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Nuclear receptors expression chart in peripheral blood mononuclear cells identifies patients with Metabolic Syndrome

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ABSTRACT

Background: Nuclear receptors are a class of 48 ligand-activated transcription factors identified as key players of metabolic and developmental processes. Most of these receptors are potential targets for pharmacological strategies in the Metabolic Syndrome. In the present study, we analyzed changes in the mRNA expression of nuclear receptors in the peripheral blood mononuclear cells of patients with Metabolic Syndrome, in order to identify novel biomarkers of disease and candidate targets for putative therapeutical approaches. *Methods and results:* We enrolled thirty healthy controls (14 M:16 F) and thirty naïve patients (16 M: 14 F; >3 criteria for Metabolic Syndrome upon *Adult Treatment Panel III*) without organ damage. Using quantitative real-time PCR, we assessed the expression patterns of nuclear receptors in peripheral blood mononuclear cells. 33/48 nuclear receptors were expressed in peripheral blood mononuclear cells. In patients with Metabolic Syndrome, we found a significant down-regulation of the entire PPAR, NR4A and RAR families, together with a repression of *RXRα*, *VDR*, and *Rev-Erbα*. Furthermore, we performed a novel statistical analysis with classification trees, which allowed us to depict a predictive core of nuclear receptor expression patterns characterizing subjects with Metabolic Syndrome. Random Forest Analysis identified *NOR1* and *PPARδ*, which were both reduced in peripheral blood mononuclear cells and specifically in $CD14^+$ cells (mostly monocytes), as classifiers of Metabolic Syndrome, with high specificity and sensitivity. *Conclusions:* Our results point to the use of PPAR and NR4A mRNA levels in the overall peripheral blood mononuclear cells as biomarkers of Metabolic Syndrome and *bona fide* putative targets of pharmacological therapy.

1. Introduction

Nuclear receptors (NRs) are a superfamily of ligand-dependent and -independent transcription factors (48 in humans and 49 in mice) [1]. NRs are intracellular sensors of different natural and/or synthetic ligands, including hormones, leukotrienes, prostaglandins, and nutritional compounds (e.g. dietary lipids, xenobiotics, and drugs) [1]. When ligands are unknown, the NRs are called "orphans". When NRs are ligand-independent, they are called "true orphans" [1]. NRs modulate coherent pathways involved in essential functions for the body, including reproduction, development, cell growth and differentiation, immune function, metabolic homeostasis, at a transcriptional level [1]. Indeed, the possibility to easily pharmacologically or nutritionally modulate the NR function highlights their value as promising pharmacological targets in different conditions, including Metabolic Syndrome (MS), cardiovascular disease and cancer [2]. NRs are involved in the regulation of a wide array of metabolic processes, ranging from fatty acid synthesis and oxidation, cholesterol and bile acid metabolism, to glucose homeostasis [2,3]. Changes in NR expression and activity are also associated with major metabolic disease, including MS [4].

MS constitutes a cluster of risk factors for an increased mortality, and includes abdominal obesity, blood hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-c) and abnormal glycemia [5,6]. Complications include diabetes mellitus type 2 and atherosclerosis, which lead to coronary artery disease (CAD) and cerebral strokes, and increased cancer risk [5,6]. The prevalence of the MS and of its complications is rapidly increasing in industrialized countries due to Western life-style [5,6]. Thus, the study of the molecular pathways involved in the development and progression of MS and its complications is

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mandatory. To date, atherosclerosis is considered secondary to complex cellular modifications involving the control of metabolism and inflammation; these imbalances result in the initiation of the pathophysiological events leading to the formation of the atheroma [7]. In this view, the inflammatory cells play a pivotal role in modulating all the pathophysiological events that drive the formation of the atheroma, since they promote the backward flux of excess cholesterol from peripheral cells to the liver for subsequent elimination, via the anti-atherogenic lipoproteins HDLs (namely Reverse Cholesterol Transport, RCT) [8,9]. Native HDL particles acquire cholesterol effluxed from peripheral cells, through the ATP-binding cassette (ABC) transporters A1 (ABCA1) and G1 (ABCG1) [10–12], that are known target genes of different NRs [2,4].

Several NRs are expressed in monocytes/macrophages and modulate the activity of the immune cells controlling cholesterol loading, and their response to inflammatory stimuli within the arterial wall [13]. As a consequence, NRs coordinate a crosstalk between the circadian rhythms, metabolic and inflammatory pathways, and are able to maintain homeostasis in immune cells (e.g. macrophages and lymphocytes) [13]. This is the case of peroxisome proliferator activated receptors (PPARs), liver X receptors (LXRs) [13], Rev-Erbs [14,15], retinoic acid receptors (RARs) [16,17], vitamin D receptor (VDR) [18–20], and of the NR4A subfamily that includes nerve growth factor IB (NGFIB), nuclear receptor related 1 protein (NURR1), and neuron derived orphan receptor 1 (NOR1) [21–23].

In inflammatory cells, PPARα inhibits the inflammatory responses (reduced expression of interferon γ, *INF-γ*; tumor necrosis factor α, *TNF-α*; tissue factor, *TF*; matrix metallopeptidase-9, *MMP-9*; and platelet-activating factor receptor) [24], thus being beneficial in the pathophysiology of atherosclerosis. Also, PPARγ exerts an anti-inflammatory activity, negatively interfering with nuclear factor κB (*NF-κB*), signal transducer and activator of transcription (*STAT*), and activator protein-1 (*AP-1*) signaling pathways, leading to a reduced expression of pro-inflammatory genes encoding interleukins and other inflammatory mediators (e.g. *IL-2*, *IL-6*, *IL-8*, *TNF-α*, and metalloproteases) [25]. PPARα and PPARγ are also known to promote cholesterol efflux (inducing ABCA1, ABCG1, the scavenger receptor CLA-1/SR-BI, and Niemann–Pick type C1 and C2-NPC1 and NPC2), thus reducing intracellular lipid accumulation and promoting RCT [24,26–30]. In addition, PPARγ activation promotes the uptake of oxidized low-density lipoprotein (oxLDL), via transcriptional induction of the scavenger receptor cluster of differentiation 36 (CD36), with subsequent differentiation of the macrophages to foam cells [13]. PPARδ is the emerging player in the modulation of inflammation and atherosclerosis. The activation of PPARδ increases the *ABCA1* expression and promotes the apolipoprotein A-I (ApoA-I)-dependent cholesterol efflux [31]. Moreover, PPARδ agonists have been shown to inhibit the expression of *TNF-α*, *IL-6* and vascular cell adhesion protein 1 (*VCAM-1*), thus leading to reduced inflammation [32].

Many other NRs have been proposed as modulators of the mechanisms involved in the pathophysiology of atherosclerosis. This is the case of retinoid sensors (RARs and RXRs), the NR4A subfamily, and VDR [18,21,23,33]. In fact, RAR ligands promote macrophage cholesterol efflux by increasing ABCA1 and ABCG1 transcription [16,17,34], while the overexpression of NR4As in human macrophages reduces the uptake of oxLDL (reduced expression of scavenger receptor A -SR-A-, CD36 and CD11b), and inhibits the production of pro-inflammatory cytokines and chemokines (*IL-1β*, *IL-6*, *IL-8*, monocyte chemoattractant protein-1 and macrophage inflammatory protein-1α and -1β), thus leading to reduced foam cell formation [21]. On the other hand, VDR, like PPARs, antagonizes the expression of different pro-inflammatory genes in macrophages [18], and the administration of vitamin D promotes the expression of the anti-inflammatory transforming growth factor (*TGF*)-*β* and *IL-4*, and decreases *INF-γ* and *TNF-α* gene expression [18].

We compared the expression patterns of all members of the NR superfamily in the peripheral blood mononuclear cells (PBMCs) of healthy subjects to those of patients with MS, in order to identify changes in the NR transcriptome developing in an early phase of MS, and to justify most of the pathophysiological events that lead to cardiovascular disease. PBMCs are circulating immune cells mainly constituted by T lymphocytes (≤70%), B lymphocytes (≤15%), natural killer cells (≤10%), monocytes (≤5%), and dendritic cells (≤1%), which have been recently proposed as carriers of genomic biomarkers of different inflammatory diseases [35]. Indeed, PBMCs play a key role in the inflammatory system, and the changes in their gene expression are considered predictors of the whole body inflammatory status in different conditions [35]. Nevertheless, PBMCs contribute to the formation of early atherosclerotic lesions, through the accumulation of cholesterol and the production of inflammatory mediators and cytokines, and their effector molecules are known to accelerate lesion progression [7].

In the present study, we identified different NRs in PBMCs that could be used as candidate biomarkers and putative targets of pharmacological therapy for the treatment of MS.

2. Methods

2.1. Study population

Patient recruitment and clinical, biochemical and instrumental assessment of MS were collected at the *Clinica Medica "A. Murri"* ("Aldo Moro"

Table 1
Clinical characterization of the study population.

Clinical variable	Control	MS	p-Value
n (M:F)	30 (14:16)	30 (16:14)	–
Weight (kg)	66.5 ± 2.2	96.2 ± 3.8	<0.01
BMI (kg/m^2)	22.5 ± 0.4	33.2 ± 0.9	<0.01
WC (cm)	85.8 ± 1.4	110.3 ± 2.3	<0.01
SBP (mm Hg)	112.3 ± 2.1	131.6 ± 2.9	<0.01
DBP (mm Hg)	69.9 ± 1.1	83.6 ± 1.7	<0.01
Glucose (mg/dl)	84.4 ± 1.1	104.0 ± 5.4	<0.01
HbA1c (%)	5.2 ± 0.1	6.1 ± 0.2	<0.01
Insulin (microUI/ml)	6.5 ± 0.4	18.3 ± 2.6	<0.01
HOMA-IR	1.4 ± 0.1	4.6 ± 0.6	<0.01
Total cholesterol (mg/dl)	184.6 ± 5.2	187.4 ± 7.1	NS
HDL-C (mg/dl)	63.3 ± 2.5	42.1 ± 2.2	<0.01
LDL-C (mg/dl)	106.6 ± 4.4	117.3 ± 6.1	NS
TG (mg/dl)	70.5 ± 5.8	156.8 ± 14.9	<0.01
AST (U/l)	26.8 ± 4.4	25.8 ± 3.0	NS
ALT (U/l)	36.0 ± 3.9	55.2 ± 6.0	<0.01
GGT (U/l)	25.3 ± 3.1	58.6 ± 22.3	0.01
ALP (U/l)	64.7 ± 4.0	86.4 ± 8.8	<0.01
BUN (mg/dl)	33.2 ± 1.3	35.3 ± 1.7	NS
Creatinine (mg/dl)	1.0 ± 0.2	0.8 ± 0.03	NS
Uric acid (mg/dl)	3.7 ± 0.2	5.2 ± 0.2	<0.01
Microalbuminuria (mg/l)	14.2 ± 1.7	23.0 ± 4.3	NS
Sodium (mEq/l)	139.0 ± 0.4	139.4 ± 0.4	NS
Potassium (mEq/l)	3.9 ± 0.04	4.1 ± 0.03	<0.01
Magnesium (mg/dl)	1.9 ± 0.04	1.9 ± 0.03	NS
Calcium (mg/dl)	8.9 ± 0.1	9.0 ± 0.1	NS
Ionized calcium (mg/dl)	3.9 ± 0.03	3.9 ± 0.03	NS
Phosphorus (mg/dl)	3.7 ± 0.1	3.6 ± 0.1	NS
ESR (mm/h)	6.3 ± 0.9	13.9 ± 1.9	<0.01
CRP (mg/dl)	0.2 ± 0	1.1 ± 0.4	<0.01
Fibrinogen (mg/dl)	205.3 ± 8.0	301.7 ± 11.7	<0.01
WBC (10^3/μl)	6.0 ± 0.3	7.3 ± 0.3	<0.01
Monocytes (%)	6. 2 ± 0.3	5.7 ± 0.2	NS
Lymphocytes (%)	35.0 ± 1.4	31.7 ± 1.7	NS
Neutrophils (%)	55.2 ± 1.5	58.0 ± 1.6	NS
Basophils (%)	0.5 ± 0.04	0.5 ± 0.1	NS
Eosinophils (%)	3.0 ± 0.3	3.0 ± 0.3	NS
Platelets (10^3/μl)	256.1 ± 11.8	277.3 ± 9.8	NS
25-OH-D (ng/ml)	32.7 ± 0.8	22.6 ± 1.5	<0.01
Cardiovascular risk (Framingham)	0.5 ± 0.2	14.3 ± 2.5	<0.01
Cardiovascular risk (*Progetto Cuore*)	0.2 ± 0.1	4.0 ± 1.0	NS

Data are presented as mean ± SEM. Abbreviations: 25-hydroxyvitamin D, 25-OH-D; alanine transaminase, ALT; alkaline phosphatase, ALP; aspartate transaminase, AST; blood urea nitrogen, BUN; Body Mass Index, BMI; C-reactive protein, CRP; diastolic blood pressure, DBP; erythrocyte sedimentation rate, ESR; gamma-glutamyltransferase, GGT; high-density lipoprotein cholesterol, HDL-c; homeostatic model assessment for insulin resistance, HOMA-IR; low-density lipoprotein cholesterol, LDL-c; non-significant, NS; systolic blood pressure, SBP; triglyceride, TG; Waist Circumference, WC.