NOS3 Genetic variants and Inflammation in Type 2 Diabetes: A Nutrigenetic Pilot Study

Thesis

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Dedication

I would like to dedicate this work to my family; my wife, my parents, and my sisters. I thank them all for all continuous help, support, encouragement, and tolerance, without which none of this would have been in the least possible.

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List of Abbreviations

Abbreviation	Full term
5-LO	5-lipoxygenase
AHA	American Heart Association
AMPK	Adenosine monophosphate-activated protein kinase
ANGPTL2	angiopoietin-like protein 2
ANOVA	Analysis of variance
APOE	Apolipoprotein E
ATP III	Adult Treatment Panel III
BMI	body mass index
Bp	base-pair
BRCA1	Breast cancer 1 gene
BRCA2	Breast cancer 2 gene
CCL2	CC-chemokine ligand 2
CDC	Centers for Disease Control and Prevention
CETP	Cholesteryl ester transfer protein
CRP	C-reactive protein
Ct	cycle threshold
CVD	cardiovascular disease

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Abbreviation	Full term
CXCL5	CXC-chemokine ligand 5
DHA	Docosahexaenoic acid
DHFR	dihydrofolate reductase
EDTA	ethyl diamine tetra-acetic
EGIR	European Group for the Study of Insulin Resistance
ELISA	Enzyme-Linked Immunosorbent Assay
eNOS	endothelial nitric oxide Synthase
EPA	eicosapentaenoic acid
FFQ	Food Frequency Questionnaire
FTO	Fat mass and obesity-associated protein
gp130	Glycoprotein 130
GPR120	G protein-coupled receptor 120
GWAS	genome wide association studies
HbA1c	Glycated Hemoglobin
HDL	high-density lipoprotein
HOMA-IR	homeostasis model assessment of insulin resistance
hsCRP	high sensitivity C-Reactive Protein
IDF	International Diabetes Federation
IFG	impaired fasting glycemia

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Abbreviation	Full term
IGR	impaired glucose tolerance
IL	Interleukin
IL-6	Interleukin-6
IL-6r	Interleukin-6 receptor
iNOS	inducible Nitric oxide Synthase
IR	insulin resistance
IRS1	Insulin receptor substrate 1
JAK	Janus kinase
Kb	kilo bases
MCP	monocyte chemoattractant protein
MetS	metabolic syndrome
MMTV	mouse mammary tumor virus
mRNA	messenger RNA
MTHFR	methylenetetrahydrofolate reductase
NAMPT	nicotinamide phosphoribosyltransferase
NCEP	National Cholesterol Education Program
NF-κB	nuclear factor kappa B
NHLBI	National Heart Lung and Blood Institute
Nrf2	Nuclear factor-erythroid 2-related factor 2
nNOS	neuronal Nitric oxide Synthase

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Abbreviation	Full term
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NOS1	Nitric oxide Synthase 1
NOS2	Nitric oxide Synthase 2
NOS3	Nitric oxide Synthase 3
NS	Non-Significant
OR	Odds ratio
PBS	phosphate buffer saline
PCR	Polymerase Chain Reaction
PMN	polymorphonuclear neutrophil
PPARγ	Proliferator-activated receptor gamma
PUFAs	polyunsaturated fatty acids
RBG	random blood glucose
RBP4	retinol-binding protein 4
S	Significant
SFRP5	secreted frizzled-related protein 5
sIL-6R	soluble IL-6 receptor
SNP	single nucleotide polymorphism
STAT	signal transducer and activator of transcription

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Abbreviation	Full term
THF	Tetrahydrofolate
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TAG	Triacylglycerol
TCFL2	Transcription factor 7-like 2
TNF	tumour necrosis factor
TNF-α	tumor necrosis factor alpha
UGT	UDP-glucuronosyltransferases
USA	United States of America
VNTR	variable number tandem repeat
WC	waist circumference
WHO	World Health Organization
WNT5a	Wingless-type MMTV integration site family, member 5a
$\mathbf{X^2}$	Chi-Square test

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INTRODUCTION

Nutrigenomics is a newly developed science domain which studies the relation between the different nutrients and the genes. The term refers to both the study of how the foods we consume affect our genes and how our genes can influence our body's response to what we eat. It has been proposed and then scientifically proved that the genes are flexible and that the genes products behave differently in the presence of certain nutrients. Recent scientific discoveries relating specific gene variants to dietary response can enable physicians to use nutrition to its fullest potential to address various health issues. These personalized diets can optimize an individual's nutritional status and specifically focus on preventing diet-related diseases ***(Fenech et al., 2011)***.

One of these genes that showed genetic variations is Nitric Oxide Synthase 3 (NOS3) gene encoded on chromosome 7q35-36. It is responsible for the production of Nitric Oxide Synthase (NOS) enzyme that mediates the production of Nitric Oxide (NO) from L-arginine in the endothelial cells ***(Liu et al., 2005)***. NO is an important endothelium-derived vascular relaxing factor ***(Hibi et al., 1998)***.

The replacement of guanine by thymine at NOS3 nucleotide 894 results in a change of amino acid from glutamate to aspartate at codon 298 of the mature NOS3 protein. Two groups of investigators have shown that NOS3 with aspartate at position 298 is subject to selective proteolytic cleavage which is predicted to result in absence or reduction of NOS activity in

homozygous carriers of the 894T allele ***(Persu et al., 2002)***. This results in defects in endothelial cell function and NO production being described for subjects with atherosclerosis, hypertension, diabetes, as well as obesity. Obesity has previously been associated with diabetes risk in several studies, and obese individuals have been reported to have reduced NO bioavailability compared to controls whose weight is in the normal range ***(Bressler et al., 2013)***. In addition, adipose tissue is one of the main sources of inflammatory mediators, with Interleukin-6 (IL6) among them ***(Eder et al., 2009)***. It has also been demonstrated that IL6 inhibits NOS activation **(*Hung et al., 2010)***.

The relation of NOS3 genetic variants and metabolic diseases has been studied**.** For example, in one study, the relationship between the G894T polymorphism and diabetes risk was modified by BMI with evidence for interaction between obesity and the minor genotype. In subsequent analyses stratified by levels of BMI, homozygosity for the NOS3 894T allele in obese individuals was shown to be significantly associated with an increased risk for diabetes when compared to the most common genotype, while no variation in susceptibility with genotype was found for individuals whose BMI was less than 30 kg/m^2 ***(Bressler et al., 2013)***. In another study, both inflammatory markers and serum lipids was lower in wild type homozygotes compared to variant carriers. Accordingly, there was a dramatic difference in the rates of arterial stiffness between the two genetic variants ***(Zineh et al., 2007)***.

Introduction

Scientific researchers believe that through exploiting the recent genomic information and better understanding of nutrient-gene interactions, better health outcomes can be achieved if nutritional requirements are customized for each individual taking into consideration the inherited genetic characteristics depending on life stage, dietary preferences and health status ***(Fenech et al., 2011)***.