY criteria as study subjects in our study and 114 non-diabetic subjects were taken as healthy controls. Our PCR-SSCP study revealed that there were 6 SSCP variants existing in exon 7 and 4 in exon 8 of the HNF1-alpha gene in case of the study subjects, which ware confirmed by sequencing. In case of the controls number of SSCP variants existing were negligible and hence it was not significant. [Fig.1-4]

Figure-1: Representing PCR Amplicons of exon 7 of HNF1-alpha gene. Products amplified were run in a 1.5% agarose gel. L3 and L4 and L5 representing PCR amplicons. L! representing Mass ruler DNA molecular marker

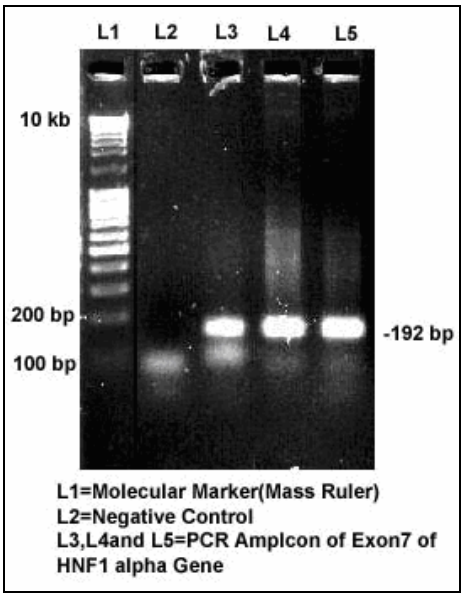


Figure-2: Representing PCR Amplicons of exon 8 of HNF1-alpha gene. Products amplified were run in a 1.5% agarose gel. L3 and L4 and L5 representing PCR amplicons. L1 representing Mass ruler DNA molecular marker

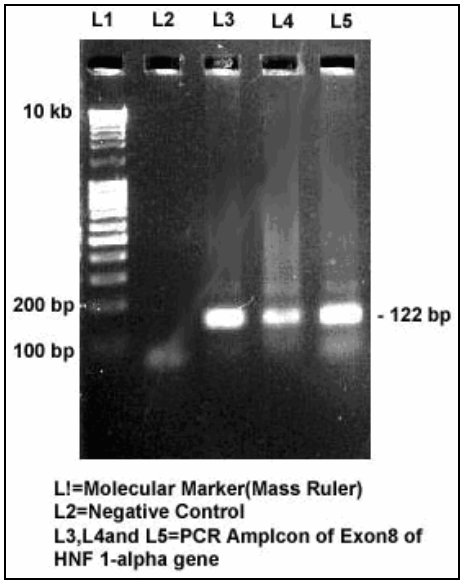


Figure-3: Representing Single strand conformational Analysis of exon 7 HNF 1-alpha gene. The denatured amplicons were run in a 6% polyacrylamide gel. L2 and L7 representing the abnormal SSCP conformers with respect to the other subjects in L3,L4,L5,L6 and L1.M shows *ΦX174* marker.

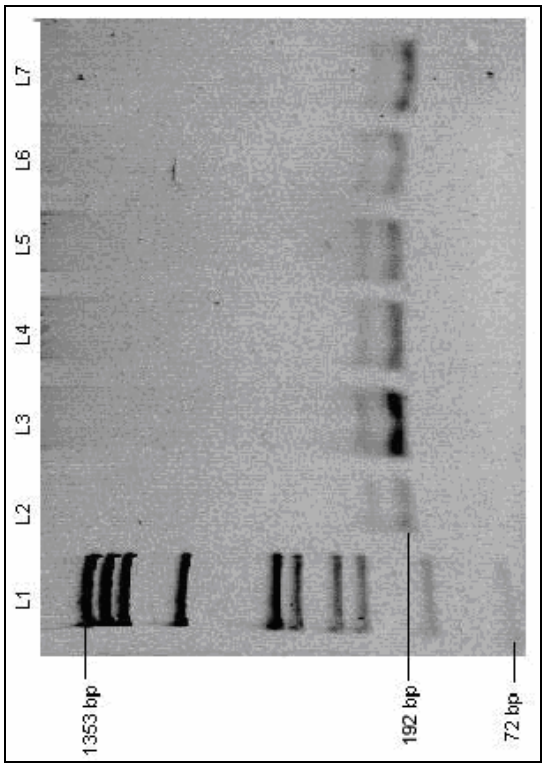
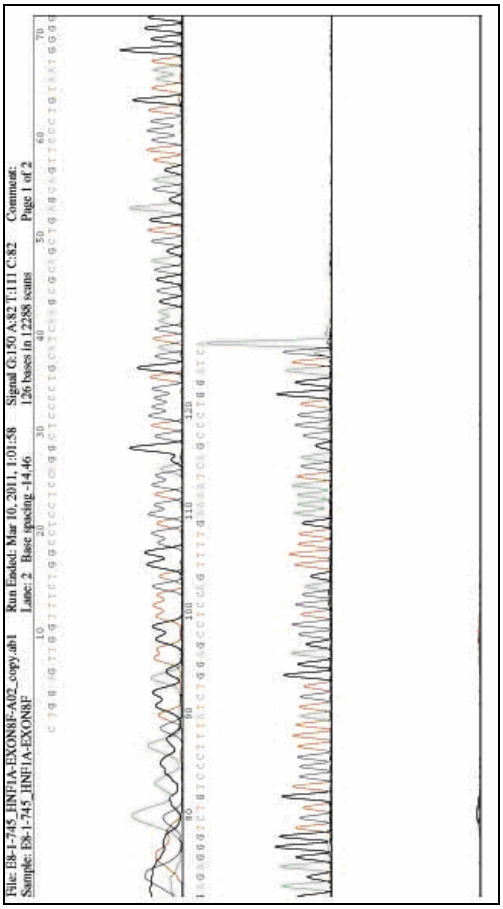


Figure-4: Representing dendogram showing sequencing profile of HNF1-alpha exon 8



Novel polymorphisms observed in study subjects in HNF1-alpha gene: After screening 98 study subjects, fulfilling MODY criteria for all the ten exons, we observed three novel polymorphisms in two different exons of the said gene. Two novel insertion polymorphisms that were identified in exon 8 were g25287_25288insA and g25341_25342insG among 4 study subjects with median onset age of diabetes 23.5 years (22-38). In case of exon seven a novel single nucleotide polymorphism was identified. The change in the nucleotide position observed was c.1398G>A, among six study subjects with median age of diagnosis of diabetes 16 years(13-22).Further statistical analysis reveals significant difference between the median age of diagnosis of diabetes among carriers of HNF1-alpha mutation in exon7 and 8 (p=0.0187) [Table2].

Table-2: Representing Age at diagnosis according to mutation position

Exon No. and change in nucleotide position	Total sample n = 98(%)	Median age of diagnosis (years)	p value
Exon 5	3 (3.06)	21(21 - 22)	
c.1104A>C			
c.1155C>G			0.0951
c.1153C>G			
Exon 7	6 (6.12)	16 (13 - 22)	
(c.1398 G>A)			0.0187 *
Exon 8	4 (4.08)	23.5 (22 - 38)	
g.25287 _ 25288 insA			
g.25341 _ 25342 insG			

n = sample No. *significant at the level of 0.05.

Discussion

Mutations in the HNF1-alpha gene are the most common cause of monogenic form of transcription factor related diabetes mellitus (MODY), in most of the population. A number of different mutations have been reported, till date in the said gene, giving rise to approximately 1-2% of the patients with this form of diabetes, although most cases are not diagnosed [10]. Patients with these form of mutations have a progressive beta cell defect HNF1-alpha being one of those several transcription factors with a complex regulatory network that is crucial for pancreatic Beta cell development and functioning [10]. HNF1-alpha mutations have high penetrance with 63%of carriers developing diabetes by 25 years of age, 79% by 35 years and 96% by 55 years. The age at diagnosis of the disease is determined in part by location of mutations; patients with mutations in terminal exons 8-10 diagnosed on average eight years later than those with mutation in exon 1-6 [4,11]. HNF-1 alpha is a transcription factor (also known as transcription factor or TCF1) which is thought to control a regulatory network (including, among other genes, HNF1α) important for differentiation of beta cells in islets of Langerhans of pancreas. Mutations of this gene lead to reduced beta cell mass or impaired function. HNF1A consists of ten exons, coding 631 amino acids and has three different isomers namely, isoform A (exons8–10), isoform AB (exon 7) and isoform ABC (exons 1–6) formed by alternative splicing and polyadenylation. The isoform A has a lower transactivation activity compared with the isoforms AB and ABC. Mutations affecting different isoforms have been associated with age at disease onset in certain studies; thus, isoforms may influence the phenotype of disease differently [4,11-12].

Here in or present study, the result of PCR-SSCP technique, revealing sequence variants in exon 7 and exon 8 of the HNF1-alpha gene, goes perfectly in agreement with the results of the sequencing study which revealed novel variants in the above mentioned exons of the said gene thereby expressing that PCR-SSCP technique can therefore be an efficient molecular genetic method for screening pathogenic mutations in MODY as described by Baroni et al 2001 [13].

During the course of our study the median of age at diagnosis in subjects with Single base substitution polymorphism, c.1398G>A in exon7, seem to vary with the median of age at diagnosis in subjects with insertion polymorphisms in exon 8 which clearly indicates that there is a significant association between the age at diagnosis of the disease and the position of the mutation with reference to the respective exons in which it is occurring. This might give a clear evidence that age at diagnosis could possibly be mediated by the variations in the different mutational positions [4,14]. Our results are consistent with the studies done in case of China [15], Japan [16], Mexico [17], UK [4,14]. Although in our population number of polymorphisms identified is relatively fewer in number, which may suggest that additional MODY gene screening is required in our population. MODY is such a type of Diabetes which certainly warrants genetic counseling [18-19]. A parent with HNF1-alpha diabetes has a 50% chance of passing on the mutation to each child. Predictive genetic testing in unaffected family members may be helpful but should be preceded by counseling to enable relatives to make an informed decision. The main advantages of knowing this genetic information include reduction in uncertainty over the risk of diabetes and increased efficiency in monitoring for early signs of diabetes [20-22].

Conclusion

In our present study we can conclude by saying that there are sequence variants existing in exon7 and exon 8 of HNF1-alpha gene, as revealed by PCR-SSCP study in our population which goes in agreement with the results of the sequencing study. The age at diagnosis of the subjects with novel single base substitution polymorphisms in exon 7 seem to be little less than that in subjects with polymorphisms in exon 8 of the HNF1-alpha gene, emphasizing that age at diagnosis is influenced by variations in mutational positions.

Acknowledgement

The authors sincerely thank M.P Birla Medical Research Centre, Kolkata, for their financial support in our study. The authors express sincere gratitude to the medical institutes for their cooperation in allowing us to contact the patients and to obtain information from their clinical records.

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