Metabolomics and Transcriptomics of Metabolic Disorders

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Abstract The current prevalence of metabolic diseases such as obesity and type 2 diabetes mellitus (T2DM) is increasing not only due to genetic causes, but also because of lifestyle factors such as lack of physical exercise and consumption of high fat and high glycemic index diets, being one of the most serious public health problems around the world. Different omic approaches such as metabolomics or transcriptomics have revolutionized the quantitative analysis of an organism's state, linking cellular pathways to biological mechanisms, and could be applicable for identifying metabolic markers, which could be useful as diagnostic tools in a personalized healthcare setting. It may also open the door to novel biomarker discovery, disease diagnosis, and novel therapeutic avenues. This review covers the metabolomics and transcriptomic studies analyzing different biomarkers involved in the development of obesity and T2DM.

Keywords Obesity · Type 2 diabetes · Omics · Transcriptome · Metabolome

Introduction

Obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome are multifactorial diseases of considerable heterogeneity [1]. However, whereas obesity and T2DM diagnostic criteria are clearly defined, this is not the case for metabolic

syndrome. Different definitions of metabolic syndrome have been proposed since 1998, when WHO defined this disease as a set of the presence of any type of diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or insulin resistance, and two additional symptoms such as hypertension, dyslipidemia, central obesity and/or microalbuminuria [2]. Different organizations such as International Diabetes Federation, European Group for the Study of Insulin Resistance, and American Heart Association have also given a definition for metabolic syndrome, and although they differ in details, all agree on the essential components. Currently, the criteria represent a combination of metabolic perturbations including central obesity, insulin resistance, dyslipidemia characterized by high triglyceride and low high-density lipoprotein concentration, and hypertension.

The emerging epidemic of metabolic disorders around the world cannot be ignored. In 2010 more than 1.5 billion adults in the world were overweight, with nearly 500 million of them being obese [3, 4]. Recent estimates predict there will be more than 400 million individuals with T2DM worldwide by the year 2030 [5]. Moreover, despite the differences between definitions, U.S. estimates show that the prevalence of metabolic syndrome among adults ranges from 34.3 % to 38.5 % depending on the criteria used [6]. Sedentary lifestyles and the high intake of foods rich in fat and sugar have raised the prevalence of these pathologies, making them the most serious public health problems around the world [7].

Due to the increasing prevalence of metabolic diseases, the identification of molecular biomarkers for assessing the risk of obesity, T2DM, and metabolic syndrome would offer a better, non-invasive way to predict patient risk and to prevent metabolic diseases in humans. Molecular biomarkers have been defined as biomarkers that can be discovered using basic and acceptable platforms such as genomics and metabolomics. This review covers the transcriptomics and metabolomics studies analyzing different biomarkers involved in the development of metabolic diseases such as obesity and T2DM.

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Thus, identifying metabolic markers and pathways of disease-associated intermediate phenotypes is very important, as these biomarkers could be applicable as diagnostic tools in a personalized healthcare setting and may also open the door to novel biomarker discovery, disease diagnosis, and novel therapeutic avenues.

Metabolomics technologies

Metabolomics is a systematic analysis of low molecular-weight biochemical compounds in a biological cell, tissue, organ, or organism, and it has been increasingly applied to discovering biomarkers, identifying perturbed pathways, diagnosing diseases, and measuring the response to treatment [8]. Metabolic profiling can give an instantaneous snapshot of the physiology of the organism and can be seen as the gap between genotype and phenotype [4]. Moreover, metabolomics brings the opportunity to discover new biomarkers important for identifying and stratifying diseases, predicting their progression, and determining the effectiveness, safety and dosage of therapeutic interventions [4].

Nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy (MS) are the two most used high-throughput tools in metabolomics studies, and both can provide complementary snapshots of the metabolome of body fluids [9]. However, the combination of both techniques has the advantage of complementary analytical outcomes and validation of the results obtained separately.

Biomarkers and metabolomics studies in metabolic disorders

Obesity

Obesity is a disorder of the whole body involving metabolic changes, but the actual alterations in metabolism during obesity and any dysfunction associated with this disease at the level of individual organs or cellular organelles are not yet clearly understood [10]. In recent years different studies have been published identifying many obesity-related metabolites implied in several pathways clarifying the metabolic intricacies of obesity (Table 1).

Glucose is the most important source of energy for most organisms, and it is known that glucose metabolism is altered in obesity. Thus, higher blood lactate levels in obese humans have been found [11]. Lactate is also the major precursor for gluconeogenesis in the body, and the rise in plasma lactate in obesity may be an indicator of perturbation of hepatic glucose production and hepatic lipid synthesis. Thus, lactate is an important obesity-related metabolite identified by metabolomics [12•].

Lipids are another important source and store of energy for metabolism, and different studies have observed changes in lipid metabolism due to obesity. Pietiläinen et al. [13] used

Ultraperformance liquid chromatography/Mass spectrometry (UPLC/MS) technology to investigate whether acquired obesity was associated with changes in global serum lipid profiles independent of genetic factors in young adult monozygotic twins. This study revealed an increase in lysophosphatidylcholines (lysoPCs) and a decrease in diethyl ether phospholipids related to obesity, independent of genetic influence. According to these results and with the same technology, Kim et al. [14] identified three lysoPCs in plasma as potential obesity biomarkers comparing overweight/obese and normal-weight men. Especially, overweight/obese subjects showed higher levels of lysoPC C14:0 and lysoPC C16:0, and lower levels of lysoPC C18:1. Obesity is also associated with elevated levels of free fatty acids (FFAs) [15] in the bloodstream; compared to conventional targeted analysis, metabolomics can provide a more unbiased model of the relationship of FFAs to other small molecules, which may help to understand obesity. For example, higher levels of stearic acid, total saturated fatty acids, palmitoleic acid, and lower linoleic acid levels, as well as the variation of amino acids and carnitine in plasma, were detected in overweight/obese men comparing to normal-weight men [14]. In a more recent study, Oberbach et al. [16] showed that obese individuals had higher levels of glycerolPC 42:0 and lower levels of diacylglycerolPC (diacylglycerolPC 32:0, 32:1 and 40:5) with MS/MS technology.

The branched chain amino acids (BCAAs) leucine, isoleucine, and valine are among the nine essential amino acids for humans. The BCAAs play important roles in protein synthesis [17], improve glucose metabolism and oxidation [18], and regulate food intake and the leptin secretion from body fat [19]. Similar to the Oberbach study, Newgard et al. [11] observed differences in the blood amino acid profiles of obese subjects compared with lean subjects. Through metabolic analysis they showed higher levels of a combination of BCAA (leucine/isoleucine and valine), glutamate/glutamine, and the aromatic amino acids phenylalanine and tyrosine in obese individuals than in lean individuals.

Type 2 diabetes mellitus

T2DM is a chronic disease characterized by high blood glucose levels, which may be due to the development of insulin resistance and subsequently the loss of β -cell function. T2DM is related to lifestyle factors including diet, insufficient physical activity, an overweight or obese state, and stress. Whereas the diagnosis and treatment of T2DM have been thoroughly investigated, the identification of novel pathways or early biomarkers related to the development of the disease is still underway.

Comparisons between the levels of various metabolites in diabetic subjects and healthy subjects revealed changes involving different metabolic pathways, as seen in the previous

Table 1 Differentially regulated metabolites in some metabolic disorders

Metabolic disorder	Pathway	Metabolite ¹	Sample	Analytical platform	Reference
Obesity	<i>Glucose metabolism</i>	Lactate (+)	Serum	MS/MS	[1]
		LysoPCs (+)	Serum	UPLC/MS	[2]
	<i>Lipid metabolism</i>	Diethyl ether phospholipids (-)	Plasma	UPLC/Q-TOF MS	[3]
		LysoPC 14:0 (+)			
		LysoPC 16:0 (+)			
		LysoPC 18:1 (-)			
		Stearic acid (+)			
		Saturated fatty acids (+)			
		Palmitoleic acid (+)			
		Linoleic acid (-)			
		Carnitine (-)			
		GlycerolPC 42:0 (+)	Serum	MS/MS	[4]
		DiacylglycerolPC 32:0 (-)			
		DiacylglycerolPC 32:1 (-)			
		DiacylglycerolPC 40:5 (-)			
	<i>Amino acid metabolism</i>	BCAA (+)	Serum	MS/MS	[1]
			Plasma	UPLC/Q-TOF MS	[3]
		Phenylalanine (+)	Serum	MS/MS	[1]
			Plasma	UPLC/Q-TOF MS	[3]
		Tryptophan (+)	Serum	MS/MS	[1]
			Plasma	UPLC/Q-TOF MS	[3]
Diabetes	<i>Glucose metabolism</i>	Glutamate/glutamine (+)	Serum	MS/MS	[1]
		Glucose (+)	Plasma	UHPLC/MS/MS2 and GC/MS	[5]
		Acetone (+)			
		Acetoacetic acid (+)			
		β -hydroxybutyrate (+)			
	<i>Lipid metabolism</i>	Phosphatidylethanolamines (+)	Plasma	UHPLC/MS/MS2 and GC/MS	[5]
		Arachidonate (-)			
		Palmitate (+)			
		Linoleate (+)	Plasma	MS/MS	[6]
		Acylcarnitines (+)			
	<i>Amino acid metabolism</i>	BCAA (+)	Plasma	MS/MS, UHPLC/MS/MS2 and GC/MS	[5, 7]
		Phenylalanine (+)			
		Tyrosine (+)			
		Alanine (+)			
		Tryptophan (+)			

¹ (+) and (-) indicate an increase or decrease in relative metabolite concentration, respectively, when comparing metabolic disease with control

PC, Phosphatidylcholine; BCAA; Branch chained amino acids; MS/MS, mass tandem spectrometry; ULPC, ultraperformance liquid chromatography; UHPLC, Ultrahighperformance liquid chromatography; GC, Gas chromatography

section (Table 1). As expected, diabetic individuals exhibited differences in glucose metabolism, showing higher levels of glucose or mannose levels [20]. Moreover, higher levels of acetone, acetoacetate, and β -hydroxybutyrate were observed in T2DM patients [20], indicating ketoacidotic metabolic decompensation.

Alterations in fatty acid metabolism have been extensively studied in patients with diabetes, using different metabolomics technologies such as ultra-high performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS2) or/and gas chromatography/mass spectrometry

(GC/MS). Thus, increased fatty acid levels were observed in T2DM individuals [20] as well as in patients with impaired glucose tolerance [21•]. Shure et al. [20] showed that arachidonate levels are, on average, lower in the diabetes group, while levels of long-chain fatty acids such as linoleate and palmitate are higher. Moreover, Mihalik et al. [22] detailed almost 20 different fatty acids including saturated, monounsaturated, and polyunsaturated fatty acids affected by T2DM, showing higher fasting levels of long-chain saturated and monounsaturated acylcarnitines in T2DM subjects compared with lean controls. It is known that

elevated levels of FFA induce insulin resistance in muscle and liver tissue by decreasing insulin-stimulated glucose uptake and glycogenesis [23]. Although the underlying mechanism is not completely understood, it has been hypothesized that it is related to oxidative stress or inflammatory lipid pathways [15].

As was previously observed in the *Obesity* section, a broad range of amino acids were also affected in diabetic patients, including the BCAA, phenylalanine, tyrosine, alanine, and tryptophan, which occur at higher levels in T2DM individuals in comparison with control subjects [20]. Similarly, a study of Asian and Chinese men demonstrated that perturbations in amino acid metabolism, including BCAA, were associated with insulin resistance [24].

Although a detailed understanding of the pathophysiology of T2DM is important, the identification of early metabolic alterations is a prerequisite for the development of preventive strategies. Thus, there are different studies that attempt to address this issue. Several biomarkers have been proposed as indicators for T2DM risk, such as fasting plasma glucose levels [25] or inflammatory markers [26], but most of them do not realize the complexity of this disease [27]. However, the predictive ability of amino acid levels, including BCAA, was revealed in different studies.

Wang et al. [28•] followed normoglycemic individuals for 12 years in the Framingham Offspring Study; 201 subjects developed diabetes. They observed that fasting plasma concentration of BCAA, tyrosine, and phenylalanine were elevated 12 years before the onset of T2DM and were also linked to a higher diabetes risk. In fact, a combination of three of the amino acids predicted future diabetes with a more than 5-fold higher risk for individuals in the top quartile of the amino acid concentration levels.

Wang-Sattler et al. [29•] also identified novel biomarkers for pre-diabetes in a recently published work. They quantified 140 metabolites in fasting serum samples of 4,297 individuals from the population-based Cooperative Health Research in the Region of Augsburg (KORA) cohort. In this study they determined that glycine, lysoPC (18:2), and acetylcarnitine, which are impaired glucose tolerance-specific metabolites, are significantly related to the pre-diabetes state, showing that low levels of glycine and lysoPC at baseline predict the risk of developing T2DM, even seven years before the onset of the disease. Moreover, those two metabolites were replicated in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cross-sectional study published in 2013 [30].

Metabolomics has revolutionized the quantitative analysis of small molecules, not only linking cellular pathways to biological mechanisms, but also for elucidating the snapshot of human health and/or disease, which could be used to yield molecular targets for therapeutic intervention in a non-invasive manner.

Transcriptomics methodology

The transcriptome is the set of all RNA molecules produced in one or a population of cells, and their quantity, for a specific developmental stage or physiological condition. Understanding the transcriptome is as essential as recognizing the metabolites discussed previously for interpreting the functional elements of the genome, revealing the molecular constituents of cells and tissues, and also for understanding development and disease [31].

The aims of transcriptomics are to catalogue different species of transcripts, to determine the transcriptional structure of the genes, and to quantify the expression levels of each transcripts during development and under different conditions [31]. In this review, we will focus on the changes in expression levels of mRNA in metabolic disorders.

Different technologies have been developed to deduce and quantify the transcriptome including hybridization or sequence-based approaches. However these techniques, although being the most affordable, have many limitations. Recently developed techniques provide novel high-throughput DNA sequencing methods for both mapping and quantifying transcriptomes. This method, termed RNA-Seq (RNA sequencing), has clear advantages over existing approaches and has already been applied to different organisms such as *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, and mouse and human cells [32–34].

Transcriptomic studies of metabolic disorders

Obesity

There is a huge amount of evidence for the genetic basis of obesity studied in different human and animal models [35]. Different independent studies and meta-analyses of existing data from genome-wide association studies have implicated a total of 18 genetic loci as relevant for body weight regulation to date [36]. Moreover, apart from DNA sequence variants, genetic influences are also manifested through differences in gene expression levels in different relevant tissues; the blood transcriptome has also been found to be a valid non-invasive biological readout to generate information of high predictive and diagnostic content [37•] (Table 2).

Ghosh et al. [37•] carried out a whole-genome expression profiling of whole blood from 17 obese subjects (body mass index (BMI) of 30–50 kg/m²) enrolled in the Ottawa Hospital Weight Management Program at the Ottawa Hospital, and 17 well matched lean subjects from the same genetic ancestry (Northern European White), with a BMI $\leq$ the 10th percentile for age and sex and no prior history of having had a BMI > 25th percentile for more than a 2-year consecutive period. Using Affymetrix GeneChip® Human Genome U133 Plus 2.0 Arrays, they detected 12,127 probe sets, among which 374 probe sets were overexpressed (2-fold or greater), and 75

Table 2 Transcriptomic studies in some metabolic disorders

Metabolic disorder	Metabolic Pathway	Transcripts	Sample	Analytical platform	Reference
Obesity	<i>Apoptosis</i>	Caspase 7	Whole blood	Affymetrix GeneChip® Human Genome U133 Plus 2.0 Arrays	[1]
		Fas			[1]
		Interleukin 1-β			[1]
	<i>Ribosome</i>	RP L31			[1]
		RP S7			[1]
		RP S24			[1]
		RP L35			[1]
		RP L7			[1]
	<i>Oxidative phosphorylation</i>	COX6C			[1]
		COX7B			[1]
		COX7C			[1]
		NADH coenzyme Q reductase			[1]
		NDUFB3			[1]
	<i>Cell cycle</i>	BMAL1	PBMC	RT-PCR	[2]
		CRY 1			[2]
		CRY 2			[2]
		PER 2			[2]
Diabetes	<i>Lipid metabolism</i>	ABCA1	PBMC	Whole human genome oligo-microarray	[3]
		GOS2			[3]
	<i>Nucleobase Nucleoside Nucleotide and Nucleic Acid Metabolic Process</i>	ILF2	Peripheral blood	Human exonic evidence-based oligonucleotide array	[4]
		NFATC4			[4]
	<i>Signal Transduction</i>	MAPK11			[4]
		GPX1			[4]

RP, Ribosome protein; COX, Cytochrome oxidase; NDUFB3, NADH dehydrogenase beta subcomplex 3; BMAL1, Brain and muscle Arnt-like protein-1; CRY, Cryptochrome; PER, Period; ABCA1, ATP-binding cassette sub-family A transporter; GOS2, G0/G1 switch 2; ILF2, nuclear factor of activated cells; NFATC4, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1; MAPK11, mitogen-activated protein kinase 11; GPX1, glutathione peroxidase 1

probe sets were underexpressed (2-fold or greater) in the obese samples compared to the leans. These results were subjected to bioinformatics, and the pathway analysis was conducted with Kyoto Encyclopedia for Genes and Genomes (KEGG) metabolic pathway database identifying the 'apoptosis', 'ribosome', and 'oxidative phosphorylation' pathways. Different genes such as Caspase 7, Fas, or Interleukin 1-β related to apoptosis pathway, and/or ribosomal protein (RP) L31, S7, S24, L35, or L7 genes related to ribosome pathway were upregulated in obese subjects. Several genes involved in the mitochondrial process of electron transfer and ATP synthesis such as cytochrome c oxidase (COX) subunits 6C, 7B and 7C, NADH coenzyme Q reductase, and NADH dehydrogenase beta subcomplex 3 (NDUFB3) demonstrated increased expression in the obese cohort, leading

to a significant enrichment of the 'oxidative phosphorylation' pathway in this group.

It is known that adipose tissue, being responsible for the storage of excess energy derived from the diet as fat, secretes a number of adipokines, which clearly exhibit daily variation in animal models and humans [38, 39]. Thus, plasma adiponectin concentrations in non-obese subjects show a 24 h rhythmic cycle, which is absent in obese subjects [40, 41]. In this context, Tahira et al. [42] investigated the association between daytime variations in gene expression related to circadian rhythm and obesity, testing peripheral blood mononuclear cells by Real-time quantitative PCR. They showed that the expression levels of clock genes such as brain and muscle Arnt-like protein-1 (BMAL1), Cryptochrome (CRY) 1 and 2, and Period (PER) 2 were significantly higher during the day compared to those in

the non-obese group. In addition, expression of BMAL1, CRY1, CRY2, and PER2 genes in blood cells increased between 12:00 and 21:00 in the obese group, suggesting a contribution of the chronotypes to fat accumulation in humans.

Type 2 diabetes mellitus

Many clinical and epidemiological studies have been carried out to elucidate the causalities of T2DM [43, 44]. However, previous studies focused on gene expression in T2DM have been restricted to adipose tissue and muscle or to animal models with limited sample size [45•]. Considering this review's objective of analyzing different biomarkers in a non-invasive way and the difficulty of obtaining permission to use human tissue samples such as liver, adipose, or muscle, the peripheral blood could be a convenient sample source for genetic studies [45•] (Table 2).

Mao et al. [45•] performed a whole human genome oligo-microarray of peripheral white blood cells from 60 healthy and 84 T2DM subjects and found 30 differentially expressed genes in T2DM patients in comparison with healthy controls. They identified ATP-binding cassette sub-family A transporter (ABCA1) and G0/G1 switch 2 (GOS2), which are reported to be associated with lipid metabolism and T2DM in gene knockout cell lines and/or animal models [46–48], as new genetic markers in human for diagnosis and pharmaceutical drug design.

Previously, Grayson et al. [49] performed a transcriptomic study on peripheral blood cells of type 2 diabetic patients, using Human Exonic Evidence-Based Oligonucleotide array. After the array analysis with Gene Ontology, they reported an increased expression of genes associated with activation, signaling, and function of T cells. One of the sets, named Nucleobase Nucleoside Nucleotide and Nucleic Acid Metabolic Process, included the overexpression of the nuclear factor of activated cells (ILF2), which modulates IL-2 expression, and the nuclear factor of activated T-cells and cytoplasmic calcineurin-dependent 1 (NFATC4), a gene involved in the inducible expression of cytokines. The Signal Transduction gene set includes the mitogen-activated protein kinase 11 gene, MAPK11, which encodes a protein activated by pro-inflammatory cytokines and is over-expressed in this gene set, along with glutathione peroxidase 1 (GPX1). These results reflected the possibility that components of the adaptive immune system may contribute to the pathogenesis of T2D.

Transcriptomic analysis is improving constantly with new technologies, better suited analysis and data interpretation tools, and by its high level of customization and automation. However, there are still technical and biological problems to be solved before changes in mRNA levels can be taken as predictive for human health or disease.

Concluding remarks

Currently the incidence of metabolic diseases worldwide is alarming. Sedentary lifestyles and the high intake of foods rich in fat and sugars have increased the prevalence of metabolic diseases. In this context, the identification of molecular biomarkers for assessing the risk of obesity, T2DM, and metabolic syndrome is crucial to predict risk and prevent metabolic diseases in humans. This review summarizes different metabolomics and transcriptomic studies focused especially on obesity and T2DM.

Metabolomics has revolutionized metabolic disease research, linking cellular pathways to biological mechanisms and providing a global picture of the organism. From a technology perspective, metabolomics has come along at precisely the right time [50]; however, the different technologies used (MS and NMR spectroscopy) to gather metabolomics data have specific advantages and disadvantages when conducting metabolomic studies. The main advantage of MS is sensitivity, but one of the major weaknesses of MS in metabolomics is the quantification. Moreover, the major weaknesses of MS are the major strengths of NMR spectroscopy. However, the combination of both techniques has the advantage of complementary analytical outcomes and validation of the results obtained separately.

Understanding the transcriptome is essential for interpreting the functional elements of the genome and revealing the molecular constituents of cells and tissues, and also for understanding development and disease [31]. However, different studies have shown that the correlation between mRNA and protein expression, which exerts the effect on the organism, can be low due to post-transcription machinery [51]. In this context, the study of metabolomics is presently considered more realistic and easier to validate and use as a biomarker analysis than transcriptomic analysis. Thus, the metabolic profile exhibits the real surrogate endpoint of obesity and T2DM and seems to be a better approach to show the global physiology snapshot.

However, in order to deep in disease biomarkers research, an integral approach of different omics could be of interest. Thus, combining metabolomic and transcriptomic data by bioinformatics approaches could show not only the obvious effect of the mRNA levels on the metabolites present in the blood, but also the effects of these metabolites on different gene expression levels in the context of a metabolic disease.

Compliance with Ethics Guidelines

Conflict of Interest Laura Paternain and Javier Campion declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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