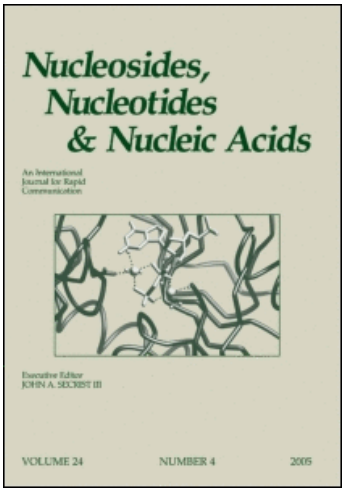

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APPLICATION OF METABOLOMIC PRINCIPLES TO DISORDERS OF NUCLEOTIDE METABOLISM REVEALS NEW METABOLIC PERTURBATIONS

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□ *A metabolomic analysis of plasma amino acids and acylcarnitines was applied to four disorders of nucleotide metabolism. Multivariate analysis gave score plots that show segregation of hypoxanthine phosphoribosyltransferase and adenine phosphoribosyltransferase deficient plasma from controls with equivocal results for adenosine deaminase and dihydropyrimidine dehydrogenase deficiencies. Loadings plots revealed the principal metabolites responsible for the discrimination between these classes. There were increases for HPRT in C4-, C6-, and C3-DC (malonyl)-carnitines, and decreased serine. For APRT there were increases in C4- to C10- and C3-DC to C6-DC-carnitines, urea, 1-methylhistidine, 3-methylhistidine, and decreased tryptophan. For ADA deficiency there were increases in C4- and C6-carnitines, taurine, and isoleucine.*

Keywords Metabolomics; nucleotide metabolism disorders; amino acids; acylcarnitines

INTRODUCTION

Metabolomics involves the global analysis of metabolites from physiological fluids followed by the application of pattern recognition statistics as a means to differentiate between states such as health and disease. Metabolomics thereby offers the possibility of revealing metabolic relationships regarding disease and physiological state, and potentially unique metabolic markers defining genetic disease. Conventional metabolite analysis has historically focused on individual metabolite comparison to norms, however, multivariate applications such as principal component analysis enables the simultaneous assessment of the contribution of a broad spectrum of metabolites, limited only by the scope of the analytical methods. The first

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compendium of the human metabolome has recently identified approximately 2,300 metabolites.[1]

In the present study, we examine the acylcarnitine and amino acid profiles of plasma from four disorders of nucleotide metabolism: hypoxanthine phosphoribosyltransferase (HPRT), adenine phosphoribosyltransferase (APRT), adenosine deaminase (ADA), and dihydropyrimidine dehydrogenase (DHPD) deficiencies. Metabolic profiles included the quantitation of 34 acylcarnitines and 39 ninhydrin positive compounds for each of the four genetic disorders in search of previously unrecognized metabolic relationships.

METHODS

Analytical Methods

Plasma acylcarnitines species from C2 to C18 were quantitated with a Waters 2795/Quattro Ultima LC-MS/MS using deuterated reference standard set B (Cambridge Isotope Labs, NSK-B, USA) as internal standards.[2,3] Plasma amino acids were quantitated using the Biochrom 20 or 30 amino acid analyzers with ninhydrin derivatisation, using physiological amino acid standards (Sigma-Aldrich, Canada) and S-(2-aminoethyl)-L-cysteine as internal standard.[4,5] Plasma methylmalonic acid was analyzed on a Hewlett-Packard GC-MS, using methyl-d3-malonic acid as an internal standard (CDN Isotopes, Canada).[6]

Data Analysis

Data was imported into SIMCA 11.5 (Umetrics, Umea, Sweden)[7] for modeling by principal component analysis (PCA) and assessment of the differentiation of genetic conditions from controls by partial least squares discriminant analysis (PLS-DA). Scores and loadings plots were examined for segregation from control data and identification of metabolites contributing to the class distinction. Individual metabolites for test and control were compared by the Mann-Whitney rank sum test and plotted as box plots (SigmaStat version 9.0). Only results significantly different from control ($p = <0.001$) are presented.

Subjects and Samples Analyzed

Plasma were from: complete HPRT deficiency (<1% activity), 4 subjects, 17 plasmas; partial HPRT deficiency,[8] 3 subjects, 5 plasmas; APRT deficiency, 4 subjects, 7 plasmas; ADA deficiency, 2 subjects, 12 plasmas; DHPD deficiency, 2 subjects, 3 plasmas. Controls consisted of 196 plasma for acylcarnitines, and 554 plasma for amino acids. HPRT and APRT

deficient subjects were on allopurinol therapy and ADA patients were receiving PEG-ADA enzyme replacement therapy.

RESULTS

Plasma metabolites were analyzed independently for acylcarnitines and amino acids. Acylcarnitine scores plot for HPRT deficiency revealed greater than half of the samples to segregate from controls (Figure 1). Analysis of the complete and partial HPRT deficient plasma profiles as distinct classes did not provide evidence for differences between these groups so they were analyzed as a single class. Loadings plot indicated minor metabolic changes with C4 (butyryl)-, C6(hexanoyl)- and C3-DC(malonyl)-carnitines showing significant increases ($p = <0.001$). The scores plot for APRT deficiency showed segregation of all samples from controls (Figure 1) with the loadings plot indicating increases in several acylcarnitine species, none of which are greater than C12. The major increases compared to controls are shown in Figure 2 and these are: C6-DC (glutaryl)- (13-fold), C5-DC (adipyl)- (3.7-fold), C4-DC (methylmalonyl)- (6-fold), and C3-DC (malonyl)- (6-fold)

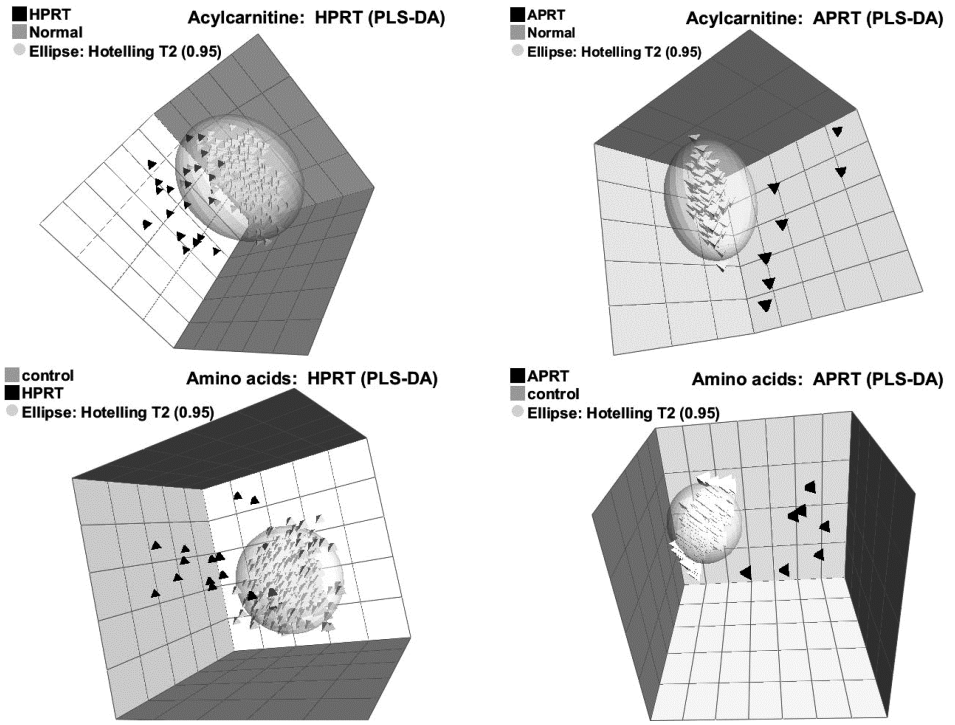

FIGURE 1 FIGURE 1 Metabolomic analysis of plasma acylcarnitine and amino acid profiles from nucleotide metabolism disorders. Score plots for partial least squares discriminant analysis for HPRT and APRT deficient subjects (black symbols) and controls (shaded symbols).

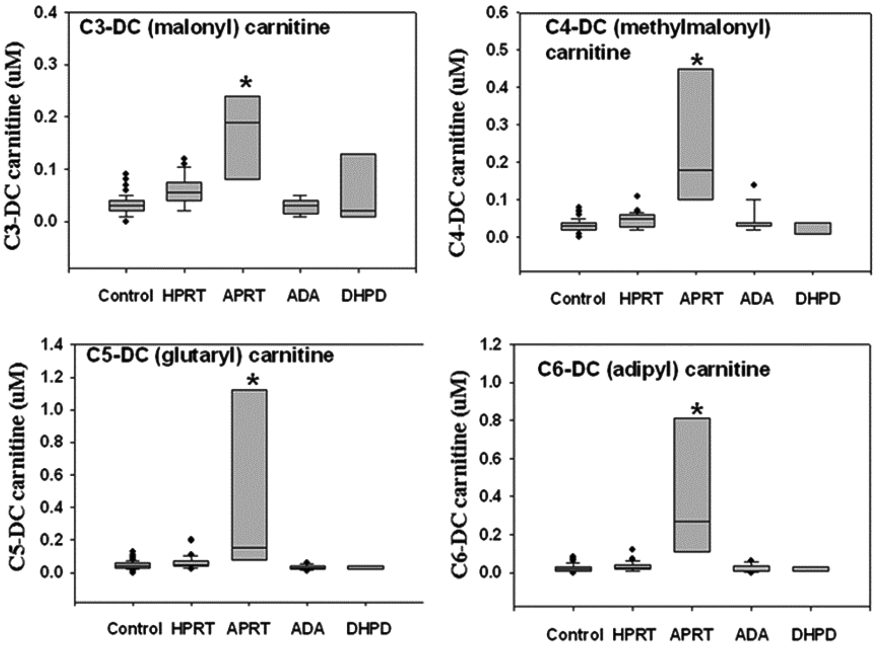

FIGURE 2 FIGURE 2 Box plots for plasma acylcarnitines from control and HPRT, APRT, ADA, and DHPD deficiency. Findings significantly different from control, p =<0.001 (*).

carnitines. The scores plots for ADA and DHPD deficiencies did not show substantial segregation from controls. In addition C4 (butyryl)- and C6 (hexanoyl)- carnitines are elevated in HPRT, APRT and ADA deficiencies and C8 (octanoyl)- and C10 (decanoyl)-carnitines in APRT deficiency only. The increase of C4-DC (methylmalonyl) acylcarnitine in APRT deficient patients directed us to examine plasma methylmalonic acid which are also increased for all specimens, ranging from 0.25 to 1.5 μM; compared to controls, <0.15 μM.

Amino acid scores plot for HPRT deficiency indicated greater than half of the samples segregated from controls (Figure 1) with the loadings plot revealing serine to be among the metabolites contributing to this finding. Amino acid scores plots for APRT deficiency show a complete segregation from controls (Figure 1) with major metabolite differences in the loadings plot due to the contributions of urea, 1-methyl- and 3-methylhistidines. As with acylcarnitines, the amino acid scores plots for ADA and DHPD deficiencies did not show substantial segregation from controls. Among the significant changes in amino acids (Figure 3) are the 4-fold increase in urea for APRT deficiency, reduced tryptophan and increased 1-methylhistidine (5-fold) and 3-methylhistidine (50-fold) levels, whereas histidine is unchanged. As valine, isoleucine and methionine are precursors to methylmalonic acid; we examined these amino acids and found no significant changes for APRT

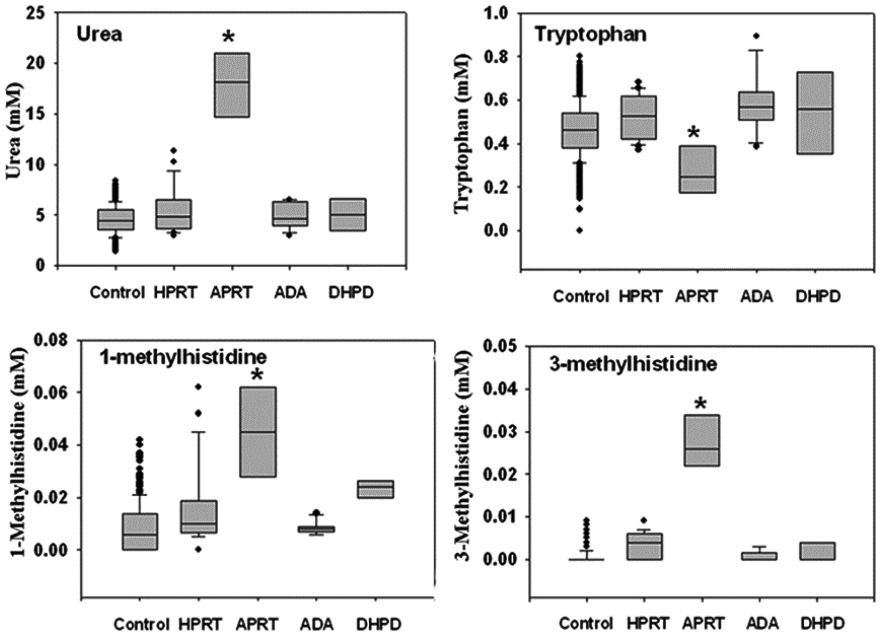

FIGURE 3 FIGURE 3 Box plots for plasma amino acids from control and HPRT, APRT, ADA, and DHPD deficiency. Findings significantly different from control, p =<0.001 (*).

deficiency, though isoleucine is elevated in ADA and HPRT deficiencies. Serine is decreased only in HPRT deficiency.

DISCUSSION

Metabolomic analysis of four disorders of nucleotide metabolism revealed major changes in APRT deficiency which have not previously been recognized though some features are common to end stage renal disease. Uremia is well characterized in the APRT deficient mouse (9) but has not been commented on for human APRT deficiency. 1-Methylhistidine is an indicator of external dietary meat but is not produced by humans whereas 3-methylthistidine is an indicator of internal muscle mass. Increased 1-methyl- and 3-methyl-histidines have been observed in end stage renal disease,[10] but are previously unrecognized in APRT deficiency. Tryptophan is also significantly reduced in APRT deficient plasma.

There are substantive increases in the dicarboxylic acylcarnitines in APRT deficiency, specifically C3-DC, C4-DC, C5-DC, and C6-DC. The increase in C4-DC (methylmalonyl) carnitine corresponded to a parallel increase in methylmalonic acid in APRT deficiency. Comparable increases in methylmalonic acid, 2- to 10-fold, are reported for end stage renal disease.[6.11]

The increased rate of purine de novo synthesis associated with elevated urate output in HPRT deficiency[12] might be expected to be reflected in the de novo pathway substrates or precursors. Serine, a precursor for both glycine and folate, is reduced only in HPRT deficiency, glycine is unchanged. Taurine was decreased in ADA deficiency.

These studies have revealed multiple metabolic perturbations outside the nucleotide metabolism pathways associated with the primary enzyme defects. The metabolites identified may serve as markers of clinical status and invite their consideration as potential markers for efficacy of treatment or disease progression. The specimens examined in this study have not been controlled for dietary manipulation or supplementation and causal relationships with the genetic disorders and disease progression require further studies. The possible benefit of supplementary metabolic therapy is raised by these findings. The increase in methylmalonic acid in APRT deficient patients raises the possibility that B12 supplementation might be beneficial. In those conditions where abnormal levels of acylcarnitines accumulate there may also be a benefit for carnitine supplementation. The metabolomic probing of disorders of nucleotide metabolism has yielded new insights into significant secondary metabolic perturbations.

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