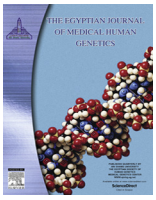
The Egyptian Journal of Medical Human Genetics xxx (2018) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com



Original article

Renalase gene polymorphisms (rs2576178 and rs10887800) in Egyptian hypertensive end stage renal disease patients

Noha S. Kandil [a,*], Rania Mohamed El Sharkawy [a], Lubna Mohamed Ibrahim Desouky [b], Lamia Said Kandil [c], I.M. Masoud [d], Noha Gaber Amin [e]

[a] Department of Chemical Pathology, Medical Research Institute, Alexandria University, Egypt
[b] Department of Human Genetics, Medical Research Institute, Alexandria University, Egypt
[c] Department of Pharmacology and Therapeutics, Faculty of Pharmacy and Drug Manufacturing, Pharos University, Egypt
[d] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Drug Manufacturing, Pharos University, Egypt
[e] Department of Internal Medicine, Faculty Of Medicine, Alexandria University, Egypt

ARTICLE INFO

Article history:
Received 6 January 2018
Accepted 26 February 2018
Available online xxxx

Keywords:
End stage renal disease
Renalasegene polymorphism
Hemodialysis
Hypertenion

ABSTRACT

Background: The highly polymorphic gene encoding human renalase (RNLS) is a 311,000 bp gene located on chromosome 10.
Aim: This study aimed at studying the possible association of the two RNLS gene polymorphisms rs2576178 and rs10887800 with chronic kidney disease in general or specifically with hypertensive nephropathy in Egyptian end stage renal disease (ESRD) patients on maintenance hemodialysis.
Subjects and method: This case control study was conducted on two hundred and eighty one individuals, divided equally into two groups; an end stage renal disease patients on maintenance hemodialysis with/without hypertension and healthy matching individuals as a control group. Full clinical examination, Biochemical analysis and Molecular genetic testing were performed to detect single nucleotide polymorphism using restriction fraction length polymorphism (RFLP) for RNLS rs2576178 and rs1088780.
Results: The results of this study demonstrated that the risk of developing ESRD was increased among carriers of AA genotype for the rs10887800 (3.05 times) p = 0.001, OR = 3.05, CI95% (1.558–5.971) and GG genotype for the rs2576178 p = 0.047, OR = 1.949, CI95% (1.028–3.694).
Conclusion: Our study revealed that the risk of developing end stage renal diseases was increased among carriers of AA genotype for the rs10887800 polymorphism and GG genotype for the rs2576178 polymorphism.

1. Introduction

The renalase, also known as monoaminooxidase-C (MAO-C), is a flavoprotein enzyme that participates in the metabolism of circulating catecholamines [1]. It metabolizes catecholamines and catecholamine-like substances via a superoxide dependent mechanism using nicotinamide adenine dinucleotide (NADH) as a cofactor [2]. At least four isoforms of renalase enzyme protein have been identified. Two of them possess an unchanged amino acid domain (h-renalase 1), whereas the other two have shortened domains (h-renalase 2) [3].

Renalase is synthesized mainly by the kidneys and is excreted directly into the blood, where it participates in the metabolism of circulating catecholamines [4]. Renalase activity is not inhibited by known monoamineoxidase (MAO) inhibitors such as pargyline and clorgyline [4,5]. Renalase plays a direct significant role in the regulation of blood pressure thus its insufficiency predisposes to higher blood pressure values. [6,7]

Renalase levels were reported to be low in chronic kidney disease patients implicating its role in development of hypertension and associated morbidity and mortality in such patients [1–3]. The observation of a significant renalase deficiency in end stage renal disease (ESRD) patients was confirmed by experimental models of uremia in rats following nephrectomies [8]. Such a deficiency leads to impaired degradation of catecholamines, causing excessive tension of the sympathetic nervous system that is related to a high risk of cardiovascular diseases [9–11]. Therefore, renalase deficiency could represent an unknown pathophysiological mechanism that might partially explain the high rates of hypertension in ESRD patients.

Peer review under responsibility of Ain Shams University.
* Corresponding author.
E-mail address: drnohakandil@yahoo.com (N.S. Kandil).

https://doi.org/10.1016/j.ejmhg.2018.02.004
1110-8630/

The gene encoding human renalase (RNLS) is a 311,000 base pairs (bp) gene located on chromosome 10 (q23.33) that consists of 10 exons [1]. Being highly polymorphic, several single nucleotide polymorphisms (SNPs) of RNLS gene have been recently described. Single nucleotide polymorphisms (SNPs) rs2576178 GG and rs2296545 CC genotypes have been found to be associated with essential hypertension in Northern Han Chinese population [12]. Also rs2296545 CC was found to be associated with cardiac hypertrophy, dysfunction and ischemia in Caucasians [11]. Furthermore, the rs2576178 and rs10887800 GG allele were demonstrated to be significantly higher in hypertensive Egyptian type 2 diabetic patients compared to normotensive diabetic patients and control group [13].

A recent study suggested an association between two SNPs (rs2576178 and rs10887800) in RNLS gene and hypertension in Polish ESRD patients [14].

2. Aim

This study aimed at studying the possible association of the two RNLS gene polymorphisms rs2576178 and rs10887800 with chronic kidney disease in general or specifically with hypertensive nephropathy in Egyptian end stage renal disease (ESRD) patients on maintenance hemodialysis.

3. Subjects and methods

This type of case control study was conducted in the period from December 2015 to October 2016 on two hundred and eighty-one individuals, divided into two groups; a 141 end stage renal disease patients on maintenance hemodialysis with and without hypertension gathered from the nephrology unit of the internal medicine department of the Medical Research Institute Teaching Hospital, and 140 apparently healthy individuals of comparable age and gender serving as a control group, gathered from the outpatient clinics of the Institute. Informed consents were obtained from all individuals undergoing this study, which were approved by the local ethics committee of the Institute in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for research involving humans.

Full clinical examination was done to all participating individuals including duration of hemodialysis, history of hypertension, presence of type 2 diabetes mellitus (DM2), and use of antihypertensive medication, along with physical examination with stress on blood pressure measurement, and the calculation of mean blood pressure and pulse pressure. Hypertension was diagnosed according to the European Society of Cardiology guidelines for the management of arterial hypertension having ≥ 140 systolic and ≥ 90 diastolic as cutoff values. [15]. Patient was at a supine position for at least 5 min prior to blood pressure measurement. The average values of predialysis, systolic and diastolic blood pressures reported in the first 4 weeks of the study were collected and used for the analysis. Mean arterial pressure was calculated from the following standard equation: 1/3 of the systolic blood pressure plus 2/3 of the diastolic blood pressure. The hypertensive group, although receiving antihypertensive drugs were not strictly controlled.

3.1. Biochemical analysis

Following a twelve hours fasting period, whole venous blood sample was obtained from each subject and divided into two portions, one portion was collected in Potassium Ethylenediaminetetraacetic acid (K_3EDTA) coated vacutainer tubes for genomic studies, while the other portion was collected in serum vacutainer tubes, left to clot 10 min and centrifuged. The obtained serum was analyzed for glucose, creatinine, alanine aminotransferase, total cholesterol, high density lipoprotein cholesterol fraction, and triglycerides. Analyses were conducted using Olympus reagents, on the Olympus AU400 clinical chemistry analyzer (Beckman Coulter Inc, Brea CA, USA). The low density lipoprotein cholesterol fraction was calculated using Friedwald formula.

3.2. Molecular genetic testing

Genomic DNA was extracted from whole EDTA blood samples using Gene JET™ genomic DNA purification kit (Thermo Fischer Scientific, USA), according to the manufacturer's instructions. The purity and integrity of the extracted DNA were assessed using the NanoDrop™ 1000 spectrophotometer, utilizing the 260 and 280 nm filters and gel electrophoresis. Polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) was carried out according to the method described by Stec et al. [14]. Separate PCR was done for both SNPs. In a 25 μL reaction volume, 10–50 ng of genomic DNA were mixed with PCR master mix and primer sets specific for the DNA sequence to be amplified (Fermentas, Thermoscientific, USA). Details of primer sequences, cycler conditions and PCR product length (bp) are supplied in Table 1. Presence of the PCR products was ascertained using electrophoresis on a 2% agarose gel, with band staining using ethidium bromide. Next was the digsteion by the restriction enzymes step, were enzymes Msp I and Pst I (Fermentas, Thermoscientific, USA) were used to identify the rs2576178 and rs10887800 polymorphisms respectively in the RNLS gene, utilizing protocols supplied by the manufacturer. Restriction products were detected by electrophoresis on a 3% agarose gel that was visualized using a UV luminescence.

In the RNLS rs2576178 polymorphism, adenine replaced by guanine in the 5′-flanking region of the gene, so restriction products of 423 and 102 bp fragments denoted GG genotype while 525 bp denoted AA wild genotype. On the other hand, the RNLS rs10887800 polymorphism was characterized by guanine

Table 1
Primer sequences, cycler conditions and RFLP enzymes and conditions for both polymorphisms.

Renalase (RNLS) gene		
Primers	Forward (sense)	Reverse (Anti-sense)
rs2576178	5′-AGC AGA GAA GCA GCT TAA CCT-3′	5′-TTA TCT GCA AGT CAG CGT AAC-3′
rs10887800	5′-CAG GAA AGA AAG AGT TGA CAT-3′	5′-AA GTT GTT CCA GCT ACT GT-3′
Polymerase chain reaction conditions for both polymorphisms (rs2576178/ rs10887800)		
Cycle components	Time	Temperature (°C)
Initial denaturation	5 min	94
30–35 Cycles:		
Denaturation	2 min	94
Annealing	30sec	60
Extension	2 min	72
Final elongation	7 min	72
Amplicon (bp)	525	554
Restriction fragment length polymorphism (RFLP)		
	rs2576178	rs10887800
Enzyme	Msp I (SMEs)	Pst I
Concentration (U)	5 U	5 U
PCR product (μL)	10 μL	10 μL
Time(hours)–Temperature (°C)	6–10 h/37 °C	
3% agarose gel Electrophoresis		

Renalase (RNLS), Restriction fragment length polymorphism (RFLP)