Iran J Public Health, Vol. 44, No.3, Mar 2015, pp.396-403

Short Communication

Frequency of *HNF4A*-P.I463V Variant in the Tunisian North-African Population and Its Relation with Diabetes Mellitus

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(Received 15 Dec 2014; accepted 25 Feb 2015)

Abstract

Background: *HNF4A*-p.I463Vvariant, reported previously in two distinct families suspected of MODY-1, is assessed in this report to determine whether it is a mutation or a polymorphism (frequency >1%).

Methods: 200 Tunisian healthy people were screened for the presence of *HNF4A*-p.I463V variant, using RFLP-PCR technique and sequencing. Then, the frequency of this variant was estimated in the Tunisian population and compared to other populations registered in genetic databases. We also performed *in-silico* analysis using PolyPhen2 and Mutation T@sting softwares to assess the probable effect of *HNF4A*-p.I463V variant.

Results: *HNF4A*-p.I463V had a rare frequency in different populations and was found in 3 control subjects (1.5%) of the studied population. PolyPhen2 predicted that it is a polymorphism, whereas mutation T@sting suggested a probably affected mutant protein.

Conclusion: *HNF4A*-p.I463V has a relatively high frequency (>1%) in our control cohort. It is also present in different ethnicities and *in- silico* analysis showed conflicting results. For these reasons, *HNF4A*-p.I463V should not be considered as a mutation responsible for MODY-1.

Keywords: MODY, *HNF4A*-p.I463V, Polymorphism, Mutation, Tunisia, in-silico, Type 2 diabetes, rs147638455

Introduction

Hepatocyte nuclear factor 4a (HNF4A) is an orphan member of the nuclear receptor family of transcription factors (1). It is expressed primarily in the liver, gut, kidney and pancreas and has important role during embryonic life in pancreatic development and maintaining of β cell function (2). Indeed, knockout studies found that Hnf4a$^{-/-}$ mice revealed impaired gastrulation and died around day E9 (3). The HNF4A protein consists of five functional domains: an N-terminal activation function (AF-1, also referred to as A/B domain), two zinc fingers responsible for DNA binding (C domain), a potential ligand binding domain (E domain) and the F domain (1).

HNF4A gene (ENSG00000101076) has many transcripts. This gene could produce nine poten-

tial isoforms (HNF4A1–HNF4A9) (4). The ENST00000316099 transcript is considered the canonical transcript that codes the isoform HNF4-Alpha-1 with 474 amino-acids (P41235-1). Mutations in *HNF4A* gene are responsible of MODY-1 (Maturity-onset diabetes of the young type 1) (OMIM#125850) and neonatal hyperinsulinism hypoglycemia (5, 6). To date, about 103 *HNF4A* inactivating mutations were reported in 173 MODY families (7). Some *HNF4A* variants were found to be associated with type 2 diabetes (T2D) (8).

T2D is a chronic disorder of glucose metabolism, characterized by a state of hyperglycemia due to defective insulin secretion, peripheral insulin resistance and increased hepatic glucose production (9). Genetic and environmental factors, contribute to the appearance of T2D (10). Whereas, MODY is a dominantly inherited form of early-onset type 2 diabetes (T2D), generally diagnosed in childhood, adolescence or young adulthood (11). It is a rare monogenic disease caused by primary defects of insulin secretion with a lack of auto-antibodies against the pancreatic cells and is rarely associated with obesity (11).

The NM_000457.4: c.1387A>G or *HNF4A* -p.I463V mutation (rs147638455 or CM993900), also known as p.I431V and p.I453V depending on the corresponding gene's isoform, was previously found in a Caucasian and a Tunisian families suspected of MODY (12, 13).

But, it remains unclear whether this variant is a mutation responsible for MODY-1 or a polymorphism. In the case of a molecular diagnosis of MODY-1, finding *HNF4A*-p.I463V would be confusing: should we consider it as a mutation?

In this report, we present a simple and easy protocol to screen *HNF4A*-p.I463V variant. We also assessed its frequency in Tunisian North-African population and discussed its probable effect.

Materials and Methods

DNA samples

Two hundred anonymous unrelated healthy controls were recruited in 2013 from the Blood Transfusion Center of Sousse (Tunisia). They were examined by the physician of the center and were found not diabetic and did not present any other disease. The mean age of the control subjects was 27.23 ± 10.28 years old. All these individuals were screened for the presence or absence of *HNF4A* -p.I463V variant.

The study was carried out after taking informed written consent from all individuals and after approval of the Ethics Committee of Farhat Hached University Hospital.

HNF4A -p.I463V mutation analysis

Genomic DNA was extracted from peripheral blood using Flexigene DNA kit (QiagenInc). The *HNF4A* -p.I463V mutation analysis was carried out by RFLP-PCR method. First, *HNF4A* exon 10 including exon-intron boundaries was amplified by PCR using the following primers F: 5' TTT ACT CCC ACA AAG GCT GG 3' and R: 5' ATC ACC AGG TGC TCT CTT AG 3'. PCR was performed in a 50 µl volume containing 50 ng of genomic DNA, 20 pmol of each primer and 1 U of Recombinant DNA polymerase (Invitrogen, Carlsbad, CA, USA). PCR conditions and thermocycler program are available on request.

Fifteen µl of PCR product were mixed with 2µl BSA, 2µl buffer, 5.75 µl sterilized water and digested by 2.5 U of FOKI restriction enzyme (TaKaRa Bio Inc, Otsu, Shiga, Japan).The digested PCR products were then separated on 2.5% agarose gel.

The *HNF4A*-p.I463V substitution abolishes the restriction site of FOKI. Thus, the wild fragment (266 bp) is cut into 164 bp and102 bp fragments. Finally, in case of mutation detection, direct sequencing was used to confirm it.

Statistical analysis

The studied population was checked for Hardy-Weinberg equilibrium.

Prediction of p.I463V-HNF4A mutation effect in-silico

The p.I463V substitution was studied *in-silico* to predict the putative effect on protein function. We performed *in-silico* analysis using two different types of software: PolyPhen2 (http://geneti-

cs.bwh.harvard.edu/pph2/index.shtml) and Mutation T@sting (http://www.mutationtaster.org/). These bioinformatics tools enable high-throughput prediction of the potential impact of residue changes and large-scale polymorphism analyses. PolyPhen and Mutation T@sting predict possible impact of an amino acid substitution on the structure and function of a human protein using straight forward physical and comparative considerations (14, 15).

Results

Carrier Frequency of p.I463V

The assessment of *HNF4A*-p.I463V variant in 200 healthy Tunisian people by RLFP-PCR method found that 3 controls (1.5%) were carriers of this mutation. The results were also confirmed by direct sequencing of *HNF4A* exon 10 (Fig. 1).The healthy carriers were aged 20, 26 and 37 years old.

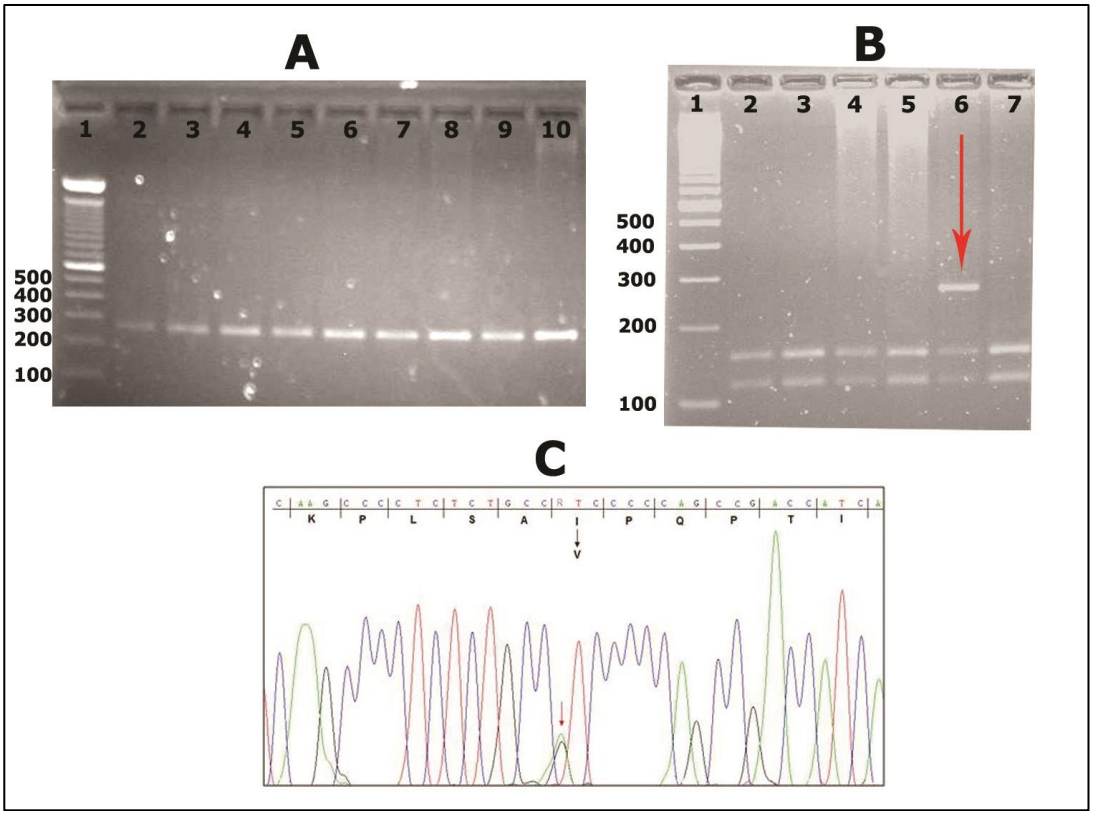

Fig. 1: *HNF4A*-p.I463V variant detected after sequencing and enzymatic digestion by FOKI restriction enzyme
A: Gel electrophoresis of *HNF4A* exon 10 (266 bp) before digestion by FOKI restriction enzyme in 9 controls.
B: Gel electrophoresis of *HNF4A* exon 10 after digestion by FOKI restriction enzyme into 2 fragments (164 and 102 bp) in 7 controls. Well 6 shows a heterozygote person for *HNF4A* p.I463V mutation.
C: Chromatograph of a part of *HNF4A* exon 10 sequence showing the c.1387A>G/p.I463V mutation

Comparison with other populations

The Tunisian population of this study is in Hardy-Weinberg equilibrium (Table 1). The frequency of *HNF4A*-p.I463V is rare in the other populations. Indeed, this variant is more frequent in the Tunisian population than the European and African American populations.

Results of in Silico Analysis

PolyPhen2 predicted that this nucleotide change is a "polymorphism" with a score of 0.144 [0-1]. While, the Mutation T@sting software indicated that this variant could be responsible of a probably affected protein.

Table 1: rs147638455 frequency in different populations

Population	Source	Sample size	Chrom sample	Mean age (years old)	Genotypes		
					G/G	G/A	A/A
European American	ESP project*	4300	8600	NA***	0	$9.3\ 10^{-4}$	0.99
African American	ESP project	2203	4406	NA	0	0	1
European American	Clinseq project**	662	1324	NA	0	0.002	0.998
North African- Tunisian[a]	This study	200	400	27.23 ± 10.28	0	0.015	0.985

*Exome sequencing project, **CSAgilent (dbSNP 138), *** Not applicable, a: the Tunisian population is in Hardy-Weinberg equilibrium (P=0.91 > 0.05)

Discussion

Performing a molecular diagnosis of monogenic diabetes is not an easy task because of its clinical and genetic heterogeneity (16). Some genetic variants could also be problematic such as *HNF4A*-p.I463V. In an earlier study, we found that *HNF4A*-p.I463V cosegregated with diabetes phenotype in a Tunisian family (Fig. 2 and Table 2). It was present in three patients from this family and was absent in two other non-affected relatives (13). Contrariwise, this variant didn't show a perfect cosegregation in a Caucasian family (12). Hitherto, there is not enough information in the literature about *HNF4A*-p.I463V variant (rs147638455). It is not clear whether it is a mutation directly causing MODY-1 or just a non-synonymous polymorphism with low effect.

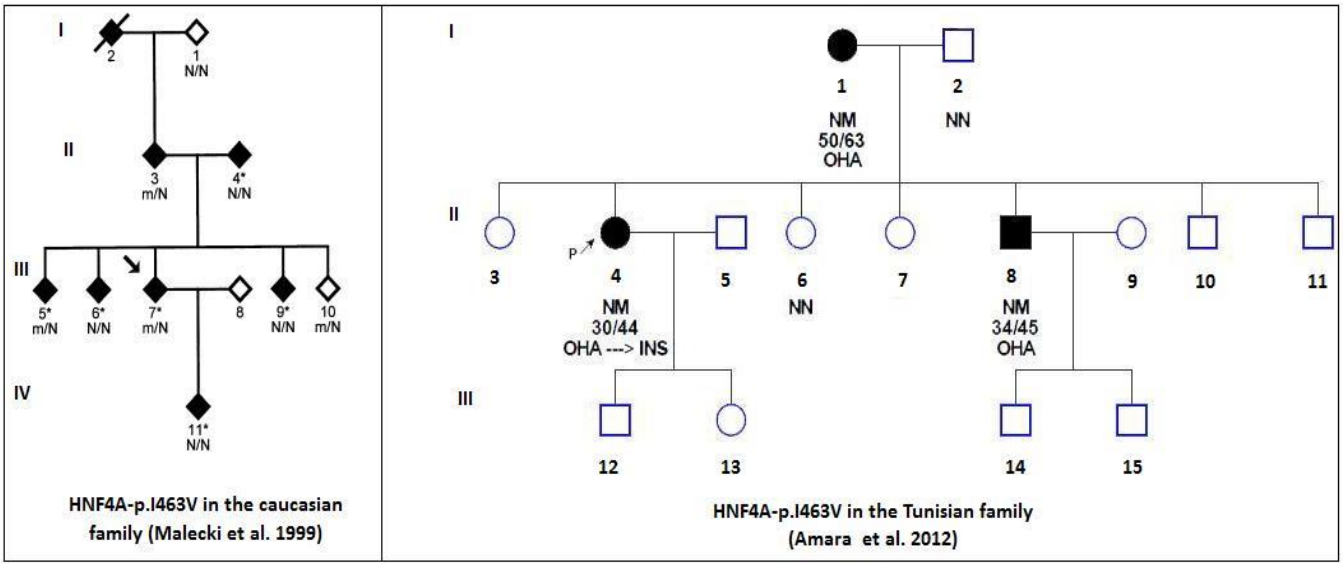

Fig. 2: The diabetic Caucasian and Tunisian families having *HNF4A*-p.I463V variant
Filled and open symbols represent diabetic subjects and normal glucose tolerance individuals, respectively. The numbers under the symbols are the identification numbers. Below the numbers, it is the genotype at codon I463V: N, normal allele (Isoleucine); m, mutant allele (valine). Below the genotype is the diagnostic age of diabetes for affected members and age at examination, followed by the treatment for diabetes (OHA: Oral hypoglycemic agents and INS: Insulin). An arrow with P letter indicates the proband.

Table 2: The clinical characteristics of the reported *HNF4A*-p.I463V carriers

Group	Individual identifier	Diabetes	Diagnosis Age	Examination Age	Fasting gly-caemia	Hb1Ac	BMI	Treatment	Reference
Tunisian family	II-1	Yes	50	63	NF	NF	NF	OHA	(12)
	II-4	Yes	30	44	17.2	13.4	35.6	OHA→ INS	
	II-8	Yes	34	45	NF	NF	30.3	OHA	
	Mean	NA	38	50.6	NA	NA	32.9	NA	
Caucasian family	3	Yes	64	64	5.43	6.2	NF	Diet	(13)
	10	No	NA	31	NA	5.7	NF	NA	
	Mean	NA	64	47.5	NA	NA	NF	NA	
Tunisian Healthy cohort	H1	No	NA	20	NA	NA	NA	NA	This study
	H2	No	NA	26					
	H3	No	NA	37					
	Mean	NA	NA	27.6					

NA: not applicable, NF: not found, OHA = Oral hypoglycemic agents, INS: Insulin

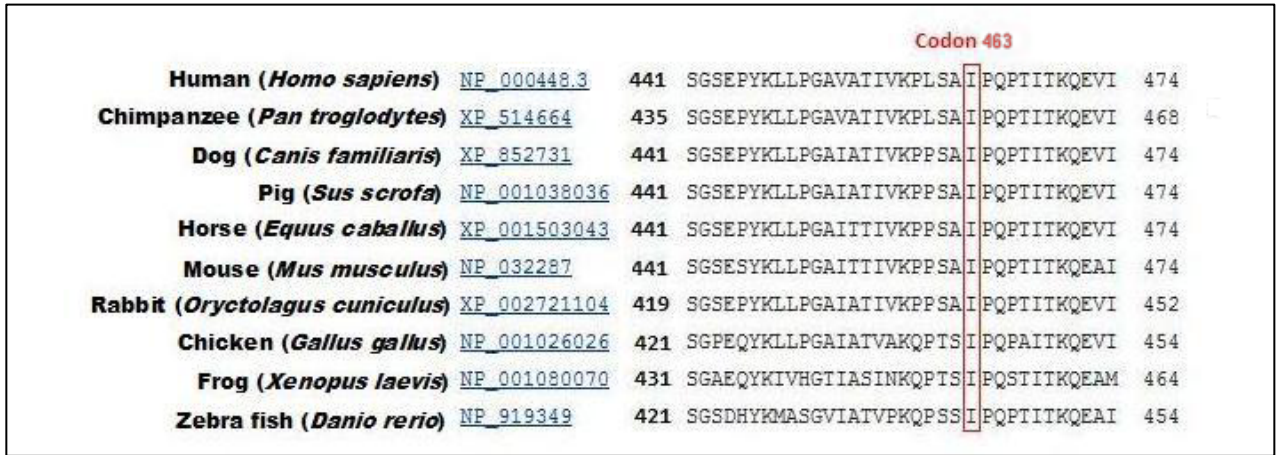

Fig. 3: Isoleucine amino-acid in position 463 is conserved across species
A portion of *HNF4A* exon 10 amino-acid sequence is compared between various species. The human Isoleucine at codon 463 and Isoleucine of other species are framed in a red box. The species are annotated in bold and the NCBI accession numbers in blue

- ***HNF4A*-p.I463V a variant with interesting characteristics**

This variant occurs in a conserved region across species (PhyloP score = 4.56 [-14.1; 6.4]) and is localized in the F domain of *HNF4A* (Fig. 3). This domain plays a role in the protein transactivation activity (17).

- ***HNF4A*-p.I463V variant could not be responsible for MODY-1**

Using a simple protocol of screening for *HNF4A*-p.I463V variant (rs147638455), we detected it in three healthy controls out of 200 Tunisian unrelated healthy individuals (400 chromosomes). Indeed, it is an unexpected high carrier frequency (1.5%) in this sample compared with the other populations (Table 1).

Commonly, MODY develops before the age of 25 years old (11). The healthy controls carriers of rs147638455 in our population are aged 20, 26 and 37 years (Table2). Even if we omit the 20 year-old control individual, the rs147638455 frequency would remain high for a mutation (1%). In other ethnic populations, rs14763845 was rarely found in healthy individuals (Table 1). If we suppose that this variant is responsible for MODY-1, which is a rare monogenic disease, Tunisian frequency, argues against this hypothesis.

On the other hand, the *in silico* analysis using mutation T@ster and polyphen2 showed conflicting results. In parallel, physicochemical difference between Isoleucine and Valine amino-acids is small (Grantham Distance = 29 [0-215]). In addition to the absence of a cosegregation of *HNF4A*-p.I463V variant in the Caucasian family, the Tunisian family suspected of MODY had a mean age of diabetes diagnosis of 38 ± 10.58 years old (13) (Table 2 and Fig. 2). Even familial, the diabetes observed in this family, could be T2D. Otherwise, a previous study showed that patients with mutations in *HNF4A* exons 9 and 10 developed diabetes later than those with mutations in exons 2–8 did(4). So, mutations in exons 9 and 10 could not be responsible for MODY-1 (1).

- **Speculations about the effect of this variant and its implication in type 2 diabetes**

HNF4A-p.I463V could act in an interactive polymorphisms network leading to T2D. At that time, the relatively young age of our healthy carriers (20, 26 and 37 years old) does not definitely mean that they will not present T2D later.

A similar missense mutation in the F domain (*HNF4A*-p.V393I localized in exon 9) was found in a French diabetic family diagnosed at the age of 45 years. *HNF4A*-p.V393I was associated with a marked reduction of transactivation activity responsible for impaired insulin secretion and co-segregated with diabetes (18).

It is of note that some non diabetic individuals aged between 30 and 40 year-old were carriers of *HNF4A*-p.V393I (18). Thus, *HNF4A*-p.I463V could have a similar effect to *HNF4A*-p.V393I. Indeed, most of the patients that carry *HNF4A*-p.I463V variant are over 30 years (Table 2). This could be a supplementary argument to associate *HNF4A*-p.I463V with T2D.

Association and functional studies should be carried out, to assess the exact effect of *HNF4A*-p.I463V variant. Meantime, we recommend to do not consider *HNF4A*-p.I463V as a mutation in case of molecular diagnostic of MODY.

This recommendation is based on the high frequency of this variant in the Tunisian population, its presence in healthy persons and the conflicting results of the *in silico* analysis.

Conclusion

We can speculate that *HNF4A*-p.I463V is a polymorphism with possible role in T2D and not a causal mutation implicated directly in MODY-1.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submiss-ion, redundancy, etc.) have been completely observed by the authors.

Acknowledgments

The authors thank all the volunteers and the staff of the Regional Center of Blood Transfusion of Sousse for their contribution to this work. Special thanks to Mrs Sihem SASSI, Mrs Ahlem M'SAKNI, Miss Safa BOUKER and Mr Hedi LAATIRI for their excellent technical assistance.
The authors declare that there is no conflict of interests.

Abbreviations

MODY: Maturity-Onset diabetes of the young
T2D: Type 2 diabetes
HNF4A: Hepatocyte nuclear factor 4a

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