

# Metabolomics Approach in Allergic and Rheumatic Diseases

Rossana Scrivo · Luca Casadei · Mariacristina Valerio ·  
Roberta Priori · Guido Valesini · Cesare Manetti

Published online: 18 April 2014  
© Springer Science+Business Media New York 2014

**Abstract** Metabolomics is the analysis of the concentration profiles of low molecular weight compounds present in biological fluids. Metabolites are nonpeptide molecules representing the end products of cellular activity. Therefore, changes in metabolite concentrations reveal the range of biochemical effects induced by a disease or its therapeutic intervention. Metabolomics has recently become feasible with the accessibility of new technologies, including mass spectrometry and high-resolution proton nuclear magnetic resonance, and has already been applied to several disorders. Indeed, it has the advantage of being a nontargeted approach for identifying potential biomarkers, which means that it does not require a preliminary knowledge of the substances to be studied. In this review, we summarize the main studies in which metabolomic approach was used in some allergic

(asthma, atopic dermatitis) and rheumatic diseases (rheumatoid arthritis, systemic lupus erythematosus) to explore the feasibility of this technique as a novel diagnostic tool in these complex disorders.

**Keywords** Metabolomics · Metabolite · Asthma · Atopic dermatitis · Rheumatoid arthritis · Systemic lupus erythematosus · Allergic diseases · Rheumatic diseases · Biomarkers · Mass spectrometry · High-resolution proton nuclear magnetic resonance

## Introduction

Systems medicine is an emerging approach in medicine in the post-genomic era. This is the translation in medicine of the systems biology approach, which overtakes the reductionism and applies methodologies and models developed in different fields of science. Through systems medicine, emerging properties due to the interactions between different elements of the systems may be discovered, and the states of health and disease and their evolution may be fruitfully described. This discipline and the digital revolution are transforming healthcare to a proactive P4 medicine that is predictive, preventive, personalized, and participatory [1]. Indeed, this vision sees the genetic, molecular, cellular, organ, and individual networks integrated to constitute a network of networks. To effectively reach the P4 objectives, the metabolomic approach is crucial, allowing for the detection of the landscape of the pathology characterizing the state of a single patient [2]. The landscape concept is used in different fields to describe system states and trajectories in multidimensional space and to study their dynamical evolution. As in the natural landscape, we can describe position of two valleys and the way to connect them by overcoming peaks; at the same manner, it is possible to describe the folding of a protein in a three-dimensional space

This article is part of the Topical Collection on *Immunologic/Diagnostic Tests in Allergy*

R. Scrivo · R. Priori (✉) · G. Valesini  
Dipartimento di Medicina Interna e Specialità Mediche,  
Reumatologia, Sapienza Università di Roma,  
Viale del Policlinico 155, 00161 Rome, Italy  
e-mail: roberta.priori63@gmail.com

R. Scrivo  
e-mail: rossana.scrivo@uniroma1.it

G. Valesini  
e-mail: guido.valesini@uniroma1.it

L. Casadei · M. Valerio · C. Manetti  
Dipartimento di Chimica, Sapienza Università di Roma,  
Piazzale Aldo Moro 5, 00185 Rome, Italy

L. Casadei  
e-mail: luk.casadei@gmail.com

M. Valerio  
e-mail: mcrivalerio@gmail.com

C. Manetti  
e-mail: cesare.manetti@uniroma1.it

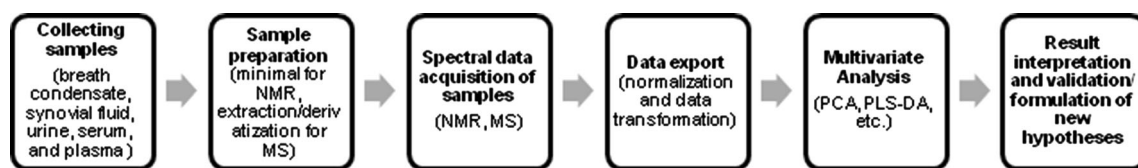


**Fig. 1** The schematic experimental procedure of a typical metabolomic study. *NMR* nuclear magnetic resonance; *MS* mass spectroscopy; *PCA* principal component analysis; *PLS-DA* partial least square discriminant analysis

spanned by the coordinates ( $x$ ,  $y$ ,  $z$ ) of atoms. Using the same metaphor, the state of a patient can be described in a multidimensional space spanned by coordinates derived by multivariate statistical analysis of metabolite levels, also including the pathways induced by treatments [2, 3]. Metabolomics is the analysis of the concentration profiles of low molecular weight compounds present in biological samples. Changes in levels of metabolic intermediates of a sequential series of reactions are often more pronounced than the changes in enzymatic kinetics or individual fluxes. For this reason, metabolomics is considered a sensitive tool for studying genotype-phenotype correlations, as well as the pharmacological and toxicological effects of drugs [4–7]. The landscape obtained is defined by multivariate analysis of spectroscopic data: patients in similar conditions are neighbors, and their movements on the surface/landscape mirror the response to treatment, which can be evaluated in different times in several biological fluids. Mass spectrometry (MS) and high-resolution proton nuclear magnetic resonance ( $^1\text{H}$  NMR) are the two main techniques used in this field, which can be used to acquire the primary experimental information of biological systems. The two methodologies are complementary and not interchangeable: although less sensitive (micromolar range) compared to MS, NMR is not destructive with regard to the specimen, and therefore, the same sample may be analyzed in vivo more than once. On the other hand, MS has a much lower detection limit, but at the cost of a more targeted analysis; it also requires a pretreatment of the sample, which has to be separated in the different components by chromatographic techniques. These include liquid chromatography (LC) and gas chromatography (GC), which need (especially in the case of GC) chemical derivation. For these reasons, MS approaches tend to be less reproducible, more platform-dependent, and susceptible to variability. Conversely, NMR and MS NMR have the advantage of being reproducible, and the sample may be evaluated without any pretreatment [8, 9].  $^1\text{H}$  NMR provides a direct fingerprint of the system status, represented by spectra containing complex metabolic information that can be simplified and explored by chemometric tools. In fact, these spectral data derive from the entire metabolome in biofluids and are extremely complex for the presence of hundreds of low-molecular-weight compounds. For this reason, multivariate analysis is an integrated part of the technique, due to its ability to provide interpretable models for complex intercorrelated data [10]. The use of multivariate

statistical techniques such as principal components analysis (PCA), partial least squares (PLS), and multidimensional scaling (MDS) is of great importance, as these are very effective in deciphering what is going on in the studied system [11]. Figure 1 displays the schematic experimental procedure of a typical metabolomic study.

A recent overview of clinical application of metabolomics presented consolidated results in type 2 diabetes and cancer, as well as in emerging areas including the functional properties of the gut microbiome and the mapping of metabolic phenotypes during surgery [12].

In the present review, we summarize the studies in which metabolomics was used in some allergic (asthma, atopic dermatitis) and rheumatic diseases (rheumatoid arthritis, systemic lupus erythematosus), to explore the feasibility of this technique as a novel diagnostic tool in these complex disorders. In Table 1, we provide the list of the main disease states for which metabolomics has shown promise, while the results of

**Table 1** Main disease states for which metabolomics has shown promise

| Disease                      | Aspect impacted by metabolomics                                                                                                                                              |
|------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Rheumatoid arthritis         | Potential for discriminating patients with different disease activity and predicting the response to a particular treatment; association with risk of cardiovascular disease |
| Spondyloarthritis            | Potential for early diagnosis and differential diagnosis (RA vs PsA)                                                                                                         |
| Systemic lupus erythematosus | Potential for differential diagnosis (especially vs RA)                                                                                                                      |
| Osteoarthritis               | Potential for discriminating patients with different degrees of disease                                                                                                      |
| Asthma                       | Potential for differential diagnosis (especially vs viral infections) and discriminating patients with different degrees of disease                                          |
| Atopic dermatitis            | Potential for early diagnosis                                                                                                                                                |
| Cancer                       | Potential for defining diagnosis and prognosis                                                                                                                               |
| Cardiovascular diseases      | Potential for predicting cardiovascular events; may discriminate between different degrees of stenosis in coronary artery disease                                            |
| Type 2 diabetes              | Potential for predicting the risk of the disease and defining diagnosis                                                                                                      |
| Ocular inflammatory diseases | Potential for diagnosis                                                                                                                                                      |
| Inflammatory bowel disease   | Potential for early diagnosis and targeted treatment                                                                                                                         |

*RA* rheumatoid arthritis, *PsA* psoriatic arthritis

sensitive analysis identifying specific altered metabolic pathways in metabolomic studies in patients with allergic and rheumatic diseases are provided in Table 2.

## Metabolomics in Allergic Diseases

### Asthma

Asthma is a serious health and socioeconomic issue all over the world, affecting over 300 million people worldwide, with an estimated additional 100 million patients by the year 2025 [13]. It is emerging as the most common chronic disease in children and is characterized by airway hyperresponsiveness, obstruction, mucus hyperproduction, and airway wall remodeling [14]. Yet, the diagnosis may be challenging, considering that there are many diseases that mimic asthma. Also, the traditional diagnostic tools are expensive, invasive, and insensitive to small changes in inflammatory status of both physiological (spirometry, peak flow measures) and functional tests (symptoms, quality of life) [15]. Furthermore, while the recruitment of eosinophils in the mucosa of asthmatics is common, a portion of them has a prevalent neutrophilic infiltration, which sustains the recent awareness that asthma is a heterogeneous disease. This may affect therapy, since inhaled corticosteroids act mainly on eosinophils in the lungs [16]. Given these challenges, in the last years, there has been a constant drive to identify key metabolites useful for diagnosis, monitoring, and treatment of asthma, and early studies on metabolomics opened encouraging perspectives for patients [17–20]. Furthermore, since objective measurements of airway inflammation produced superior therapeutic results compared with traditional measures alone [21, 22], metabolomics continues to be investigated also with the aim of improving the outcome of patients. Preliminary studies focused on identifying diagnostic biomarkers in the urine or breath and indeed were able to discriminate subjects with asthma from healthy populations [17–20]. The earliest of these studies was carried out in exhaled breath condensate (EBC), a noninvasive and easy to perform technique, by means of  $^1\text{H}$  NMR spectroscopy [17]. EBC is obtained by cooling exhaled air, and it contains several biocompounds that are believed to reflect airway lining fluid composition [22]. However, since EBC analysis enables only the detection of known metabolites, in this study, metabolomics was used to integrate EBC and allowed the prediction of unknown metabolites and novel biomarkers, providing insight into disease mechanisms. Twenty-five children with controlled allergic asthma and 11 age-matched healthy controls were studied, showing that NMR-based metabolomic analysis could be effectively combined with EBC to characterize asthmatic children. In particular, within the NMR spectrum, some profiles indicative of acetylated and oxidized compounds that significantly

distinguished children with asthma from healthy children were identified, paving the way for the study of new metabolic pathways possibly implicated in asthma pathophysiology [17]. The same authors lately applied metabolomics in EBC to study asthmatic children with different degrees of disease severity, finding that metabolomic profiling enabled a clear separation between children with nonsevere asthma, children with severe asthma, and healthy children. Furthermore, severe asthma phenotype could be fully discriminated, suggesting that this approach may be used to develop new targeted therapies [23••]. Recently,  $^1\text{H}$  NMR spectroscopy was found to discriminate the EBC metabolic profile of a relatively large adult cohort of patients with asthma from healthy controls [24]. However, since different equipments were used, it was not possible to reproduce the results obtained in children.

Most studies were carried out in urine, based on the idea that the effect of airway inflammatory cells on the airways would produce in the body a unique pattern of metabolites cleared by urine excretion. Urine is considered one of the most informative, accessible, and stable body fluids, due to noninvasive collection, low protein and cellular levels, and richness in metabolites. Hence, urine seems to be an ideal biofluid for NMR-based metabolomic investigations. Two of these studies were performed by the same Canadian group, one in murine models and the second in humans [18, 19]. In the former, guinea pigs were considered as a reliable model of asthma [18]: indeed, these animals have an airway physiology similar to that of humans [25] and present allergic airway dysfunction which in part reflects what occurs during a human asthma exacerbation [26]. In particular, five groups of guinea pigs were considered: control, control treated with dexamethasone, sensitized (ovalbumin, administered intraperitoneally), sensitized and challenged (ovalbumin, administered intraperitoneally, plus ovalbumin aerosol), and sensitized challenged with dexamethasone. Using a library of known urine metabolites, the concentrations of 50 metabolites were measured in the urine of all animals by multivariate statistical analysis of NMR spectra, and each group was compared by PLS-DA to discriminate the subtypes of animals. Challenged guinea pigs developed airway hyperreactivity (AHR) and increased inflammation compared with sensitized or control animals.  $^1\text{H}$  NMR spectroscopic analysis of urine could differentiate animals with or without airway inflammation and AHR by a combination of more than one metabolite, with linear discriminant analysis (LDA)-based classification demonstrating 80–90 % separation of the animal groups [18]. These promising results led to investigate the performance of  $^1\text{H}$  NMR analysis in human urine samples [19]. To this purpose, a total of 135 children aged 4–16 years were enrolled, divided into three groups according to their clinical status (stable asthma, unstable asthma, and healthy controls). This study provided evidence that the analysis of excreted urine metabolites measured by  $^1\text{H}$  NMR can be used to differentiate stable asthma from

**Table 2** Human metabolomic studies in allergic and rheumatic diseases

| Disease | Objective of the study                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Participants                                                                                                                                                                                                                                                                | Specimen types   | Analytical methods | Main metabolite associated with pathology                                                                                                                                                                                                               | Study                        |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|
| Asthma  | To assess the feasibility of NMR-based metabolomic analysis applied to exhaled breath condensate                                                                                                                                                                                                                                                                                                                                                                           | <ul style="list-style-type: none"> <li>• 17 children with persistent asthma treated with inhaled corticosteroids</li> <li>• 8 children with intermittent asthma inhaled corticosteroid naïve</li> <li>• 11 healthy children as controls</li> </ul> Carraro et al. 2007 [17] | Exhaled breath   |                    | condensate                                                                                                                                                                                                                                              | <sup>1</sup> H NMR           |
| Asthma  | Presence of acetylated and oxidized compounds<br>To discriminate different asthma phenotypes, with a particular focus on severe asthma in children                                                                                                                                                                                                                                                                                                                         | <ul style="list-style-type: none"> <li>• 31 children with no severe asthma (treated with inhaled steroids or not)</li> <li>• 11 children with severe asthma</li> <li>• 15 healthy children as controls</li> </ul> Carraro et al. 2013 [23]                                  | Exhaled breath   |                    | condensate                                                                                                                                                                                                                                              | LC-MS                        |
| Asthma  | Compounds related to retinoic acid, adenosine and vitamin D were relevant for the discrimination between groups<br>• To determine and validate discriminatory metabolomic profiles in adult asthma<br>• To identify asthmatic subgroups based on sputum eosinophilia, neutrophilia, asthma control, and inhaled corticosteroid use<br>Asthmatics vs control subjects:<br>5 spectral regions: 0.16–0.18 ppm, 0.78–0.84 ppm, 0.88–0.94 ppm, 7.36–7.42 ppm, and 7.44–7.52 ppm | <ul style="list-style-type: none"> <li>• 82 asthmatic patients</li> <li>• 35 healthy controls</li> </ul> Ibrahim et al. 2013 [24]                                                                                                                                           | Exhaled breath   |                    | condensate                                                                                                                                                                                                                                              | <sup>1</sup> H NMR           |
| Asthma  | To assess the feasibility to measure differences between children with asthma exacerbation, children with stable asthma, and healthy children                                                                                                                                                                                                                                                                                                                              | <ul style="list-style-type: none"> <li>• 73 children with stable asthma</li> <li>• 20 children with unstable asthma</li> <li>• 42 healthy children as controls</li> <li>• 41 atopic asthmatic children</li> <li>• 12 controls</li> </ul>                                    | Urine            | <sup>1</sup> H NMR | Metabolites related to citric acid cycle, stress on energy metabolism, protein and amino acids metabolism                                                                                                                                               | Saude et al. 2011 [19]       |
| Asthma  | To identify the most representative metabolites of asthma                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                             | Urine            | LC-MS              | (L) Urocanic acid, methyl-imidazoleacetic acid and a metabolite resembling the structure of an Ile-Pro fragment                                                                                                                                         | Mattarucchi et al. 2011 [20] |
| Asthma  | • To investigate the altered metabolic pattern in sera from patients with asthma<br>• To identify the mechanism underlying asthma and potential biomarkers                                                                                                                                                                                                                                                                                                                 | <ul style="list-style-type: none"> <li>• 39 asthmatic patients</li> <li>• 26 healthy controls</li> </ul>                                                                                                                                                                    | Serum            | <sup>1</sup> H NMR | <ul style="list-style-type: none"> <li>• (T) Methionine, glutamine, and histidine;</li> <li>• (L) Formate, methanol, acetate, choline, O-phosphocholine, arginine, and glucose</li> </ul>                                                               | Jung et al. 2013 [29]        |
| AD      | To explore the possibility of detecting metabolite-level changes correlated with AD in children                                                                                                                                                                                                                                                                                                                                                                            | <ul style="list-style-type: none"> <li>• 20 children with AD</li> <li>• 12 children without any clinical manifestation of AD as controls.</li> </ul>                                                                                                                        | Urine            | <sup>1</sup> H NMR | <ul style="list-style-type: none"> <li>• (T) VLDL/LDL products in severe asthma patients</li> <li>• (T) Creatinine, creatine, citrate, formate, 2-hydroxybutyrate, dimethylglycine, and lactate</li> <li>• (L) Betaine, glycine, and alanine</li> </ul> | Assfalg et al. 2012 [33]     |
| RA      | To profile lipids in synovial fluid of RA patients                                                                                                                                                                                                                                                                                                                                                                                                                         | 5 patients with RA                                                                                                                                                                                                                                                          | Synovial fluid   | LC-MS/MS           | Identification and quantification of MaR1, 5S, 12S-diHETE, RvD5, and LXA <sub>4</sub>                                                                                                                                                                   | Giera et al. 2012 [41]       |
| RA      | To characterize phenotypes of RA patients classified by Chinese medicine expert as cold or heat type according to Chinese medicine theory<br>Wietmarshchen et al. 2012 [42]                                                                                                                                                                                                                                                                                                | 39 RA patients (20 cold and 19 heat type)                                                                                                                                                                                                                                   | Urine and plasma | LC-MS              | Cold vs heat patients:<br>(L) 11 acylcarmines and dehydroepiandrosterone sulfate (DHEAS)                                                                                                                                                                | van                          |
| RA      | To identify biomarkers in the plasma of patients with RA                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                             | Plasma           | <sup>1</sup> H NMR | RA active patients vs healthy controls:                                                                                                                                                                                                                 | Lauridsen et al. 2010 [45]   |

**Table 2** (continued)

| Disease | Objective of the study                                                                                                   | Participants                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Specimen types | Analytical methods | Main metabolite associated with pathology                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Study                   |
|---------|--------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|
| RA      | To assess metabolic fingerprints in serum from patients with established RA and those with early arthritis               | <ul style="list-style-type: none"> <li>• 47 patients with RA (23 with active disease at baseline and 24 in remission)</li> <li>• 51 healthy subjects</li> <li>• 16 patients with established RA who were naive for disease-modifying anti-rheumatic drugs</li> <li>• 14 healthy controls</li> <li>• 2 groups of patients with synovitis (<math>N=89</math> and <math>N=127</math>) of <math>\leq 3</math>-month duration whose outcomes were determined at clinical follow-up</li> <li>• 38 patients with early RA</li> <li>• 20 healthy controls</li> </ul> | Serum          | $^1\text{H}$ NMR   | <ul style="list-style-type: none"> <li>• (†) Cholesterol, lactate, acetylated glycoprotein and unsaturated lipid</li> <li>• (‡) HDL and an unassigned signal</li> </ul> <p>For both groups of patients with synovitis: Lactate and lipids are the metabolites most strongly correlated with inflammation</p>                                                                                                                                                                                                                                                                                                         | Young et al. 2013 [46]  |
| RA      | To identify the major serum biomarkers predicting the response to methotrexate (MTX) treatment in patients with early RA | <ul style="list-style-type: none"> <li>• 16 RA patients</li> <li>• 20 PsA patients</li> <li>• Both groups were evaluated before and during therapy with infliximab or etanercept</li> <li>• 25 patients with RA</li> <li>• 20 patients with PsA</li> <li>• 10 healthy controls</li> <li>• For the validation study were enrolled 14 RA patients and 20 healthy controls</li> </ul>                                                                                                                                                                           | Serum          | $^1\text{H}$ NMR   | <p>For effective group:</p> <ul style="list-style-type: none"> <li>• (†) Uric acid, taurine, glycine, histidine, hypoxanthine, and methionine</li> <li>• (‡) Uracil, TMAO, tryptophan, aspartate, and <math>\alpha</math>-oxoglutarate</li> </ul> <p>Baseline urinary metabolites most strongly correlated with response to anti-TNF therapy:</p> <ul style="list-style-type: none"> <li>• (†) Histamine, glutamine xanthurenic acid</li> <li>• (‡) Ethanolamine</li> </ul>                                                                                                                                          | Wang et al. 2012 [47]   |
| RA      | To determine if the patient's metabolic fingerprint prior to therapy could predict responses to anti-TNF agents          | <ul style="list-style-type: none"> <li>• 16 RA patients</li> <li>• 20 PsA patients</li> <li>• Both groups were evaluated before and during therapy with infliximab or etanercept</li> <li>• 25 patients with RA</li> <li>• 20 patients with PsA</li> <li>• 10 healthy controls</li> <li>• For the validation study were enrolled 14 RA patients and 20 healthy controls</li> </ul>                                                                                                                                                                           | Urine          | $^1\text{H}$ NMR   | <p>Baseline urinary metabolites most strongly correlated with response to anti-TNF therapy:</p> <ul style="list-style-type: none"> <li>• (†) Histamine, glutamine xanthurenic acid</li> <li>• (‡) Ethanolamine</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                            | Kapoor et al. 2013 [48] |
| RA      | To assess the feasibility of diagnosing early RA by measuring selected metabolic biomarkers                              | <ul style="list-style-type: none"> <li>• 25 patients with RA</li> <li>• 20 patients with PsA</li> <li>• 10 healthy controls</li> <li>• For the validation study were enrolled 14 RA patients and 20 healthy controls</li> </ul>                                                                                                                                                                                                                                                                                                                              | Serum          | LC-MS              | <p>RA patients vs healthy controls:</p> <ul style="list-style-type: none"> <li>• (†) Glycemic acid, D-ribofuranose, and hypoxanthine</li> <li>• (‡) Histidine, threonine acid, methionine, cholesterol, asparagine and threonine</li> </ul> <p>RA patients vs PsA patients</p> <ul style="list-style-type: none"> <li>• (†) Glutamine, heptanoic acid, succinate, pseudouridine, inosine, guanosine, arabinol, cysteine, cysteine, and phosphoric acid</li> <li>• (‡) Aspartic acid, glutamic acid, glutamate, histidine, serine, arachidonic acid, cholesterol, threonine acid, and 1-monooleoylglycerol</li> </ul> | Madsen et al. 2011 [49] |
| RA      | To find reliable biomarkers in the synovial fluid to differentiate between septic and nonseptic arthritis                | <ul style="list-style-type: none"> <li>• 59 patients with OA, gout, calcium pyrophosphate disease, spondyloarthritis, septic arthritis, and RA were analyzed</li> <li>• 64 patients with SLE</li> <li>• 30 patients with RA</li> <li>• 35 healthy controls</li> </ul>                                                                                                                                                                                                                                                                                        | Synovial fluid | $^1\text{H}$ NMR   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Hügler et al. 2012 [51] |
| SLE     | To establish a human SLE serum metabolic profile                                                                         | <ul style="list-style-type: none"> <li>• 64 patients with SLE</li> <li>• 30 patients with RA</li> <li>• 35 healthy controls</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                       | Serum          | $^1\text{H}$ NMR   | <ul style="list-style-type: none"> <li>• (‡) Valine, tyrosine, phenylalanine, lysine, isoleucine, histidine, glutamine, alanine, citrate, creatinine, creatine, pyruvate, high-density lipoprotein, cholesterol, glycerol, formate</li> <li>• (†) N-acetyl glycoprotein, very low-density lipoprotein, and low-density lipoprotein</li> </ul>                                                                                                                                                                                                                                                                        | Ouyang et al. 2011 [53] |

**Table 2** (continued)

| Disease | Objective of the study                                                                                                                                                                 | Participants                                                                                                                                                                                                                                                          | Specimen types | Analytical methods | Main metabolite associated with pathology                                                                                                                                                                                                                                                                                                                                                                   | Study                            |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| SLE     | To identify urinary metabolites that discriminated between proliferative and pure membranous lupus nephritis (LN) and between LN and primary focal segmental glomerulosclerosis (FSGS) | <ul style="list-style-type: none"> <li>• 7 patients with proliferative LN without membranous features (class III/IV)</li> <li>• 7 patients with pure membranous LN (class V)</li> <li>• 10 patients with primary FSGS and proteinuria as disease controls.</li> </ul> | Urine          | <sup>1</sup> H NMR | <ul style="list-style-type: none"> <li>• Class V LN had 8-fold lower levels of citrate compared to class III/IV who had normal levels</li> <li>• Class III/IV LN patients had &gt;10-fold lower levels of taurine compared to class V patients who had mostly normal levels</li> <li>• Class V LN patients had normal hippurate levels compared to FSGS patients who completely lacked hippurate</li> </ul> | Romic-Rosendale et al. 2011 [54] |
| SLE     | To search potential disease markers                                                                                                                                                    | <ul style="list-style-type: none"> <li>• 20 SLE patients</li> <li>• 9 healthy controls</li> <li>• 38 SLE patients for validation</li> </ul>                                                                                                                           | Serum          | LC/MS and GC/MS    | (†) Lipid peroxidation products, MDA, gamma-glutamyl peptides, leukotriene B <sub>4</sub> , and 5-HETE                                                                                                                                                                                                                                                                                                      | Wu et al. 2012 [59]              |

(†) and (‡) indicates up- and downregulation of metabolites, respectively

AD atopic dermatitis, RA rheumatoid arthritis, PsA psoriatic arthritis, OA osteoarthritis, SLE systemic lupus erythematosus, <sup>1</sup>H NMR high-resolution proton nuclear magnetic resonance, LC-MS liquid chromatography (LC) mass spectrometry (MS), GC-MS gas chromatography (GC) mass spectrometry

healthy controls and patients with asthma undergoing disease exacerbation, suggesting that metabolomics has the potential to be a useful clinical tool for physicians treating asthma [19]. Finally, in an Italian study, urine samples from 41 asthmatic children and 12 healthy controls were profiled by LC-MS using two models: in the first, asthmatics were compared with healthy controls, while in the second, patients taking controller drugs (mainly corticosteroids) were compared with those who did not [20]. Both models proved to be very efficient. Interestingly, the discrimination of the asthmatics proved to be uncorrelated with possible metabolic effects due to the chronic assumption of controller drugs. Within the dataset of the first model, the most significant difference between asthmatics and healthy controls was given by a significantly reduced concentration of urocanic acid, an intermediate in the catabolism of histidine, in patients' urines. Since this compound is known to be an immune suppressor [27], it was hypothesized that this occurrence can have a negative influence on the resolution of the inflammation process in asthma [20]. Overall, these studies highlight the possible role of urine as a target for metabolomic studies on pulmonary diseases, even if this biofluid is generally considered a suitable target only for systemic disorders or with a direct involvement of the secretory apparatus.

In a very recent study, metabolic changes were directly investigated in the bronchoalveolar lavage fluid (BALF) from murine models with ovalbumin-induced asthma by using an integrated approach combining LC and GC MS. This technology enabled discrimination of diseased animals from healthy control animals, demonstrating substantial alterations in energy, lipid, and sterol metabolism, possibly related to an increased respiratory burden in supplying energy to recruited inflammatory cells and reversible upon treatment with dexamethasone [28].

Finally, another application of <sup>1</sup>H NMR metabolomics was reported in sera from adult patients with asthma: again, clear distinct profiles between patients and healthy subjects were found, and the levels of serum metabolites from patients correlated with disease severity [29•].

In summary, all of these studies show promising results for metabolomics as a novel diagnostic tool for asthma irrespective of the biological specimen used.

## Atopic Dermatitis

Atopic dermatitis (AD) is an inflammatory disorder of the skin representing a major health impact in pediatrics. Indeed, it is a common condition and frequently presents with comorbidities including allergic rhinitis, asthma, and immunoglobulin E-mediated food reactions [30]. The pathogenesis has not been clearly defined, probably due to a high degree of genetic background and pathophysiological heterogeneity, mirrored by the high variability of clinical phenotype severity [31]. This

requires tailored prevention and treatment to optimize the management strategies [31], and therefore, the need for one or more specific biomarkers is a compelling issue [32]. Metabolomics has been investigated as a potential diagnostic tool in AD in a single explorative study to date, in which this analysis was used to explore metabolite-level changes in 20 children aged 6–10 months [33]. The approach consisted of a noninvasive analysis of urine samples by  $^1\text{H}$  NMR spectroscopy, an ideal method when children are involved. The calculation of combined standardized loadings from both the PCA and canonical analysis (CA) transformations was used to analyze the contribution of the original variables in discriminating the children with AD and 12 age-matched children without any clinical manifestation of AD or other known acute or chronic disease [33]. In this study, the application of the unsupervised pattern recognition approach PCA did not allow to identify metabolic features associated with the disease. Conversely, by applying the supervised approach CA, an overt separation was obtained. In particular, the overall analysis demonstrated increased levels of creatinine, creatine, citrate, formate, 2-hydroxybutyrate, dimethylglycine, and lactate in AD urine samples, as well as decreased levels of betaine, glycine, and alanine. These altered metabolic contents may be an indirect consequence of the main pathogenic molecular mechanism underlying the disease. However, the authors recognized that the insufficient level of understanding of AD biochemistry should represent a warning for an unequivocal interpretation of these data, also considering that the genetically heterogeneous nature of the disease may result in different metabolic phenotypes [33].

Therefore, further investigation is needed to ascertain the feasibility of metabolomic analysis to provide new perspectives for the characterization of different clinical phenotypes, early differential diagnosis, and targeted treatment of AD.

## Metabolomics in Rheumatic Diseases

### Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease affecting about 1 % of the population and characterized by systemic inflammation and synovitis in the diarthrodial joints potentially leading to joint destruction [34]. In recent years, two main concerns have gained special attention, both addressing the purpose to ameliorate the outcome of patients: the need to diagnose the disease as early as possible [35] and the capability to predict the response to therapy [36]. This is supported by data demonstrating that an appropriate early therapeutic intervention dramatically improves the prognosis of RA [37]. In addition, markers for prediction of response to therapy would be helpful to further the success of the treatment approach [38]. The analysis of metabolic profiling seems to be

promising for RA in regard to all of these aspects both in murine models and humans. In the only study performed in a murine model, a metabolite analysis by  $^1\text{H}$  NMR spectroscopy was used which permitted to identify, among 59 serum metabolites, a significant upregulation of uracil and a significant downregulation of xanthine and glycine [39]. Interestingly, these three metabolites could discriminate arthritic from control animals and may therefore be considered as potential candidate biomarkers for diagnosis. In patients with RA, metabolomic analysis has been producing relevant clues. In an early study, an important cause of cardiovascular disease in RA was identified in the elevated plasma lipoprotein (a) levels observed in 93 patients relative to healthy controls despite decreased levels of cholesterol, triglycerides, and high-density lipoprotein cholesterol [40]. The lipid profile in RA patients was also studied in the synovial fluid by a capillary LC-MS/MS screening platform, allowing the demonstration of high amounts of 5S,12S-diHETE, an isomer of pro-resolving mediator LTB<sub>4</sub> with anti-inflammatory properties [41]. Another recent report focused on traditional Chinese medicine (TCM) to investigate metabolites in urine and plasma samples in RA subtypes as defined by symptom profiles. Indeed, according to TCM, RA patients may suffer from either cold RA or heat RA, each characterized by specific symptoms in addition to the upregulation of apoptosis resistance genes in the former and the upregulation of apoptosis-related genes in the latter [42]. Interestingly, significant biochemical differences were found between cold and heat RA both in urine and plasma [42]. These data were confirmed in other studies, in which higher anti-citrullinated protein/peptide antibody levels in the RA heat subtype were also observed [43, 44]. As described in patients with asthma, metabolomic analysis seems to be useful also in discriminating patients with different disease activity. Indeed, comparison of patients with active RA and those in remission provided different baseline metabolic profiles, suggesting that efficacious treatment may affect biological changes [45]. The capability of metabolomics to measure the inflammatory extent of RA has been confirmed in a more recent study, in which patients with established disease not yet treated with disease-modifying anti-rheumatic drugs were compared with healthy controls and patients with undifferentiated arthritis of  $\leq 3$ -month duration [46]. The analysis was carried out in serum samples by  $^1\text{H}$  NMR spectroscopy and showed a significantly different metabolic fingerprint in patients with active established RA with respect to that of healthy controls as well as different serum metabolic fingerprint in patients with early arthritis depending on the level of inflammation as measured by serum C-reactive protein level [46].

Moreover, evidence exists that metabolic changes may predict the response to treatment, including traditional immunosuppressants, such as methotrexate (MTX) [47••], and biological agents [48••]. In the first study, the  $^1\text{H}$  NMR-based metabolomic approach was applied to 38 patients with early

active RA: after 24 weeks, patients responding to MTX taken as monotherapy showed statistically significant elevated uric acid, taurine, methionine, glycine, histidine, and hypoxanthine serum levels coupled with statistically significant decreased uracil, TMAO,  $\alpha$ -oxoglutarate, aspartate, and tryptophan serum levels with respect to patients for whom MTX was not effective [47]. The only study currently published in patients treated with biological agents suggests that metabolomic profile may be a potential predictor of responses also to TNF antagonists [48]. This study, evaluating patients with RA and psoriatic arthritis (PsA) by  $^1\text{H}$  NMR spectroscopy, showed several interesting findings: first of all, there were clear differences in the metabolic profiles of baseline urine samples of patients with RA who responded well to anti-TNF therapy (etanercept or infliximab) at 12 months compared with those who did not. Several metabolites contributed to this difference; in particular histamine, glutamine, xanthurenic acid, and ethanolamine were identified by all three analytic methods performed (PLS-DA, GALGO, and PLS regression models). There was also a correlation between baseline metabolic profiles and the magnitude of change in the disease activity from baseline to 12 months in RA patients. Interestingly, within the responders, the same metabolites were altered in the urine samples from patients with RA and PsA. This observation may support the hypothesis that chronic inflammatory diseases respond by a common mechanism to TNF antagonists [48].

Finally, metabolomics has been tested in its capability to differentiate RA from other diseases, including PsA and osteoarthritis (OA). Patients with RA or PsA with predominant peripheral arthritis could be distinguished with a sensitivity of 90 % and a specificity of 94 % using both GC-MS and LG-MS. Surprisingly, the analysis of the results indicated larger differences between the RA and PsA patients than between the RA patients and healthy controls [49]. Likewise, an early study demonstrated that comparison of RA and OA patient metabolite profiles from knee synovial fluid samples allowed for clear distinction between the two diseases when evaluated by  $^1\text{H}$  NMR [50]. These results were not confirmed in a more recent study, albeit the low number of included individuals, especially for RA, could have affected the results [51].

### Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic, autoimmune, and often systemic disorder of the connective tissue characterized by involvement of the skin, joints, kidneys, nervous system, and serosal membranes [52]. Until now, accumulated evidence suggests that the metabolite profiling is altered in patients with SLE and may be associated with disease activity. In one study, metabolic profile was evaluated in serum from SLE patients in comparison with healthy controls and RA patients using the  $^1\text{H}$  NMR spectroscopy-based

approach. Peak intensities differentiated the three groups, even if SLE and RA patients shared some features, including decreased citrate, pyruvate, phosphocholine, and amino acid levels [53]. Relevant data emerged when metabolomic analysis was applied in SLE patients with kidney and brain involvement, representing potentially challenging complications of the disease due to the severity of clinical manifestations. Two urinary metabolites, taurine and citrate, were found to accurately distinguish between proliferative lupus nephritis (class III/IV) and pure membranous lupus nephritis (class V) patients. Moreover, urinary hippurate levels accurately distinguished between class V patients, who had normal levels of urinary hippurate, compared with focal segmental glomerulosclerosis patients, who completely lacked urinary hippurate [54]. Interestingly, since previous studies suggested that taurine and citrate are both measures of tubular cell function, it is conceivable that kidney disease can impair the filtration of small metabolites such as taurine and citrate through the glomerulus and their subsequent reabsorption in the renal tubules leading to alterations in metabolic profiles [55]. Metabolites in SLE patients have also been studied in 12 patients with psychiatric symptoms, showing significantly decreased regional cerebral metabolic rates for glucose in the prefrontal, inferior parietal, and anterior cingulate regions compared to patients without psychiatric manifestations [56]. These results are interesting in view that the brain is dependent exclusively on glucose as its sole source of energy. Therefore, in-depth investigations were carried out in mice with lupus to determine the rates of incorporation of glucose into amino acids and lactate via cell-specific pathways [57]. By using  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy, it was found that the MRL/lpr mice had a significant increase in total brain glutamine, glutamate, and lactate concentrations, as compared with control mice. Furthermore, a significant increase in total brain water content was observed, indicative of possible edema [57]. These results support a role for lactate in the neurological disturbances of patients with SLE: indeed, severe lactic acidosis can result in complete infarction of the tissue, and given its vasodilating effect on cerebral vessels, even at neutral pH levels, it may contribute to changes in cerebral blood flow [58]. More recently, LC/MS and GC/MS platforms were applied to detect serum metabolites of 20 SLE patients and 9 healthy controls. This study revealed that medium chain fatty acids, lipid peroxidation products (9-HODE, 13-HODE, and MDA), several  $\gamma$ -glutamyl peptides, and two eicosanoid metabolites in the n6-polyunsaturated fatty acids (PUFA) pathway, leukotriene B4 and 5-HETE, were increased in SLE patients [59]. By contrast, glycerol-3 phosphate, pyruvate, lactate, malate, citrate,  $\alpha$ -ketoglutarate, 1,2 propanediol, 3-hydroxybutyrate, ketogenic and glucogenic amino acids, all long-chain FA and acyl-carnitines, PUFA (both n3-PUFA and n6-PUFA), methionine, cysteine, choline, and phosphocholines were all decreased compared with healthy

controls. These findings were validated using an independent platform and cohort of 38 SLE patients [59]. Finally, in a SLE mouse model, specific disorders in the metabolic pathway of unsaturated fatty acids (UFAs), tryptophan, and phospholipid were observed. The therapeutic effects of Jieduquyuziyan, a traditional Chinese medicine, were also evaluated, which showed a good effect on SLE repairing the UFA and phospholipid metabolism [60].

## Conclusions

As complex interactions between genetic, epigenetic, and environmental factors are at the basis of allergic and rheumatic diseases, new systemic approaches, including metabolomics, are required for a better understanding of the pathogenesis, diagnosis, and treatment of these conditions.

The above-mentioned studies on allergic and rheumatic diseases show that the use of metabolomic approaches can be very useful to further the understanding of systemic diseases, allowing for the detection of the landscape characterizing the state of a single patient.

Metabolomics of breath condensate, synovial fluid, urine, serum, and plasma has demonstrated the capabilities of differentiating patients with allergic or rheumatic diseases from healthy subjects and characterizing several biochemical phenotypes in patients with these inflammatory disorders.

Changes in metabolite concentrations may also reflect disease progression; furthermore, some studies suggest that the metabolic fingerprint prior to therapy may predict responses to drugs such as MTX and anti-TNF agents in patients with RA. Moreover, global metabolic profiles can provide new insights into the complex pathophysiology of allergic and rheumatic diseases.

Overall, these studies suggest that metabolomics could be a powerful tool for discovering novel diagnostic biomarkers and developing strategies for disease monitoring and treatment evaluation.

However, the preliminary findings obtained in these studies need to be validated with further works, including larger sample sizes and multicenter trials more representative of the patient populations.

In the near future, downstream of the metabolomic analysis, set of biomarkers potentially specific to a particular state will be monitored by point-of-care tests (POCT) to detect the position on the patient maps: a sort of “metabolomic navigator” to localize the patient on the interconnected networks of disease states, allowing for a dynamic vision about grading of a pathology.

This kind of analytical devices, integrated to digital networking connecting patients, doctors, and databases, are crucial to translate systems biology approach into the proactive

P4 medicine that will be predictive, preventive, personalized, and participatory.

## Compliance with Ethics Guidelines

**Conflict of Interest** Rossana Scrivo, Luca Casadei, Mariacristina Valerio, Roberta Priori, Guido Valesini, and Cesare Manetti 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.

## References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Hood L, Flores M. A personal view on systems medicine and the emergence of proactive P4 medicine: predictive, preventive, personalized and participatory. *N Biotechnol*. 2012;29:613–24.
2. Priori R, Scrivo R, Brandt J, Valerio M, Casadei L, Valesini G, et al. Metabolomics in rheumatic diseases: the potential of an emerging methodology for improved patient diagnosis, prognosis, and treatment efficacy. *Autoimmun Rev*. 2013;12:1022–30.
3. Goodacre R, Broadhurst D, Smilde AK, Kristal BS, Baker JD, Beger R, et al. Proposed minimum reporting standards for data analysis in metabolomics. *Metabolomics*. 2007;3:231–41.
4. Fiehn O. Metabolomics—the link between genotypes and phenotypes. *Plant Mol Biol*. 2002;48:155–71.
5. Nicholson JK, Connelly J, Lindon JC, Holmes E. Metabonomics: a platform for studying drug toxicity and gene function. *Nat Rev Drug Discov*. 2002;1:153–62.
6. Griffin JL. Metabolic profiles to define the genome: can we hear the phenotypes? *Philos Trans R Soc Lond B Biol Sci*. 2004;359:857–71.
7. Griffin JL. The Cinderella story of metabolic profiling: does metabolomics get to go the functional genomics ball? *Philos Trans R Soc Lond B Biol Sci*. 2006;361:147–61.
8. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. *Mass Spectrom Rev*. 2007;26:51–78.
9. Shushan B. A review of clinical diagnostic applications of liquid chromatography-tandem mass spectrometry. *Mass Spectrom Rev*. 2010;29:930–44.
10. Holmes E, Antti H. Chemometric contributions to the evolution of metabonomics: mathematical solutions to characterising and interpreting complex biological NMR spectra. *Analyst*. 2002;127:1549–57.
11. Trygg J, Holmes E, Lundstedt T. Chemometrics in metabonomics. *J Proteome Res*. 2007;6:469–79.
12. Nicholson JK, Holmes E, Kinross JM, Darzi AW, Takats Z, Lindon JC. Metabolic phenotyping in clinical and surgical environments. *Nature*. 2012;491:384–92.
13. Masoli M, Fabian D, Holt S, Beasley R, Program G. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*. 2004;59:469–78.
14. Bisgaard H, Szefer S. Prevalence of asthma-like symptoms in young children. *Pediatr Pulmonol*. 2007;42:723–8.

15. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet*. 2002;360:1715–21.
16. Djukanović R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, et al. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis*. 1992;145:669–74.
17. Carraro S, Rezzi S, Reniero F, Heberger K, Giordano G, Zanconato S, et al. Metabolomics applied to exhaled breath condensate in childhood asthma. *Am J Respir Crit Care Med*. 2007;175:986–90.
18. Saude EJ, Obiefuna IP, Somorjai RL, Ajamian F, Skappak C, Ahmad T, et al. Metabolomic biomarkers in a model of asthma exacerbation urine nuclear magnetic resonance. *Am J Respir Crit Care Med*. 2009;179:25–34.
19. Saude EJ, Skappak CD, Regush S, Cook K, Ben-Zvi A, Becker A, et al. Metabolomic profiling of asthma: diagnostic utility of urine nuclear magnetic resonance spectroscopy. *J Allergy Clin Immunol*. 2011;127:757–64.
20. Mattarucchi E, Baraldi E, Guillou C. Metabolomics applied to urine samples in childhood asthma; differentiation between asthma phenotypes and identification of relevant metabolites. *Biomed Chromatogr*. 2011;26:89–94.
21. Brightling CE, Green RH, Pavord ID. Biomarkers predicting response to corticosteroid therapy in asthma. *Treat Respir Med*. 2005;4:309–16.
22. Horvath I, Barnes P, Hunt J. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J*. 2005;26:523–48.
23. Carraro S, Giordano G, Reniero F, Carpi D, Stocchero M, Sterk PJ, et al. Asthma severity in childhood and metabolomic profiling of breath condensate. *Allergy*. 2013;68:110–7. *Provided evidence that metabolomic profiling may allow a full discrimination of asthma phenotype, suggesting that this approach may be used to develop new targeted therapies.*
24. Ibrahim B, Marsden P, Smith JA, Custovic A, Nilsson M, Fowler SJ. Breath metabolomic profiling by nuclear magnetic resonance spectroscopy in asthma. *Allergy*. 2013;68:1050–6.
25. Canning BJ. Modeling asthma and COPD in animals: a pointless exercise? *Curr Opin Pharmacol*. 2003;3:244–50.
26. Evans CM, Fryer AD, Jacoby DB, Gleich GJ, Costello RW. Pretreatment with antibody to eosinophil major basic protein prevents hyperresponsiveness by protecting neuronal M2 muscarinic receptors in antigen-challenged guinea pigs. *J Clin Invest*. 1997;100:2254–62.
27. Gibbs NK, Tye J, Norval M. Recent advances in urocanic acid photochemistry, photobiology and photoimmunology. *Photochem Photobiol Sci*. 2008;7:655–67.
28. Ho WE, Xu YJ, Xu F, Cheng C, Peh HY, Tannenbaum SR, et al. Metabolomics reveals altered metabolic pathways in experimental asthma. *Am J Respir Cell Mol Biol*. 2013;48:204–11.
29. Jung J, Kim SH, Lee HS, Choi GS, Jung YS, Ryu DH, et al. Serum metabolomics reveals pathways and biomarkers associated with asthma pathogenesis. *Clin Exp Allergy*. 2013;43:425–33. *Provided evidence that 1H NMR metabolomics may distinguish between adult patients with asthma and healthy subjects; the levels of serum metabolites from patients correlated with disease severity.*
30. Bieber T. Atopic dermatitis. *N Engl J Med*. 2008;358:1483–94.
31. Bieber T. Atopic dermatitis 2.0: from the clinical phenotype to the molecular taxonomy and stratified medicine. *Allergy*. 2012;67:1475–82.
32. Hanifin JM. Evolving concepts of pathogenesis in atopic dermatitis and other eczemas. *J Invest Dermatol*. 2009;129:320–2.
33. Assfalg M, Bortoletti E, D'Onofrio M, Pigozzi R, Molinari H, Boner AL, et al. An exploratory (1) H-nuclear magnetic resonance metabolomics study reveals altered urine spectral profiles in infants with atopic dermatitis. *Br J Dermatol*. 2012;166:1123–5.
34. Scott DL, Wolfe F, Huizinga TWJ. Rheumatoid arthritis. *Lancet*. 2010;376:1094–108.
35. Zeidler H. The need to better classify and diagnose early and very early rheumatoid arthritis. *J Rheumatol*. 2012;39:212–7.
36. Atzeni F, Antivalle M, Pallavicini FB, Caporali R, Bazzani C, Gorla R, et al. Predicting response to anti-TNF treatment in rheumatoid arthritis patients. *Autoimmun Rev*. 2009;8:431–7.
37. van Nies JA, Krabben A, Schoones JW, Huizinga TW, Kloppenburg M, van der Helm-van Mil AH. What is the evidence for the presence of a therapeutic window of opportunity in rheumatoid arthritis? A systematic literature review. *Ann Rheum Dis*. 2013 Apr 9 [Epub ahead of print].
38. Miossec P, Verweij CL, Klareskog L, Pitzalis C, Barton A, Lekkerkerker F, et al. Biomarkers and personalised medicine in rheumatoid arthritis: a proposal for interactions between academia, industry and regulatory bodies. *Ann Rheum Dis*. 2011;70:1713–8.
39. Weljie AM, Dowlatabadi R, Miller BJ, Vogel HJ, Jirik FR. An inflammatory arthritis-associated metabolite biomarker pattern revealed by <sup>1</sup>H NMR spectroscopy. *J Proteome Res*. 2007;9:3456–64.
40. Rantapää-Dahlqvist S, Wallberg-Jonsson S, Dahlen G. Lipoprotein (a), lipids, and lipoproteins in patients with rheumatoid arthritis. *Ann Rheum Dis*. 1991;50:366–8.
41. Giera M, Ioan-Facsinay A, Toes R, Gao F, Dalli J, Deelder AM, et al. Lipid and lipid mediator profiling of human synovial fluid in rheumatoid arthritis patients by means of LC-MS/MS. *Biochim Biophys Acta*. 2012;1821:1415–24.
42. van Wietmarshchen HA, Dai W, van der Kooij AJ, Reijmers TH, Schroen Y, Wang M, et al. Characterization of rheumatoid arthritis subtypes using symptom profiles, clinical chemistry and metabolomics measurements. *PLoS One*. 2012;7:e44331.
43. Jiang M, Xiao C, Chen G, Lu C, Zha Q, Yan X, et al. Correlation between cold and hot pattern in traditional Chinese medicine and gene expression profiles in rheumatoid arthritis. *Front Med*. 2011;5:219–28.
44. Gu Y, Lu C, Zha Q, Kong H, Lu X, Lu A, et al. Plasma metabolomics study of rheumatoid arthritis and its Chinese medicine subtypes by using liquid chromatography and gas chromatography coupled with mass spectrometry. *Mol BioSyst*. 2012;8:1535–43.
45. Lauridsen MB, Bliddal H, Christensen R, Danneskiold-Samsøe B, Bennett R, Keun H, et al. 1H NMR spectroscopy-based interventional metabolic phenotyping: a cohort study of rheumatoid arthritis patients. *J Proteome Res*. 2010;9:4545–53.
46. Young SP, Kapoor SR, Viant MR, Byrne JJ, Filer A, Buckley CD, et al. The impact of inflammation on metabolomic profiles in patients with arthritis. *Arthritis Rheum*. 2013;65:2015–23.
47. Wang Z, Chen Z, Yang S, Wang Y, Yu L, Zhang B, et al. (1)H NMR-based metabolomic analysis for identifying serum biomarkers to evaluate methotrexate treatment in patients with early rheumatoid arthritis. *Exp Ther Med*. 2012;4:165–71. *Provided evidence that metabolic changes may predict the response to methotrexate in patients with rheumatoid arthritis.*
48. Kapoor SR, Filer A, Fitzpatrick MA, Fisher BA, Taylor PC, Buckley CD, et al. Metabolic profiling predicts response to anti-tumor necrosis factor  $\alpha$  therapy in patients with rheumatoid arthritis. *Arthritis Rheum*. 2013;65:1448–56. *Provided evidence that metabolic changes may predict the response to biological agents in patients with rheumatoid arthritis.*
49. Madsen RK, Lundstedt T, Gabrielsson J, Sennbro CJ, Alenius GM, Moritz T, et al. Diagnostic properties of metabolic perturbations in rheumatoid arthritis. *Arthritis Res Ther*. 2011;13:R19.
50. Meshitsuka S, Yamazaki E, Inoue M, Hagino H, Teshima R, Yamamoto K. Nuclear magnetic resonance studies of synovial fluids from patients with rheumatoid arthritis and osteoarthritis. *Clin Chim Acta*. 1999;281:163–7.

51. Hügler T, Kovacs H, Heijnen IA, Daikeler T, Baisch U, Hicks JM, et al. Synovial fluid metabolomics in different forms of arthritis assessed by nuclear magnetic resonance spectroscopy. *Clin Exp Rheumatol*. 2012;30:240–5.
52. Wallace D, Hahn BH. *Dubois' lupus erythematosus and related syndromes*. 8th ed. Philadelphia: Sanders Elsevier; 2013.
53. Ouyang X, Dai Y, Wen JL, Wang LX.  $^1\text{H}$  NMR-based metabolomic study of metabolic profiling for systemic lupus erythematosus. *Lupus*. 2011;20:1411–20.
54. Romick-Rosendale LE, Brunner HI, Bennett MR, Mina R, Nelson S, Petri M, et al. Identification of urinary metabolites that distinguish membranous lupus nephritis from proliferative lupus nephritis and focal segmental glomerulosclerosis. *Arthritis Res Ther*. 2011;13:R199.
55. Quaggin SE, Kreidberg JA. Development of the renal glomerulus: good neighbors and good fences. *Development*. 2008;135:609–20.
56. Komatsu N, Kodama K, Yamanouchi N, Okada S, Noda S, Nawata Y, et al. Metabolic rate for glucose in systemic lupus erythematosus patients with psychiatric symptoms. *Eur Neurol*. 1999;42:41–8.
57. Alexander JJ, Zwingmann C, Quigg R. MRL/lpr mice have alterations in brain metabolism as shown with  $[^1\text{H}-^{13}\text{C}]$  NMR spectroscopy. *Neurochem Int*. 2005;47:143–51.
58. Laptook AR, Peterson J, Michael PA. Effects of lactic acid infusions and pH on cerebral blood flow and metabolism. *J Cereb Blood Flow Metabol*. 1988;8:193–200.
59. Wu T, Xie C, Han J, Ye Y, Weiel J, Li Q, et al. Metabolic disturbances associated with systemic lupus erythematosus. *PLoS One*. 2012;7:e37210.
60. Ding X, Hu J, Wen C, Ding Z, Yao L, Fan Y. Rapid resolution liquid chromatography coupled with quadrupole time-of-flight mass spectrometry-based metabolomics approach to study the effects of Jieduquyuziyin prescription on systemic lupus erythematosus. *PLoS One*. 2014;9:e88223.