Molecular Genetics and Metabolism Reports 1 (2014) 158–169

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/molecular-genetics-and-metabolism-reports/

Differential hexosamine biosynthetic pathway gene expression with type 2 diabetes

Megan Coomer, M. Faadiel Essop *

Cardio-Metabolic Research Group (CMRG), Department of Physiological Sciences, Stellenbosch University, Stellenbosch 7600, South Africa

ARTICLE INFO

Article history:
Received 19 March 2014
Accepted 19 March 2014
Available online 17 April 2014

Keywords:
Hexosamine biosynthetic pathway
Fasting blood glucose
Pre- and overt diabetes
Leukocytes
Gene expression

ABSTRACT

The hexosamine biosynthetic pathway (HBP) culminates in the attachment of *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) onto serine/threonine residues of target proteins. The HBP is regulated by several modulators, i.e. *O*-linked β-N-acetylglucosaminyl transferase (OGT) and β-N-acetylglucosaminidase (OGA) catalyze the addition and removal of *O*-GlcNAc moieties, respectively; while flux is controlled by the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFPT), transcribed by two genes, *GFPT1* and *GFPT2*. Since increased HBP flux is glucose-responsive and linked to insulin resistance/type 2 diabetes onset, we hypothesized that diabetic individuals exhibit differential expression of HBP regulatory genes. Volunteers (n = 60; n = 20 Mixed Ancestry, n = 40 Caucasian) were recruited from Stellenbosch and Paarl (Western Cape, South Africa) and classified as control, pre- or diabetic according to fasting plasma glucose and HbA1c levels, respectively. RNA was purified from leukocytes isolated from collected blood samples and *OGT*, *OGA*, *GFPT1* and *GFPT2* expressions determined by quantitative real-time PCR. The data reveal lower *OGA* expression in diabetic individuals ($P < 0.01$), while pre- and diabetic subjects displayed attenuated *OGT* expression vs. controls ($P < 0.01$ and $P < 0.001$, respectively). Moreover, *GFPT2* expression decreased in pre- and diabetic Caucasians vs. controls ($P < 0.05$ and $P < 0.01$, respectively). We also found ethnic differences, i.e. Mixed Ancestry individuals exhibited a 2.4-fold increase in *GFPT2* expression vs. Caucasians, despite diagnosis ($P < 0.01$). Gene expression of HBP regulators differs between diabetic and non-diabetic individuals, together with distinct ethnic-specific gene profiles. Thus differential HBP gene regulation

Abbreviations: HBP, hexosamine biosynthetic pathway; *O*-GlcNAc, *O*-linked β-*N*-acetylglucosamine; OGT, *O*-linked β-N-acetylglucosaminyl transferase; OGA, β-N-acetylglucosaminidase; GFPT, glutamine:fructose-6-phosphate amidotransferase.

* Corresponding author.
E-mail address: mfessop@sun.ac.za (M.F. Essop).

http://dx.doi.org/10.1016/j.ymgmr.2014.03.003

may offer diagnostic utility and provide candidate susceptibility genes for different ethnic groupings.

1. Introduction

The hexosamine biosynthetic pathway (HBP) culminates in the attachment of *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) onto target proteins. *O*-GlcNAcylation is a dynamic and reversible post-translational modification where *O*-GlcNAc moieties are attached to the hydroxyl groups of serine/threonine residues of target cytoplasmic and nuclear proteins [1]. The HBP usually functions as a nutrient sensor under normal physiological conditions and plays a fundamental role in modulating intracellular signaling and gene transcription [2,3]. Crosstalk between phosphorylation and *O*-GlcNAcylation is abundant since there is a competition for similar binding sites on target proteins [3,4]. However, while phosphorylation is controlled by a multitude of kinases and phosphatases, *O*-GlcNAcylation is regulated by only two known enzymes, *O*-linked β-N-acetylglucosaminyl transferase (OGT) and β-N-acetylglucosaminidase (OGA) that catalyze the addition and removal of *O*-GlcNAc moieties, respectively [5,6]. Regulation of HBP flux is also controlled by the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFPT; or sometimes referred to as GFAT) that catalyzes the reaction of glucosamine with fructose-6-phosphate to form glucosamine-6-phosphate [7,8]. There are two GFPT isoforms – *GFPT1* and *GFPT2* – that are transcribed by separate genes with differing tissue distributions [9] raising the possibility of distinct roles.

It is well established that enhanced HBP activation – by augmented intracellular oxidative stress – is robustly linked to hyperglycemia and insulin resistance, two hallmarks of type 2 diabetes [10,11]. In support, recent clinical studies linked greater HBP flux and subsequent upregulation of global *O*-GlcNAcylation to the onset of type 2 diabetes [12,13]. Furthermore, a single mutation in the *OGA* gene (also referred to as *MGEA5*) of a Mexican-American population resulted in the early termination of *OGA* translation leading to decreased expression and increased susceptibility to diabetes [14]. OGT dysregulation is also implicated in the onset of insulin resistance. For example, hepatic OGT overexpression impairs the expression of insulin-responsive genes and causes insulin resistance and dyslipidemia [15]. In support, OGT can trigger hepatic gluconeogenesis thus confirming the importance of the HBP in the development of glucose intolerance [16]. Since *O*-GlcNAcylation of target proteins occurs in a glucose-responsive manner [12,13] and persistently higher HBP flux is strongly associated with the development of insulin resistance/type 2 diabetes [11,17], diabetic individuals are likely to display differential HBP gene expression. However, there are very limited clinical studies that investigated gene expression of HBP regulators with the development of type 2 diabetes, and to our knowledge none that examined *OGT* and *OGA* mRNA levels within this context. As we previously found greater leukocyte *O*-GlcNAcylation with the onset of type 2 diabetes and since all the HBP regulatory genes are known to be expressed in white blood cells [13,18–20], we here hypothesized that the *OGA*, *OGT*, *GFPT1*, and *GFPT2* genes are differentially expressed in leukocytes isolated from pre-diabetic and diabetic individuals compared to matched controls. Thus the key objective of this study is to focus on gene expression analysis of various HBP modulators in order to determine whether any variability can be exploited to assist with type 2 diabetes detection.

2. Materials and methods

2.1. Participant recruitment

Study participants (n = 60; n = 20 Mixed Ancestry, n = 40 Caucasian) were recruited from two neighboring metropolitan regions, namely Stellenbosch and Paarl (Western Cape, South Africa). All recruited participants were personally informed about the study and were requested to sign a written consent form detailing the study aims and procedures. This study was approved by the Committee for Human Research at Stellenbosch University (reference number: S12/03/074) and was conducted according to the ethical guidelines and principles of the International Declaration of Helsinki, the Medical Research