Molecular and Cellular Endocrinology 391 (2014) 41–49

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Blood microRNA profile associates with the levels of serum lipids and metabolites associated with glucose metabolism and insulin resistance and pinpoints pathways underlying metabolic syndrome
The cardiovascular risk in Young Finns Study

Emma Raitoharju [a,*], Ilkka Seppälä [a], Niku Oksala [a,b], Leo-Pekka Lyytikäinen [a], Olli Raitakari [c,d], Jorma Viikari [e], Mika Ala-Korpela [f,g,h,i], Pasi Soininen [f,g], Antti J. Kangas [f,g], Melanie Waldenberger [j], Norman Klopp [j,k], Thomas Illig [j,k], Jaana Leiviskä [l], Britt-Marie Loo [l], Nina Hutri-Kähönen [m], Mika Kähönen [n], Reijo Laaksonen [a], Terho Lehtimäki [a]

[a] Department of Clinical Chemistry, Pirkanmaa Hospital District, Fimlab Laboratories, University of Tampere, School of Medicine, Tampere, Finland
[b] Division of Vascular Surgery, Department of Surgery, Tampere University Hospital, Tampere, Finland
[c] Department of Clinical Physiology and Nuclear Medicine, University of Turku and Turku University Hospital, Turku, Finland
[d] Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku and Turku University Central Hospital, Turku, Finland
[e] Department of Medicine, University of Turku and Turku University Hospital, Turku, Finland
[f] Computational Medicine, Institute of Health Sciences, University of Oulu, Oulu, Finland
[g] NMR Metabolomics Laboratory, School of Pharmacy, University of Eastern Finland, Kuopio, Finland
[h] Oulu University Hospital, Oulu, Finland
[i] Computational Medicine, School of Social and Community Medicine and the Medical Research Council Integrative Epidemiology Unit, University of Bristol, Bristol, UK
[j] Research Unit of Molecular Epidemiology, Helmholtz Zentrum, German Research Center for Environmental Health, Munich, Germany
[k] Hannover Unified Biobank, Hannover Medical School, Hannover, Germany
[l] Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki and Turku, Finland
[m] Department of Pediatrics, University of Tampere and Tampere University Hospital, Tampere, Finland
[n] Department of Clinical Physiology, Tampere University Hospital, School of Medicine, University of Tampere, Tampere, Finland

ARTICLE INFO

Article history:
Received 30 January 2014
Received in revised form 27 March 2014
Accepted 22 April 2014
Available online 28 April 2014

Keywords:
MicroRNA
Metabolic syndrome
Diabetes
MRNA expression
NMR metabolomics

ABSTRACT

Since metabolic syndrome (MetS) is a collection of cardiovascular risk factors involving multiple signaling systems, we related the metabolic abnormalities associated with MetS with circulating microRNA profiles to pinpoint the affected signaling pathways. The blood microRNA profile, genome wide gene expression and serum NMR metabolomics were analyzed from 71 participants of the Young Finns Study.

We found nine microRNAs that associated significantly with metabolites connected to MetS. MicroRNA-144-5p concentration correlated with glucose levels, hsa-1207-5p with glycosylated hemoglobin and hsa-miR-484 with metabolites related to insulin resistance. Hsa-miR-625-3p correlated with cholesterol levels, hsa-miR-1237-3p and hsa-miR-331-3p expression with certain fatty acids levels and hsa-miR-129-1-3p, -129-2-3p, and -1288-3p with glycerol levels. The down-regulated targets of miR-1207-5p and -129-2-3p were enriched in PI3K and MAPK pathways and 8 out of the 12 enriched pathways were down-regulated in individuals with MetS. In conclusion microRNAs associated with several aspects of MetS, possibly regulating glucose and lipid metabolism.

1. Introduction

Metabolic syndrome (MetS) is a cluster of risk factors for cardiovascular diseases (CVD) and type 2 diabetes mellitus (T2D) (Eckel et al., 2010). Irrespective of the several definitions, MetS generally involves various combinations of dyslipidemia (hypertriglyceridemia [TG], low high-density lipoprotein [HDL] cholesterol), hypertension, peripheral insulin resistance, and increased waist circumference, body mass index (BMI), or waist-to-hip ratio (WHR) (Alberti et al., 2005). The prevalence of the factors contributing to MetS is high and ever-growing. It has been estimated that one third of adult Americans and 20–30% of Europeans meet the

* Corresponding author. Address: Clinical Chemistry, Pirkanmaa Hospital District, Biokatu 8, Tampere, P.O. Box 2000, 33521 Tampere, Finland. Tel.: +358 331174052; fax: +358 331174168.
E-mail address: emma.raitoharju@uta.fi (E. Raitoharju).

http://dx.doi.org/10.1016/j.mce.2014.04.013
0303-7207/

MetS criteria. Although the existence of MetS as a specific syndrome as opposed to a collection of individual risk factors is debatable, it can be used as an umbrella term for metabolic and physiological abnormalities that increase the risk of CVDs and T2D.

MicroRNAs (miRNAs, miRs) are small non-coding regulatory RNAs. They primarily regulate their target gene expression at the post-transcriptional level by inhibiting translation or causing degradation of the target messenger RNA (mRNA) (Silvestri et al., 2009). MicroRNAs can also be transported between cells and tissues via circulation. Both membrane-free microRNAs stabilized by proteins (Wang et al., 2010; Arroyo et al., 2011); and microRNAs associated with lipid vesicles can be found in blood. Circulating microRNAs have also been found in high-density lipoprotein (HDL) (Kosaka et al., 2010; Vickers et al., 2011). These circulating microRNAs have been shown to participate in cell-to-cell communication (Valadi et al., 2007); potentially contributing to disease progression. These findings support the potential role of microRNAs in systemic diseases involving the dysfunction of several organs. Humans are predicted to have 2579 mature microRNAs (mirBase, release 20) that can control several genes, and individual genes can be bound by several microRNAs—microRNAs can thus establish wide regulatory networks affecting several processes.

MicroRNAs have been widely studied in cancers, cardiovascular diseases, cholesterol metabolism, and diabetes (Lujambio and Lowe, 2012; Latronico et al., 2008; Vickers and Remaley, 2010; Raitoharju et al., 2013;Guay and Regazzi, 2013). MicroRNA-33 in particular has been shown to have a crucial role in cholesterol metabolism and a potential also in clinical use (Rayner et al., 2011). Interestingly miR-143 and -24 have been associated to increased incidence of MetS in Chinese population through polymorphisms in miRNA binding sites in APOL6 3'UTR, connecting these microRNAs to lipid metabolism (Ye et al., 2013). In addition miR-24 and miR-126 have been associated to fatty acid metabolism (Liang et al., 2013) while miR-204-5p associates with the oxidation of fatty acids (Civelek et al., 2013). MicroRNAs in metabolism and metabolic disorders are reviewed by Rottiers et al. indicating an important regulatory role of microRNAs in the key organs involved in lipid and glucose metabolism (Rottiers and Naar, 2012). Serum circulating microRNA profile has been shown to be dysregulated in prepubertal obese children (Koch, 2013) and Pescador et al. have predicted that some serum miRNAs could be used as potential predictive tools for obesity and type 2 diabetes (Pescador et al., 2013). With regard to human metabolic syndrome (MetS), seven candidate microRNAs in peripheral blood have been found to associate with the risks involved in the manifestation of MetS (Karolina et al., 2012) and let-7g and miR-221 have been shown to be up-regulated in women with MetS (Wang et al., 2013).

MicroRNAs have also proven powerful tools in finding disease-associated pathways. For example, researchers have been able to identify Dupuytren's-contracture-related pathways in a sample set containing only 29 well-characterized patients by using blood microRNA profiling (Mosakhani et al., 2010). These results were later confirmed in a genome-wide association study (GWAS) involving thousands of patients (Dolmans et al., 2011). A recent GWAS on MetS and its components in 4 independent Finnish cohorts suggested that genes from lipid metabolism pathways play a key role in the genetic background of MetS. However, no gene variant with a genome-wide significant association with MetS as an entity could be identified by using this method (Kristiansson et al., 2012). Therefore, new more sensitive tools for the identification of related pathways for different diseases are needed.

We hypothesized that the components of MetS (harmonized criteria) and the previously MetS-associated metabolic traits (Würtz et al., 2012a) correlate with peripheral blood microRNA expression profiles. To reflect this, we (1) compared the microRNA profiles of individuals from the general population with and without MetS or its components; (2) correlated blood microRNA expression with nuclear magnetic resonance (NMR) metabolomics as well as molecular and physiological features previously associated with MetS; (3) analyzed the expression of predicted microRNA targets found to be associated with MetS features; and (4) studied whether these microRNA targets are involved in known biological pathways and whether these pathways are dysregulated in individuals with MetS. The rationale was that the analysis could support the notion of microRNA being involved in the systemic organ dysfunction characteristic of MetS.

2. Materials and methods

2.1. Young Finns Study

The Cardiovascular Risk in Young Finns Study (YFS) is a large multicenter follow-up study on cardiovascular risk from childhood to adulthood. The YFS was launched in 1980, with over 3500 children and adolescents (3–18 years old) from all around Finland participating in the baseline study (Raitakari et al., 2008). Thereafter, the subjects have been followed with several examinations including comprehensive risk factor assessments. The 30-year follow-up was performed in 2011–2012, with 2046 adults, now aged 33–48 years, participating in the study. The examinations have included comprehensive data collection using questionnaires, physical measurements, and blood tests. The present study has been approved by the Ethical Review Committee of Turku University Hospital. All study subjects have given an informed consent and the study has been conducted according to the principles of the Declaration of Helsinki. For the current study, 72 individuals were selected independently of their metabolic status or variables related to that and samples were a part of two case/control studies, not focusing on metabolic status or diabetes. The subpopulation however does not precisely represent the whole Young Finns Study, as the selected cases are older and have higher percentage of diabetes and metabolic syndrome than the whole YFS population. The demographics of the study population used in the final microRNA analysis are presented in Table 1.

2.2. Anthropometry and blood pressure

Weight was measured with a Seca weighing scale to the nearest 0.1 kg and height with a Seca anthropometer to the nearest centimeter. BMI was calculated as weight(kg)/(height(m))^2. Waist circumference was measured using an anthropometric tape at the midpoint between the iliac crest and the lowest rib to the nearest 0.1 cm. The average of two measurements was used. Blood pressure was measured three times after a 5-min rest with a random zero sphygmomanometer. Blood pressure was estimated as the average of the three measurements of systolic and diastolic blood pressure.

2.3. Blood samples

Venous blood samples were drawn from the right antecubital vein after an overnight fast, and serum was separated, aliquoted, and stored at −70 °C until analysis. The serum triglyceride concentration was assayed using the enzymatic glycerol kinase–glycerol phosphate oxidase method (Triglyceride reagent, Beckman Coulter Biomedical). Serum total cholesterol levels were measured by the enzymatic cholesterol esterase–cholesterol oxidase method (Cholesterol reagent, Beckman Coulter Biomedical). The same reagent was used for estimating HDL cholesterol levels after the precipitation of LDL and VLDL with dextran sulfate-Mg^{2+}. Serum glucose