) [5]). Two such metabolites were pseudouridine, a modi“ed nucleotide present in t- and rRNA and a marker of cell turnover, as well as the tricarboxylic acid cycle intermediate 2-oxoglutarate. Furthermore, 3 further new compounds were also excellent discriminators between patients and controls: 2-hydroxy, 2-methylpropanoic acid, erythritol and 2,4,6-trihydroxypyrimidine. Although renal disease may be associated with heart failure, and metabolites associated with renal disease and other markers were also elevated (e.g. urea, creatinine and uric acid), there was no correlation within the patient group between these metabolites and our heart failure biomarkers, indicating that these were indeed biomarkers of heart failure and not renal disease*per se* These “ndings demonstrate the power of data-driven metabolomics approaches to identify such markers of disease.

KEY WORDS: heart failure; metabolomics; biomarkers; pseudouridine; 2-oxoglutarate.

1. Introduction

Biomarkers are essential tools in diagnosing disease and monitoring progression as well as response to therapy. In heart failure, BNP has proved its usefulness as a diagnostic marker and there are some limited data on monitoring response to therapy (Troughton

There is intense interest in the identi“cation of further biomarkers in the syndrome of heart failure. Distinct patterns of the ensemble of several such markers may eventually help in identifying speci“c classes of the syndrome with improved predictive power in terms of diagnosis, prognosis and treatment options.

In a “rst step towards this goal, we here present a new approach to identifying biomarkers by focusing on metabolic changes during heart failure. Metabolomics is the data-driven study of the di erent patterns of metabolites within living organisms, in which we seek measurements that are as comprehensive as possible. Conceptually, metabolomics lends itself ideally to studying heart failure and other diseases. In contrast say to malignancy, where tissue is readily available for transcriptomic and proteomic analysis, routine laboratory tests for heart failure are limited to serum samples. Furthermore, metabolic alterations have been well documented in the heart (Neubauer

The present metabolomic study of serum from patients with heart failure and appropriate controls detected 272 candidate metabolite peaks, of which 38 showed highly signi“cant di erences between cases and controls. At least two of these metabolites, pseudouridine and 2-oxoglutarate, have the potential, alone or together, to improve on or add to BNP in terms of their sensitivity and speci“city as biomarkers.

2. Methods

2.1. Participants

The study was approved by the local ethics committee for clinical studies. Patients attending the heart failure clinic at the South Manchester University Hospital Regional Cardiothoracic Centre and Heart Transplant Unit were invited to participate in the study by providing samples of venous blood. 52 patients were recruited with an established diagnosis of left ventricular dysfunction (LVD, EF < 40%) and heart failure. Ejection fraction was determined echocardiographically. All patients belonged to NYHA (New York Heart Association) classes II-IV. The age-matched control group were mainly taken from an ENT clinic, though some were from an ophthalmology clinic and others were the healthy partners of the patients. They comprised subjects with no history of cardiac disease, and were examined clinically to this end at recruitment. Subjects with acute or chronic in”ammatory conditions, malignancies and signi“cant respiratory pathology were excluded. See Table

2.2. Sample collection

Venous blood was collected from the antecubital veins by venepuncture, left to coagulate for 10 min, followed by centrifugation (2200 g, 15 min). The supernatant serum was then aliquoted into 2.5 mL cryotubes and stored at) 80 C until required.

Table

2.3. Sample preparation and GC-TOF-MS analysis

Serum samples were prepared for Gas Chromatography-Time of Flight-Mass Spectrometry (GC-tof-MS) analysis as described (Kenny

2.4. Pro-BNP

2.5. Urea and creatinine

Urea and creatinine were measured enzymatically in serum using a Roche analyser according to the manufacturer’s instructions.

2.6. Statistical analyses

Univariate statistical analysis was performed in order to assess the characteristics of each independent metabolite peak generated using the above protocol. As the experimental design employed in this study was that of a matched case…control study (Rothman and Greenland,

Table

Fig-

ure

Figure

All statistical analyses were carried out using the Matlab scripting language (http://www.mathworks.com/). All algorithms used are implemented such that any missing values are ignored. Scripts are available upon request.

Figure

3. Results

Of the 272 metabolite peaks produced by the pre-processing protocol, 38 showed a signi“cant di erence between case and control (

Table

Table

Table

Bias or the in”uence of uncontrolled variables can present a major headache in many omics studies, including those seeking biomarkers (Ransoho ,

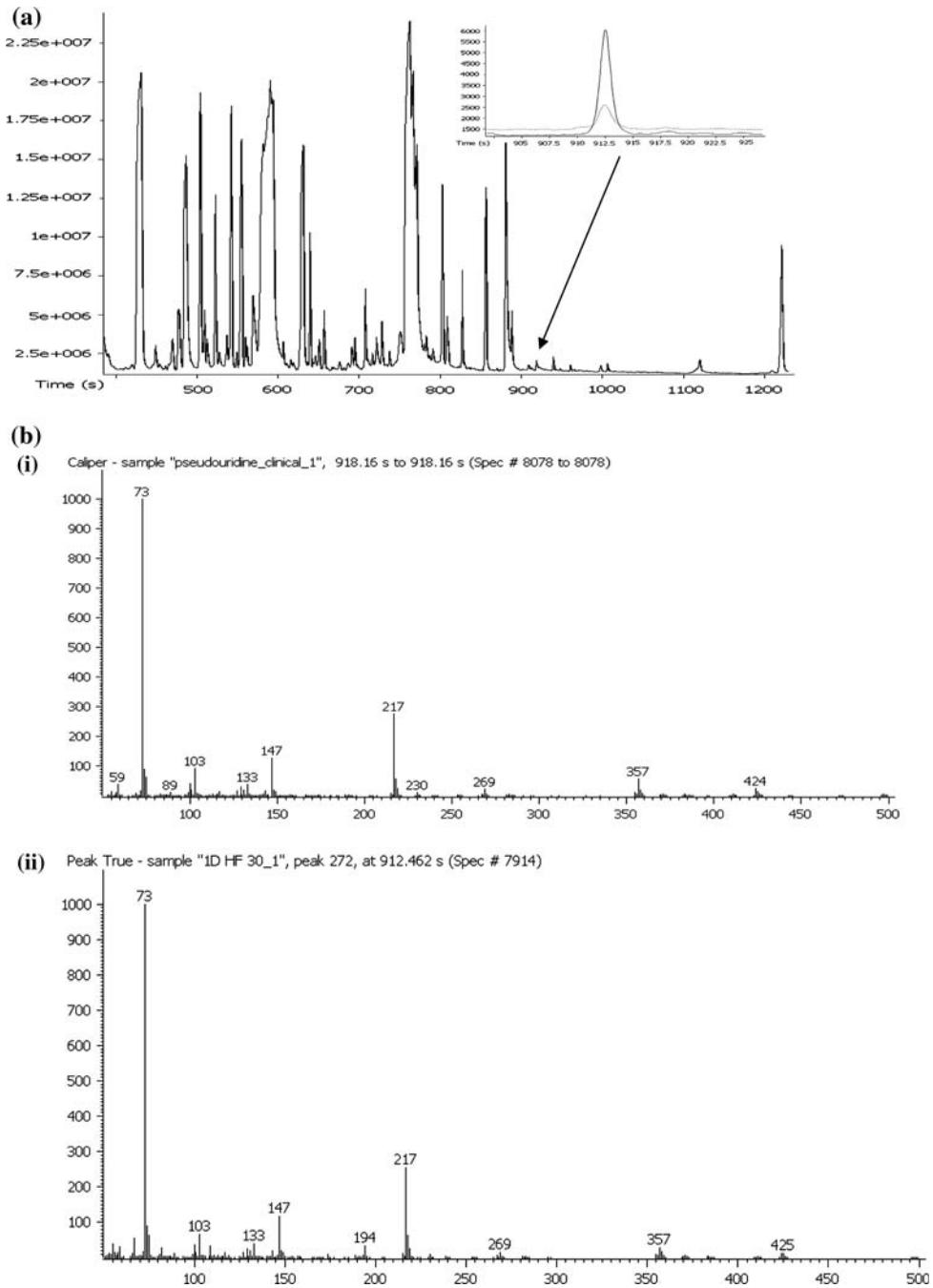
(a)
2.25e+007
2e+007
1.75e+007
1.5e+007
1.25e+007
1e+007
7.5e+006
5e+006
2.5e+006
Time (s)
500
600
700
800
900
1000
1100
1200
(b)
(i)
Caliper - sample "pseudouridine_clinical_1", 918.16 s to 918.16 s (Spec # 8078 to 8078)
73
217
147
103
59
89
133
230
269
357
424
(ii)
Peak True - sample "1D HF 30_1", peak 272, at 912.462 s (Spec # 7914)
73
217
147
103
133
194
269
357
425

Figure 1

Table

(a)

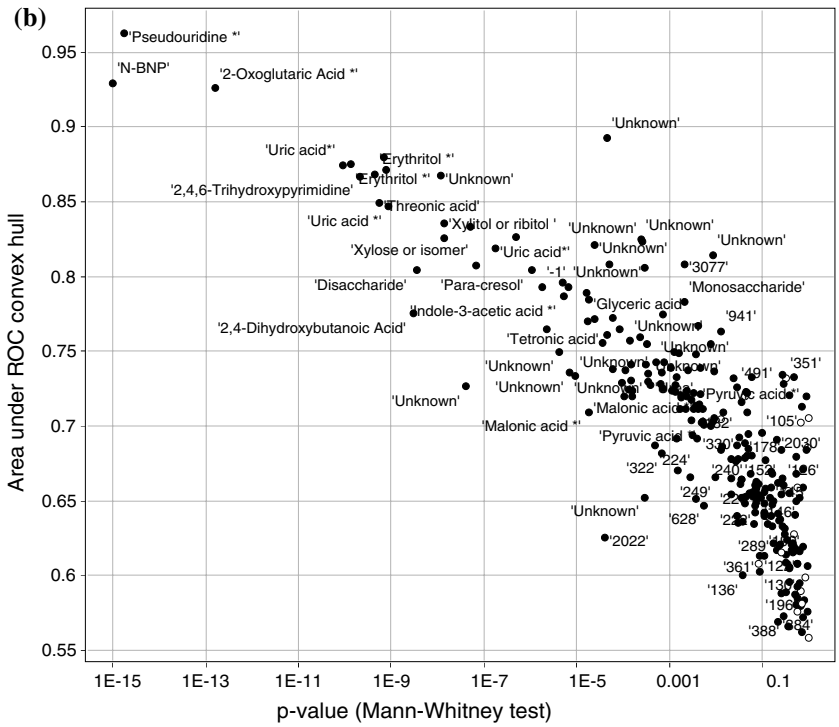

Figure 2

Table

Another kind of potential for bias or misinterpretation can come where A causes B and A causes C and it might then be interpreted that C causes B as they

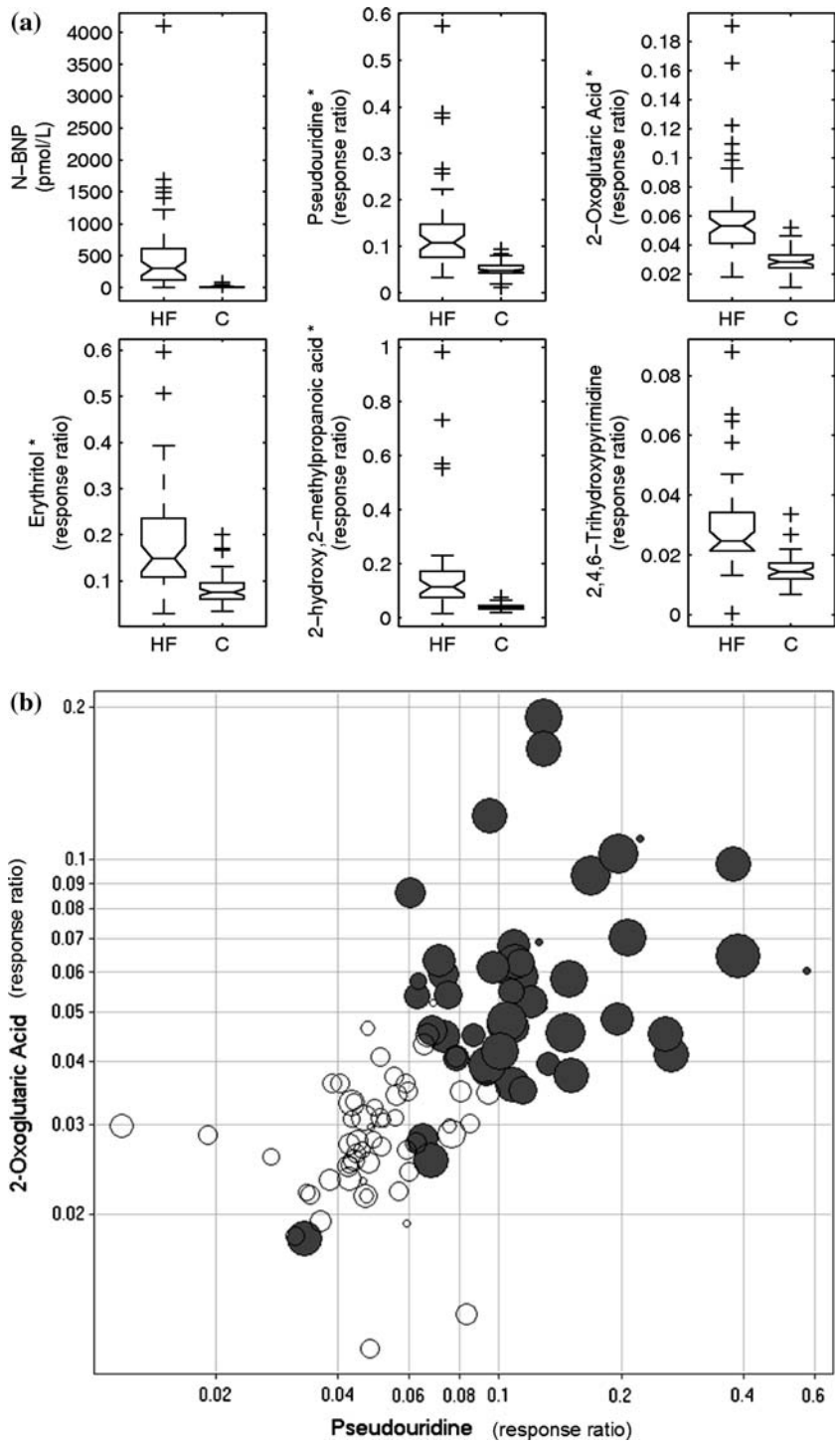
(a)
N–BNP (pmol/L)
Pseudouridine * (response ratio)
2–Oxoglutaric Acid * (response ratio)
Erythritol * (response ratio)
2–hydroxy,2–methylpropanoic acid * (response ratio)
2,4,6–Trihydroxypyrimidine (response ratio)
HF
C
(b)
2-Oxoglutaric Acid (response ratio)
Pseudouridine (response ratio)

Figure 3

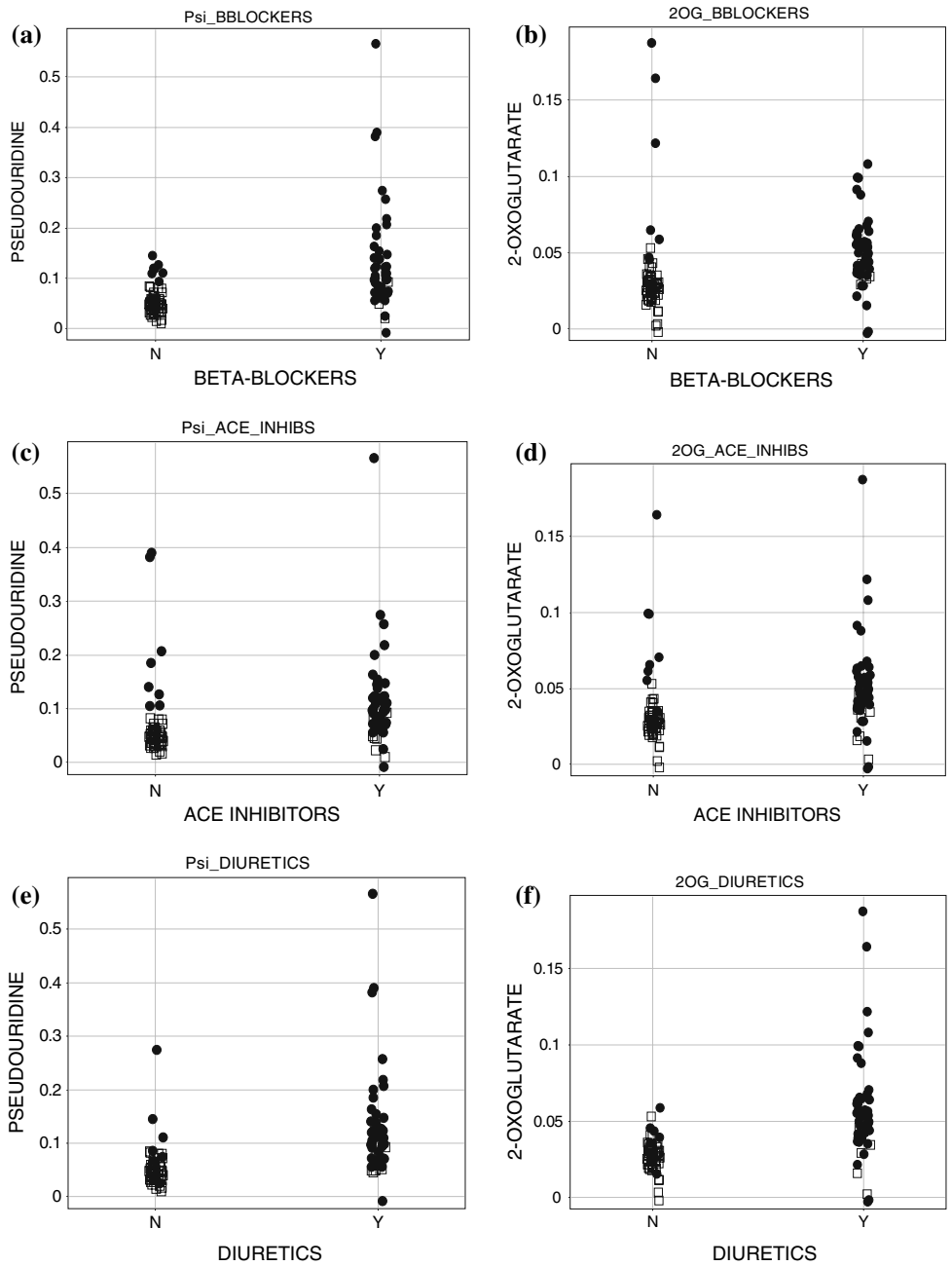

Figure 4

will tend to be correlated (Pearl,

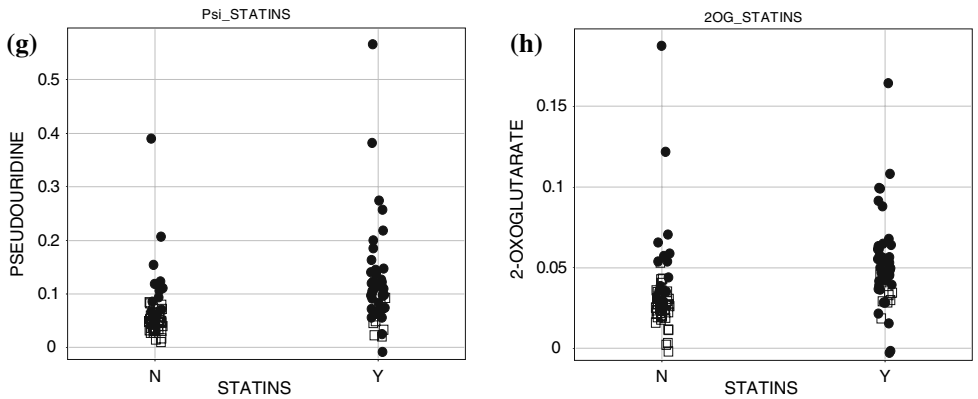

Figure 4

4. Discussion

The principal “ndings of this study are represented by the identi“cation of a “ngerprint of serum metabolites that characterize heart failure, and stress the potential of pseudouridine and 2-oxoglutaric acid (α-ketoglutarate in the traditional nomenclature) as novel diagnostic markers of heart failure. Furthermore, this study highlights the potential of metabolomic analysis and, more generally, of the systems biology approach (Kell,

An advantage of the metabolomic approach in the identi“cation of novel biomarkers is that it is not limited by our current knowledge, and instead seeks to measure the maximum number of metabolites in a given sample; the total number in the native human metabolic network is unknown (although leaving aside combinatorial lipids a number around 3000 is a reasonable starting estimate (Kell,

Uric acid has been suggested as a marker of cardiovascular mortality and its level is known to increase in patients with heart failure (Leyva

Table

Pseudouridine is a modi“ed nucleoside that is found in ribosomal and transfer RNA and is produced post-transcriptionally (Charette and Gray,

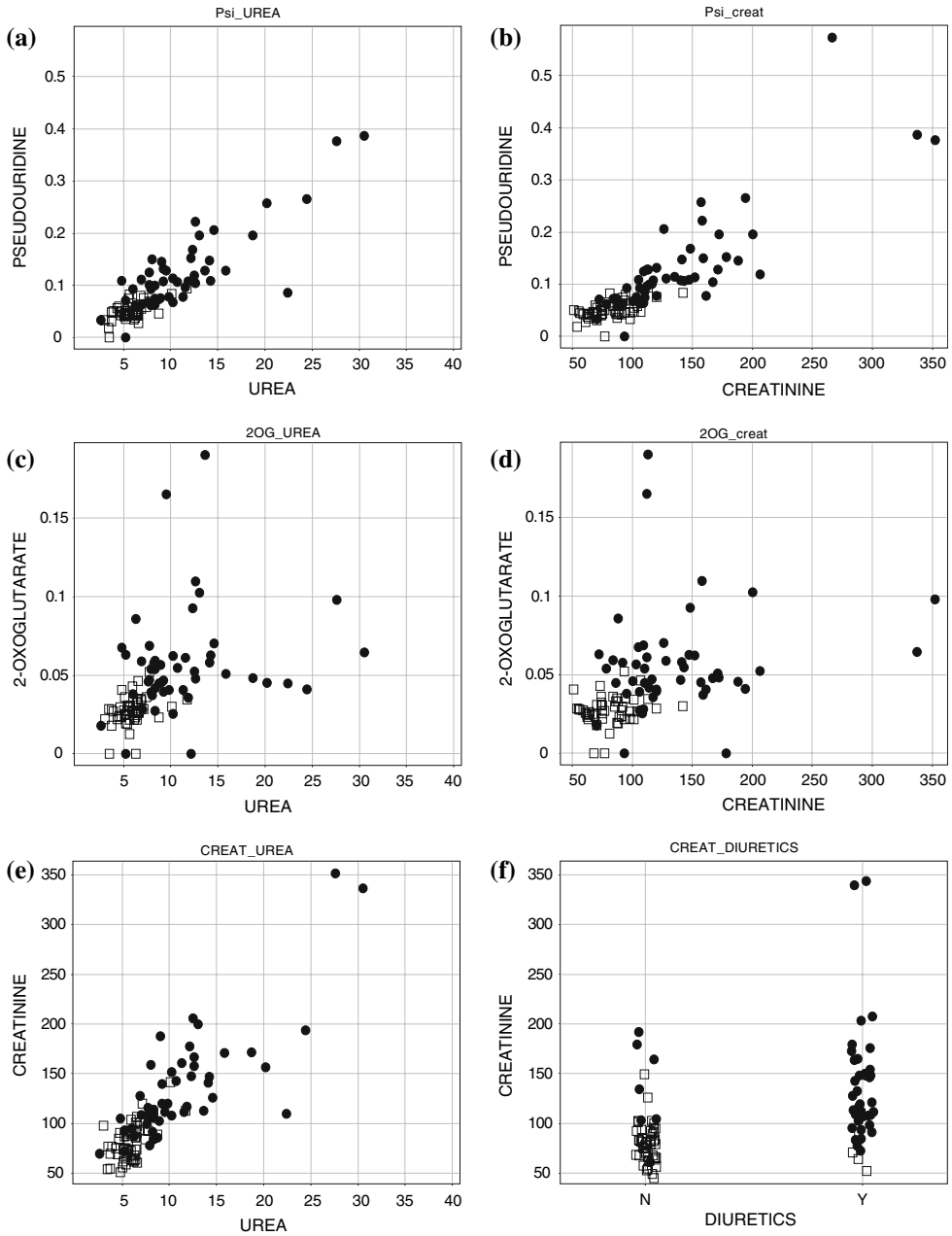
(a)
Psi_UREA
PSEUDOURIDINE
UREA
(b)
Psi_creat
PSEUDOURIDINE
CREATININE
(c)
2OG_UREA
2-OXOGLUTARATE
UREA
(d)
2OG_creat
2-OXOGLUTARATE
CREATININE
(e)
CREAT_UREA
CREATININE
UREA
(f)
CREAT_DIURETICS
CREATININE
N
Y
DIURETICS

Figure 5

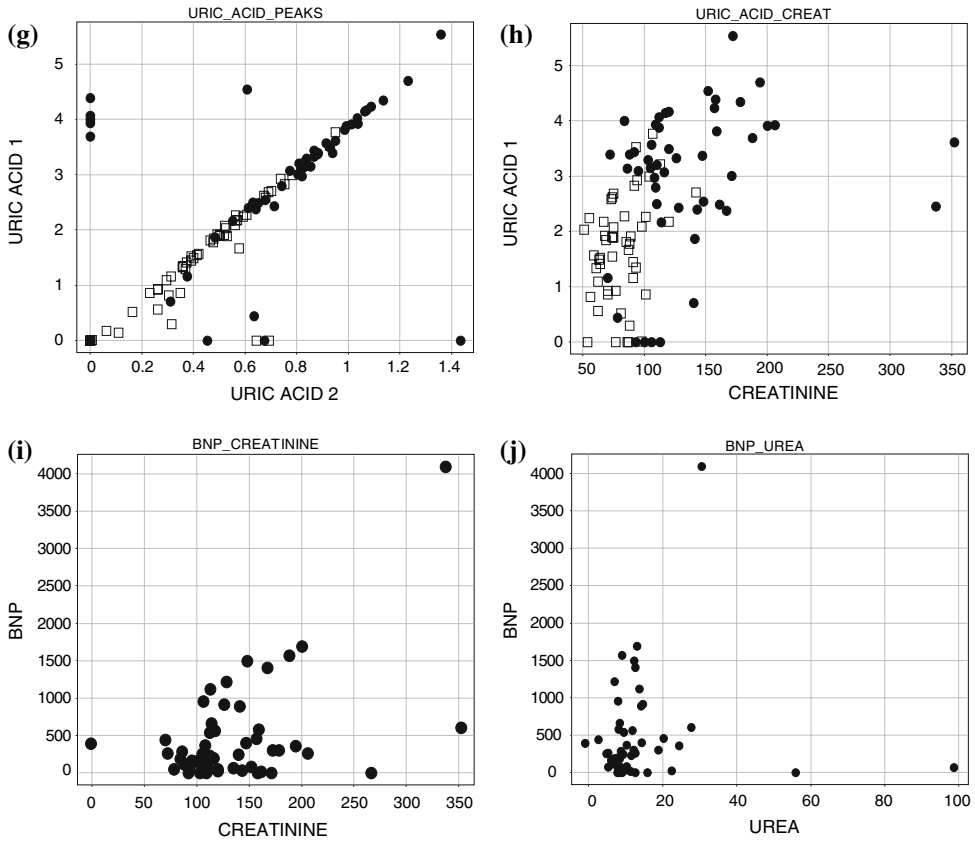

Figure 5

We note that 2,4,6-trihydroxypyrimidine is often displayed and described as its tautomer malonylurea or barbituric acid. Its origin in mammals has not been fully elucidated (http://www.genome.ad.jp/dbget-bin/show_pathway?map00240±C02067), but the fact that we identify it as a marker in an important syndrome such as heart failure points to the value of further research into this issue.

We note, too, that it is also possible that pseudouridine and other metabolites whose concentrations are

Table

raised in heart failure are not merely innocent marker metabolites but participate in the pathophysiology of the syndrome, e.g. by inducing cellular alterations in the heart or in peripheral tissues. Such may be the case for uric acid (Sakai

Pseudouridine is also related to creatinine in our overall dataset. This might be expected, given that a reduction in renal function is part of the pathophysiology of heart failure and indeed, reduced renal function has been considered to contribute to the increase in BNP in heart failure (Tsutamoto

Conversely, reduced renal function contributes to the progression of heart failure. The mechanisms of the intimate link between renal and cardiac function, sometimes referred to as the 'cardiorenal syndrome' (Bongartz

Overall, however, when we look at the heart failure class alone, the correlation between BNP and creatinine was weak (Table

As with other marker studies, it cannot be completely excluded

2-oxoglutarate is a major intermediate of the tricarboxylic acid cycle (also known as citric acid or Krebs cycle) that occupies a central place in energy metabolism and is one of the 12 major precursors for the synthesis of most biochemical substances (Csete and Doyle,

The origin of the sugar alcohol erythritol in serum is unclear. Although there are a few reports of its measurement in serum and urine (e.g. (Bultitude and Newham,

Table

In conclusion, an unbiased, hypothesis-generating (Kell and Oliver,

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