Genotypic and functional association of apolipoprotein E and lipoprotein (a) gene polymorphisms with diabetic nephropathy in Bangladeshi population

M. Phil. Thesis

A thesis is submitted to the University of Dhaka in partial fulfillment of the requirements for the degree of Masters of Philosophy in Biochemistry and Molecular Biology

Department of Biochemistry
and Molecular Biology
University of Dhaka
Dhaka-1000, Bangladesh

Submitted by:
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Registration no: 206
Session: 2013-2014

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Declaration

This dissertation has been submitted to the University of Dhaka in partial fulfillment of the requirements for the degree of Master of Philosophy (M. Phil.) in Biochemistry and Molecular Biology. This study has been carried out in the Laboratory of the Department of Biochemistry and Molecular Biology, University of Dhaka. No part of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other learning institutes.

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M. Phil Student

Certificate

This is to certify that Bartholomia Keya Byapari, is a student ofMasters of Philosophy (M.Phil.) of the session 2013-2014 at the Department of Biochemistry and Molecular Biology, University of Dhaka. Her registration No. is 206.She has submitted her thesis work entitled “Genotypic and functional association of apolipoprotein E and lipoprotein (a) gene polymorphisms with diabetic nephropathy in Bangladeshi population” for the partial fulfillment for her M.Phil. degree. This original research work was entirely performed by her using the departmental research facilities.

I wish her all the success in life.

A.H. M. Nurun Nabi, *PhD*

Abstract

Background: Type 2 diabetes mellitus (T2DM) is one of the most common diseases with high incidence and prevalence throughout the world. Lipoprotein related metabolism associated with the damage of micro-and macro- vascular disease in T2DM. Apolipoprotein E (ApoE) and lipoprotein (a) (apolipoprotein A1, ApoA1) genes that affect the clearance of lipoproteins and consequently lipid profile in our body are the most important candidate genes, which have been reported to be associated with the diabetes related complications like nephropathy. Thus, the aim of the present study was to find out the genotypic and functional association of apolipoprotein E and lipoprotein(a) gene polymorphisms with diabetic nephropathy in Bangladeshi population and thus, to evaluate the possibility of these genes for their involvement as the independent risk factor for the development of diabetic nephropathy.

Methods: A total of 349 unrelated Bangladeshi individuals were enrolled in this. Individuals having HbA1c level $\geq$ 6.5% were considered as type 2 diabetic (T2D-DN) patients while individuals having HbA1c level $\geq$ 6.5% and microalbumin level > 30 mg/L were considered as type 2 diabetic patients with nephropathy (T2D +DN). Different anthropometric, demographic and biochemical parameters were recorded and measured from the study participants. Genomic DNA was extracted from the white blood cells of the collected blood samples. The amplification-refractory mutation system (ARMS) polymerase chain reaction was used to identify apolipoprotein E gene polymorphism and TaqMan SNP genotyping assay was used to analyze lipoprotein(a) (apolipoprotein A1) gene polymorphism through Real-time polymerase chain reaction. Anthropometric and biochemical parameters were evaluated according to the genotypic frequencies of respective gene of interests studied. Lipid profile measured in the participants was considered as the functional outcome according to their respective genotypes.

Result: Out of the total study participants, 123 and 122 individuals were diagnosed as type 2 diabetic patients without and with nephropathy, respectively. Rest of 104 participants was healthy individuals. In this study, healthy individuals were not age and BMI matched with that of patients without or with nephropathy. Systolic and diastolic blood pressure also varied significantly between the study groups ($p<0.05$). The levels of HbA1c and microalbumin varied significantly between healthy individuals and patient groups confirming their status of T2D without and with nephropathy. Also, levels of microalbumin and albumin creatine ratio (ACR)

differentiate the two patient groups: T2D-DN and T2D + DN (7.79 ± 4.68 vs 238.58 ± 316.07 and 10.22 ± 6.07 vs 313.66 ± 519.87, respectively). Levels of lipid profiles varied significantly between the healthy individuals and two groups of patients ($p<0.05$). Out of three isoforms of ApoE, frequency of isoform E3 allele was higher in all the participants (77.4% in healthy individuals, 85.4% in T2D-DN and 87.3% in T2D +DN patients) followed by E4 and E2 allele (22.1%, 12.2%, 11.9% and 0.5%,2.4%, 0.8%, respectively). Out of six genotypes with respect to ApoE gene, E2/E4 was not identified in any of the study participants. We did not find any association of neither allele nor genotypes with respect to ApoE gene with the risk of T2D with and without nephropathy. Rather E4 allele and E3/E4 genotype were found to be associated in developing resistance against type 2 diabetes without (OR=0.5, X^2 =7.44, $p<0.00$ and OR=0.38. X^2 =10.96 $p<0.00$, respectively) and with nephropathy (OR=0.31, X^2 =17.08, $p<0.00$ and OR=0.29, X^2 =16.25, $p<0.00$, respectively). However, with respect to rs121912717 within apolipoproteinA1 gene, no association of genotypic and allelic frequencies was found without and with nephropathy. Different distribution pattern of biochemical parameters of glucose, HbA1c, lipid profiles and ACR were observed in different genotypes of ApoE and ApoA1 genes.

Conclusion: This study conclude that ApoE gene polymorphism does not determine genetic susceptibility for the development of nephropathy and T2D rather E4 allele and E3/E4 genotype have protective role against T2D with or without nephropathy, while no such association was found in case of ApoA1 gene. Thus E3 allele and E3/E4 genotype can be an important marker to enumerate whether an individual does have any possibility of developing nephropathy with type 2 diabetes. However, a wide scale study with large number of sample is warranted to establish the association of genetic and allelic variations with diabetic nephropathy with respect to ApoE and ApoA1 gene in Bangladeshi population.

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List of abbreviation

ALT	: Alanin transaminase
Arg	: Arginine
ADP	: Adenosine di aminase
ATP	: Adenosine tri aminase
BMI	: Basal Metabolic Index
bp	: Base pair
BP	: Blood pressure
cm	: Centimete
Cys	: Cysteine
CO2	: Carbon di oxide
Chol	: Cholesterol
CMIA	: Chemiluinescent microparticle immunoassay
DBP	: Diastolic blood pressure
dL	: Deciliter
DMSO	: Dimethyl sulfoxide
dH_2O	: Distilled water
dHDL	: Direct High density Lipoprotein
DNA	: Deoxyribo nucleic acid
EDTA	: Ethylenediaminetetraacetic acid
Et. Br	: Ethidium bromide
F	: Female
FPG	: Fasting plasma glucose
Gln	: Glutamine
GDM	: Gastetional diabetes mellitus

Glu	: Glucose
g	: Gram
HDL	: High density lipoprotein
Hb	: Hemoglobin
Kg	: kilogram
IGF	: impaired Fasting glucose
IDF	: International diabetes federation
IGT	: Impaired glucose tolerance
IU/ml	: International unit/milliliter
L	: Liter
LDL	: Low density lipoprotein
LDH	: Lactate dehydrogenase
M	: Male
mL	: Mililiter
mg/dL	: Miligram/deciliter
mg	: Miligram
Mg	: Magnesium
MgCl	: Magnesium chloride
mALB	: Microalbumin
mg	: milligram
mmol/L	: Milimole/Liter
mmHg	: Milimeter mercury
N	: Normality
nm	: Nanometer
NDM	: Neonatal diabetes mellitus

ng	: Nano gram
NaCl	: Sodium Chloride
NH3	: Amonia
NADH	: Nicotinamide adenine dinucleotide
O.D	: Optical density
PBS	: Phosphate buffered saline
PDH	: Pyruvate dehydrogenase
p.s.i	: Pounds per square inch
PCR	: Polymerase chain reaction
r.p.m	: Rotation per minute
RT	: Real time
RBC	: Red blood cell
RNA	: Ribonucleic acid
SBP	: Systolic blood pressure
SD	: Standard Deviation
SNP	: Single nucleotide polymorphism
SER	: Serine
TG	: Triglycerides
T2D	: Type 2 diabetes
TAE	: Tris- acetate-EDTA
TE	: Tris-EDTA
UV	: Ultra violet
U/L	: Unit/ liter
VLDL	: Very low density lipoprotein
WHO	: World health organization

WBC	: White blood cell
Yr	: Year
µg	: microgram
µg/L	: Microgram/liter
µL	: Microliter
µm/L	: Micromole/liter
%	: Percent
^{O}C	: Degree Centigrade
>	: greater than
<	: Less than
≤	: Less than or equal to
≥	: Greater than or equal to