Mutations in HNF1A Gene are not a Common Cause of Familial Young-Onset Diabetes in Iran

Meysam Moghbeli¹ · Bahram Naghibzadeh² · Martha Ghahraman^{2,3} · Sedigheh Fatemi⁴ · Morteza Taghavi⁵ · Rahim Vakili⁶ · Mohammad Reza Abbaszadegan^{2,3}

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Abstract Mutations in hepatocyte nuclear factor-1 alpha (HNF1A) as a homeodomain transcription factor which regulates variety of genes, are the most common cause of maturity-onset diabetes of the young (MODY). Detection of HNF1A mutations not only classifies the subtype, but also predicts the likely clinical course and may alters the method of treatment from insulin to the oral sulphonylureas, which is shown to improve glycemic control. The coding and promoter regions of *HNF1A* gene were screened for mutations in 34 unrelated Iranian MODY patients. We identified one novel missense mutation (C49G) and two novel polymorphisms and 8 recently identified SNPs in the *HNF1A* gene. It is possible that in Iran, other yet to be identified genes are responsible for the

familial young onset diabetes. Hence, there is a need for more extensive genetic analyses in Iranian patients with familial young onset diabetes.

Keywords Hepatocyte nuclear factor-1 alpha · Maturity-onset diabetes of the young · Sulphonylureas

Introduction

Monogenic defects in beta cell function also are known as maturity-onset diabetes of the young (MODY). MODY are a heterogeneous group of autosomal dominant hyper glycemia which are characterized by early age onset (before 25 years) [1]. MODY is the most common type of monogenic diabetes which affects the development of pancreas and beta cells function [2]. It involves 1–5% of all diabetes in the United States and Europe [3]. At least 13 genes such as HNF4A, GCK, HNF1A, PDX1, TCF2, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8 and KCNJ are involved in inherited diabetes with MODY phenotype [4]. Mutations in hepatocyte nuclear factor-1 alpha (HNF1A) gene (also called MODY3) are considered the commonest form of monogenic diabetes [1]. Patients with HNF1A mutation shave to have young-onset and non-insulin dependent hyperglycemia which tends to increase over time [5]. In such patients, the achieved glycemic control by sulphonylureas is better than the metformin or insulin [1]. Detection of HNF1A mutations not only classifies the subtype, but also predicts the likely clinical course and may alters the method of treatment from insulin to the oral sulphonylureas, which is shown to improve glycemic control [6, 7]. Moreover, finding a HNF1A mutation in a proband could be predictive for the asymptomatic first relative carriers [3]. In the present study, we aimed to

✉ Rahim Vakili
vakilir@mums.ac.ir

✉ Mohammad Reza Abbaszadegan
abbaszadeganmr@mums.ac.ir

¹ North Khorasan University of Medical Sciences, Bojnurd, Iran
² Medical Genetics Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
³ Division of Human Genetics, Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
⁴ Immunogenetics Department, Immunology Research Center, Bu-Ali Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
⁵ Endocrinology and Metabolism Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
⁶ Department of Pediatric Endocrinology, School of Medicine, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran

assess the prevalence of HNF1A mutations in clinically suspected diabetic patients for the first time in Iranian population, and hopefully change the method of treatment from insulin to the oral hypoglycemic agents [8].

Methods

Patients and Sampling

Thirty-four unrelated patients with clinical features of MODY 3, who were below 25 years old and had strong family history of diabetes (at least in three generations) were selected from out-patient clinics as well as from major hospitals mainly located on Northeastern Iran between 2008 and 2012 [3, 9]. The other cases with type 1 diabetes (history of ketoacidosis, negative c-peptide, and detectable markers of the immune destruction of the beta cell) or type 2 diabetes (marked obesity and acanthosis nigricans) were excluded. All patients were Iranian Caucasians. Written informed consent forms were obtained from all of the participants or their parents. This study was carried out in accordance with the Declaration of Helsinki and was approved by the ethics committee of Mashhad University of Medical Sciences. Ten milliliters of venous blood was drawn from the patients' antecubital fossa according to World Health Organization's Guidelines on Drawing Blood.

Anthropometric Measurements

Anthropometric measurements were performed according to the World Health Organization's STEP wise Approach (STEPS) Guide to Physical Measurements. The patients were weighed wearing light clothes and no footwear on a mechanical scale and

with results given to the nearest 0.1 kg. Height was measured to the nearest 0.5 cm using a wall mounted measuring tape. Body mass index (BMI) was calculated as patient's weight divided by the square of their height. Hip and waist diameters were measured to the nearest 0.5 cm using a non-extendable fiberglass body tape measure.

DNA Extraction and Amplification of HNF1A Gene

Genomic DNA was extracted from the peripheral whole blood using the standard salting-out method (26). A polymerase chain reaction (PCR) amplified the promoter region, coding exons of the HNF1A gene and their intron-exon junctions. Eleven pairs of previously described primers were used for the promoter and 10 exons of the HNF1A gene [10] (Table 1). PCR reactions were performed in 50 µl reaction tubes using the 100–200 ng of template DNA, 10 pmol of each primers (Table 1), 1.5 mMol MgCl₂, 200 µmol dNTPs, 4U prime Taq DNA polymerase, and 10X buffer (GeNet Bio Co, Chungnam, Korea). PCR products were purified using a DNA Gel Extraction Kit (Invitex Co, Berlin, Germany) according to the manufacturer's instructions. PCR products were assayed for size and purity by separation on 2% agarose gels (Fig. 1).

Sequencing and Analysis

The promoter, coding regions and intron-exon boundaries of HNF1A gene were screened for mutations by Sanger bidirectional sequencing. The sequencing was performed by Macrogen Institute (Seoul, South Korea) using ABI3730 automated DNA Sequencer (Applied Biosystems, Foster City, California, USA). Each fragment was sequenced with the original primers and compared to the human GenBank

Table 1 Primers used for amplification and sequencing of HNF1A gene

Fragment	Forward primer (5' → 3')	Reverse primer (3' → 5')	Product size (bp)
Promoter	TCCCATCGCAGGCCATAGCTC	CCGTCTGCAGCTGGCTCAGTT	385
1	GGCAGGCAAACGCAACCCACG	GAAGGGGGGCTCGTTAGGAGC	483
2	CATGCACAGTCCCCACCCTCA	CTTCCAGCCCCACCTATGAG	390
3	GGGCAAGGTCAGGGGAATGGA	CAGCCCAGACCAAACCAGCAC	304
4	CAGAACCCTCCCCTTCATGCC	GGTGACTGCTGTCAATGGGAC	397
5	GGCAGACAGGCAGATGGCCTA	GCCTCCCTAGGGACTGCTCCA	346
6	TGGAGCAGTCCCTAGGGAGGC	GTTGCCCATGAGCCTCCAC	322
7	GGTCTTGGGCAGGGGTGGGAT	CTGCAATGCCTGCCAGGCACC	347
8	GAGGCCTGGGACTAGGGCTGT	CTCTGTCACAGGCCGAGGGAG	229
9	CCTGTGACAGAGCCCCTCACC	CGGACAGCAACAGAAGGGGTG	287
10	GTACCCCTAGGGACAGGCAGG	ACCCCCAAGCAGGCAGTACA	248

Fig. 1 PCR Products of promoter and 10 exons of HNF1A gene on 2% agarose gel

sequence for the HNF1A gene (OMIM 142410) using Sequencher sequence alignment software (Version 4.10.1 Build 5828; Gene Codes Corporation, Ann Arbor, Michigan, USA).

Statistical Analysis

Analysis of anthropomorphic measurements and biochemical assays was performed using Statistical Product and Service Solutions (SPSS) software (SPSS, Chicago, IL, USA) version 15.

Results

All of our patients were Iranian Caucasians mainly living in Northeastern Iran. Thirteen out of thirty-three (39.4%) were male, and 20 (60.6%) were female. The age of patients ranged from 7 to 25 with mean age of 16.57 ± 6.45 years old, at the time of investigation. The mean BMI was 21.50 ± 3.86 . We identified only C49G mutation which is a new missense mutation in exon one in one of the cases. The proband carrying the homozygote C49G mutation was a 12 year-old lean girl who was diagnosed with diabetes type one 2 months before the genetic analysis. Insulin was started for her at the time of diagnosis. Her parents and brother were diagnosed with diabetes 2 years ago and is now treated without any insulin injection. Abdominal ultrasonography showed no sign of any liver adenomas. We have easily transferred from insulin to glyburide in this carrier of the HNF1A mutation. Her HbA1C before starting glyburide and after 3 months of taking glyburide was 64 mmol/mol and 40 mmol/mol, respectively. We have also identified 8 known single nucleotide polymorphisms (SNPs) and 2 novel SNPs in patients (Table 2).

Table 2 Identified single nucleotide polymorphisms (SNPs) in patients

Reference SNP	Allele change	Position	Residue change
rs1169289	CTC $\Rightarrow$ CTG	17	Leu $\Rightarrow$ Leu
rs1169288	ATC $\Rightarrow$ CTC	27	Ile $\Rightarrow$ Leu
rs1800574	GCC $\Rightarrow$ GTC	98	Ala $\Rightarrow$ Val
Novel SNP	GAG $\Rightarrow$ GAA	105	Glu $\Rightarrow$ Glu
rs56348580	GGG $\Rightarrow$ GGC	288	Gly $\Rightarrow$ Gly
Novel SNP	AGC $\Rightarrow$ AGT	388	Ser $\Rightarrow$ Ser
rs118028009	CTG $\Rightarrow$ CTA	459	Leu $\Rightarrow$ Leu
rs2259820	CTG $\Rightarrow$ TTG	459	Leu $\Rightarrow$ Leu
rs2464196	AGC $\Rightarrow$ AAC	487	Ser $\Rightarrow$ Asn
rs55834942	ACG $\Rightarrow$ ACA	515	Thr $\Rightarrow$ Thr

Discussion

It seems that there is a huge discrepancy in the case of role of HNF1A in MODY, in which about half of some European families harbor the HNF1A mutation whereas HNF1A mutations are rare in Asian families [11]. Monogenic defects in beta cell function are characterized by onset of hyperglycemia at an early age, autosomal dominant mode of inheritance and impaired insulin secretion [5]. To date, at least thirteen MODY subtypes with distinct genetic etiologies have been reported in the literature [4, 12–14]. Estimations show that up to 80% of patients with MODY are currently undiagnosed or misdiagnosed with type 1 or young-onset type 2 diabetes [15]. When MODY is suspected in a patient, based on the clinical features, the commonest candidate genes (HNF1A, GCK and HNF4A) are selected and tested by PCR amplification and Sanger sequencing [9]. Heterozygous mutations of HNF1A gene are considered the commonest cause of familial young-onset diabetes in most populations [9]. To date, more than 200 mutations have been identified in patients of all racial and ethnic backgrounds

[16, 17]. Molecular diagnosis of HNF1A mutation is crucial as it leads to accurate disease treatment with sulfonylureas and care management of the patient and his family, and estimation of diabetes risk for the asymptomatic relatives [4, 18]. Cysteine as a polar amino acid is involved in hydrogen bonds, whereas the Glycine is found on the surface of proteins within the loops. Glycine is responsible for the loop flexibility. C49G is located between the 2nd and 3rd helices of HNF1a where is not involved in dimerization and DNA interaction. Therefore, it seems that this mutation does not have any significant role in the protein functions. However, some reports mention that mutations in this region can be resulted in benign or malignant manifestation [19]. Although, this region is not directly related to the DNA binding, it has been reported that the mutations in this region can influences indirectly DNA binding of HNF1a [19]. Prevalence of this disease is different among populations, or even within a country. This form of diabetes is more common among Europeans, but reports from Mexico [20], South Korea [10], and India [21] show that prevalence of HNF1A mutations among patients with familial young-onset diabetes is less than 10%. Our study, in which prevalence of HNF1A mutations is about 3% concurs with the latter reports and shows that mutation of HNF-1A is not a major cause of early-onset type 2 DM in Iran regardless of strong family history. Studies conducted in Germany [22], Norway [23], Denmark [2], Italy [24], Czech Republic [25], Japan [26], and China [27] suggested that a majority of MODY patients cannot be explained by known MODY genes. Considering the limited budget for our study, a great deal of effort has been made to carefully select patients with stringent clinically defined MODY. Two other studies conducted in Mashhad University of Medical Sciences analyzed GCK and HNF4A mutations in the same population and found that prevalence of these mutations among patients with young onset familial diabetes to be 36.6 and 26.6%, respectively [28]. Patients with characteristic findings of MODY in whom there is no mutation found in common known MODY genes are sometimes referred to as MODYX cases in literature [29, 30].

In conclusion, it is possible that in Iran, other yet to be identified genes are responsible for the familial young onset diabetes. Hence, there is a need for more extensive genetic analyses in Iranian patients with familial young onset diabetes. Exome sequencing is an outstanding method to identify disease mutations in monogenic disorders, thus making it a valuable investigation where Sanger sequencing comes short.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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