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Untargeted metabolomic profiling of serum from dogs with chronic hepatic disease

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Abstract

Background: Chronic hepatopathies present a diagnostic challenge, with different diseases being associated with similar clinical and laboratory findings. Characterization of dogs with chronic hepatopathies can be difficult and require costly diagnostic procedures such as acquisition of a liver biopsy specimen. Noninvasive and inexpensive biomarkers that reliably characterize chronic hepatopathies such as chronic hepatitis or a congenital portosystemic vascular anomaly may decrease the need for costly or invasive diagnostic testing and guide novel therapeutic interventions.

Objective: To investigate differences in the serum metabolome among healthy dogs, dogs with congenital portosystemic shunts, and dogs with chronic hepatitis.

Animals: Stored serum samples from 12 healthy dogs, 10 dogs with congenital portosystemic shunts, and 6 dogs with chronic hepatitis were analyzed.

Methods: The serum metabolome was analyzed with an untargeted metabolomics approach using gas chromatography–quadrupole time of flight mass spectrometry.

Results: Principal component analysis and heat dendrogram plots of the metabolomics data showed clustering among individuals in each group. Random forest analysis showed differences in the abundance of various metabolites including increased aromatic amino acids and xylitol in dogs with congenital portosystemic shunts. Based on the univariate statistics, 50 metabolites were significantly different among groups.

Conclusions and Clinical Importance: The serum metabolome varies among healthy dogs, dogs with congenital portosystemic shunts, and dogs with chronic hepatitis. Statistical analysis identified several metabolites that differentiated healthy dogs from dogs with vascular or parenchymal liver disease. Further targeted assessment of these metabolites is needed to confirm their diagnostic reliability.

KEYWORDS

chronic hepatitis, congenital portosystemic shunt, biomarker, liver disease, metabolome

Abbreviations: AAA, aromatic amino acid; BCAA, branched chain amino acid.

1 | INTRODUCTION

Chronic hepatopathies in dogs represent a spectrum of diseases that include idiopathic chronic hepatitis, copper-associated chronic hepatitis, drug-associated chronic hepatitis, breed-associated metabolic errors, congenital portosystemic vascular anomalies, lobar dissecting hepatitis, and granulomatous hepatitis.[1] Further characterization of metabolic changes that occur in these diseases will increase our understanding of disease pathophysiology, which may improve disease diagnosis, management, or treatment. Although an early accurate diagnosis is important for an improved clinical outcome, achieving a definitive diagnosis can be cost prohibitive and invasive with the examination of a liver biopsy specimen regarded as the gold standard. The identification of noninvasive biomarkers that can reliably characterize chronic hepatopathies is desirable and may have clinical implications.

The liver is a central organ for regulating metabolism, and a variety of metabolic disturbances are seen in patients with chronic liver disease.[2,3] Data from studies in human and animal models have documented alterations in hepatic lipid metabolism, protein metabolism, energy metabolism, cytokine metabolism, and increased generation of reactive oxygen species in patients with chronic liver disease.[4–6] Global metabolomic profiling, the untargeted quantification of small molecules in biologic samples, allows for a comprehensive analysis of changes in several metabolic and signaling pathways and their interactions.[7–9] This platform utilizes nuclear magnetic resonance spectroscopy or mass spectrometry to measure low-molecular-weight metabolites, permitting the formation of a metabolite profile. Metabolite profiles can be altered by a variety of physiological and pathological processes, and therefore global changes in such profiles may signal the presence of a particular disease.[10,11] Characteristic metabolite profiles that can discriminate among various types of liver disease have been identified in studies of humans.[12–17] To our knowledge, no previous studies have evaluated serum metabolomics in dogs with chronic liver diseases, but a similar study examined the plasma metabolome in chronic liver disease of dogs and found significant differences.[18]

We examined the serum metabolome of dogs with chronic hepatitis, dogs with congenital portosystemic shunts, and clinically healthy dogs. We hypothesized that differences would be found in serum metabolites among the groups and that the 3 groups would have significantly different metabolomes.

2 | MATERIALS AND METHODS

2.1 | Animals

Serum was collected from dogs examined at the Texas A&M University Veterinary Medical Teaching Hospital between September 2011 and August 2017 with histologically confirmed chronic hepatitis or an ultrasonographic diagnosis of a congenital portosystemic shunt. Informed client consent was obtained for each patient and the study was approved by the Texas A&M University Institutional Animal Care and Use Committee (Animal Use Protocols #2014-0142, 2014-0320, and 2015-0043). Patients with chronic hepatitis underwent a laparoscopic liver biopsy, and 5 to 8 closed-cup forceps liver biopsy specimens of 5 mm were collected. One liver biopsy specimen was collected in a sterile tube for copper quantification by atomic absorption spectroscopy (expressed as μg/g dry weight), and another was collected and stored in a sterile specimen cup for aerobic and anaerobic culture and susceptibility testing. The remaining liver tissue from dogs with chronic hepatitis was fixed in neutral buffered formalin for routine histological processing. Histological sections from formalin-fixed paraffin embedded tissue were stained with hematoxylin and eosin, picrosirius red, and rhodamine. The diagnosis of chronic hepatitis was based on clinical signs, routine serum biochemistry test results, and histological assessment of liver specimens by a board-certified veterinary pathologist according to the World Small Animal Veterinary Association Liver Standardization Group Guidelines.[19] Dogs with hepatic copper content >400 μg/g dry weight, centrilobular copper accumulation, and an associated inflammatory infiltrate were characterized as having copper-associated chronic hepatitis. Stage of hepatic fibrosis, grade of necroinflammatory activity, and semiquantitative assessment of copper content were assessed using a previously published scoring scheme.[20,21] Dogs with suspected congenital portosystemic shunting based on compatible clinical signs and clinicopathologic findings were definitively diagnosed on the basis of 2-dimensional, gray-scale ultrasonography as previously described.[22] Dogs with a history of current systemic disease such as hyperadrenocorticism, cancer, or others were excluded from the study. All patients with chronic hepatitis or a congenital portosystemic shunt had a complete abdominal ultrasound examination and were assessed for acquired portosystemic shunting sonographically as described previously.[23] Dogs with acquired portosystemic shunting were excluded from the study. Dogs presented for a wellness examination to the Small Animal Hospital at Texas A&M University, College Station, Texas, were used as healthy controls after normal physical examination. Informed client consent was obtained for each dog, and sample collection was approved by the Texas A&M University Institutional Animal Care and Use Committee Animal Use Protocol #2014-0251.

2.2 | Serum sample collection

Serum from all dogs was obtained from non-heparinized whole-blood samples that were submitted to the clinical pathology service or the gastrointestinal laboratory of Texas A&M University College of Veterinary Medicine where serum was separated by centrifugation at 2150 *g* for 10 minutes at 4°C. Serum biochemical analysis was performed using an Ortho Vitros 4600 analyzer, and the remaining serum was stored at 4°C for 72 hours before retrieval by study investigators for storage at −80°C. All serum samples were stored for a similar length of time. Food was withheld for a minimum of 12 hours before blood sample collection.

2.3 | Medical record data collection

The information recorded from the medical record of dogs with chronic hepatitis and congenital portosystemic shunting included age,

breed, sex, diet, medical treatment at the time of enrollment, serum biochemical markers of liver disease, and histologic or sonographic diagnosis, respectively. For control dogs, recorded medical record data included age, breed, sex, diet, medical treatment at the time of enrollment, and serum biochemical markers of liver disease.

2.4 | Serum metabolomics analysis

Untargeted metabolomics analysis was performed by the West Coast Metabolomics Center at the University of California (Davis, California) on a fee-for-service basis. Serum aliquots were extracted by degassed acetonitrile. Internal standards C8-C30 fatty acid methyl ethers were added, and the samples were derivatized by methoxyamine hydrochloride in pyridine and subsequently by N-methyl-N-trimethylsilyltrifluoroacetamide for trimethylsilylation of acidic protons. Analytes were separated using an Agilent 6890 gas chromatograph (Santa Clara, California), and mass spectrometry was performed on a Leco Pegasus IV time of flight mass spectrometer (St. Joseph, Michigan) following a published protocol.[24] Unnamed peaks were excluded from statistical analysis.

2.5 | Statistical analysis

2.5.1 | Univariate analysis

Differences in the abundance of serum metabolites among the chronic hepatitis, congenital portosystemic shunt, and healthy control groups were evaluated using a Kruskal-Wallis test. Univariate analysis was performed using JMP Pro 13 (JMP Software, Marlow, England). *P*-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate and significance was set at $q < 0.10$.[25] An estimate of the false discovery rate (*q*-value) was calculated to take into account the effect of multiple comparisons. A small *q*-value ($q < 0.10$) is an indication of high confidence, whereas a larger *q*-value indicates decreased confidence of a result. Post hoc testing was performed using Dunn's Test of multiple comparisons using JMP Pro 13, and significance was set at $P < 0.05$.

2.5.2 | Multivariate analysis

Data reported as peak height were log transformed before multivariate analysis. Principal component analysis, random forest analysis, and hierarchical cluster analysis were performed using MetaboAnalyst 4.0 (http://www.metaboanalyst.ca).[26]

3 | RESULTS

3.1 | Selection criteria of case and control dogs

Twelve healthy control dogs enrolled in the study based on normal physical examination and the absence of any reported clinical signs. Three of the 10 dogs with portosystemic shunts had an intrahepatic anomaly, whereas the other 7 had an extrahepatic anomaly. All 10 dogs had blood ammonia concentrations >50 μ/dL, but compatible clinical signs only were reported in 4. Three of the 6 dogs with chronic hepatitis had copper-associated chronic hepatitis and 3 had idiopathic chronic hepatitis based on histopathologic findings and a quantitative liver copper determination. No evidence of acquired portosystemic shunting was identified in any of the dogs enrolled in the study.

3.2 | Animal population

The demographics of the healthy control dogs, congenital portosystemic shunt dogs, and chronic hepatitis dogs enrolled in the study are summarized in Table 1. Breeds of dogs included in each study group, diet, and medical treatment are summarized as supporting information (Table S1). No significant difference was found in the distribution of sex among groups. A statistically significant difference in age was found with chronic hepatitis dogs being older than healthy control or congenital portosystemic shunt dogs. A statistically significant difference in weight was found with chronic hepatitis dogs weighing more than healthy control or congenital portosystemic shunt dogs.

3.3 | Laboratory and pathology findings

Serum biochemistry results relevant to hepatic inflammation, cholestasis, and the blood ammonia concentration from the dogs with congenital portosystemic shunting are summarized in Table 2. Serum biochemistry results relevant to hepatic inflammation, cholestasis, and the liver copper concentration from the dogs with chronic hepatitis are summarized in Table 3. Histologic diagnosis and anatomic pathology scores from the dogs with chronic hepatitis are provided as supporting information (Table S2). Serum biochemistry results relevant to hepatic inflammation and cholestasis from control dogs are provided as supporting information (Table S3).

3.4 | Effect of congenital portosystemic shunting and chronic hepatitis on serum metabolomics

A total of 126 named serum metabolites were identified, of which 50 differed significantly ($P \leq 0.05$; $q < 0.10$) among healthy control

TABLE 1 Control (n = 12), congenital portosystemic shunt (n = 10), and chronic hepatitis (n = 6) dog demographics

Heading	Control (n = 12)	Congenital portosystemic shunt (n = 10)	Chronic hepatitis (n = 6)	*P*-value
Age in years (mean ± SD)	3.75 ± 1.9	3.3 ± 2.0	8.67 ± 4.0	.0006
Sex (male/female)	6/6	4/6	2/4	.78
Weight in kilogram (median/range)	15.5/3-32	6.5/2-35	30.3/10-48.6	.02

TABLE 2 Serum biochemistry variables pertinent to hepatic inflammation and cholestasis and blood ammonia in dogs with a congenital portosystemic shunt (n = 10)

Clinical pathological variable	Median	Range	Number (%) of dogs with a result outside reference interval	Reference interval
Alkaline phosphatase (IU/L)	126.5	38–343	3/10 (30)	24–147
Alanine transaminase (IU/L)	181.5	53–407	6/10 (60)	10–130
Gamma-glutamyl transferase (IU/L)	12	<10–15	0/10 (0)	0–25
Total Bilirubin (mg/dL)	0.35	<0.1–0.5	0/10 (0)	0–0.8
Ammonia (μg/dL)	136.5	73–696	10/10 (100)	<50

dogs, congenital portosystemic shunt dogs, and chronic hepatitis dogs (Table 4). Principal component analysis plots showed clustering of variables based on disease classification (Figure 1). The distribution of the most significant metabolites separating the 3 experimental groups was visualized with a heat map (Figure 2). Every column represents a different sample and each box represents a metabolite in the sample. Increased abundances are shaded red, whereas decreased abundances are shaded blue. The congenital portosystemic shunt group showed greater abundances of the aromatic amino acids (AAAs) tyrosine and phenylalanine and decreased abundances of the branched chain amino acids (BCAAs) leucine, isoleucine, and valine when compared to healthy dogs and dogs with chronic hepatitis. Random forest analysis identified metabolites that had the highest discriminatory power among the 3 groups (Figure 3). Individual compounds in serum that contributed most to the accuracy of disease classification are shown in Figure 3. A subset of those highly discriminatory metabolites was selected, and individual sample results were plotted (Figures 4 and 5).

4 | DISCUSSION

We identified extensive metabolic abnormalities in the serum of dogs diagnosed with congenital portosystemic shunting or chronic hepatitis, with significant alterations in 50 of 126 named serum metabolites. These metabolites are known to be involved in a variety of processes, including aminoacyl-tRNA biosynthesis, branch chain amino acid biosynthesis and degradation, proline metabolism, phenylalanine metabolism, citrate cycle, and pantothenate and coenzyme A biosynthesis.

4.1 | Abnormal amino acid metabolism

Significant abnormalities were observed in the state of conjugation and relative amounts of individual amino acids in dogs with congenital portosystemic shunts and dogs with chronic hepatitis compared to healthy control dogs. Among these findings was a significant increase in the abundance of the AAAs tyrosine and phenylalanine, and a significant decrease in the BCAAs, leucine, isoleucine, and valine in dogs with congenital portosystemic shunts (Figure 4). The decreased serum ratio of BCAAs to AAAs is a hallmark of liver cirrhosis in humans and is attributable to several factors, including decreased nutritional intake, hypermetabolism, and ammonia detoxification by skeletal muscle.[27] A low serum BCAA/AAA ratio decreases biosynthesis and secretion of albumin by hepatocytes and also is associated with a less favorable prognosis in human patients with chronic liver disease.[28,29] The BCAAs are not only a constituent of protein but also a source of glutamate, which detoxifies ammonia by glutamine synthesis in skeletal muscle.[27] The changes in metabolism of BCAAs and AAAs that occur as an epiphenomenon of liver disease also play a role in the pathogenesis of complications of end-stage liver disease, such as hepatic encephalopathy and hypoalbuminemia.[30] Additionally, the serum concentration of the AAA tyrosine has been found to increase early in the course of chronic liver disease in humans and to positively correlate with histologic fibrosis scores.[31] A low serum BCAA/AAA ratio was only observed in dogs with congenital portosystemic shunting and may represent decreased hepatic function because of hypoperfusion, consistent with previous studies.[32–34] A low BCAA/AAA ratio was not observed in the dogs in our study with chronic hepatitis, but none of these dogs had any evidence of acquired portosystemic shunting. The BCAA/AAA ratio is considered an indicator of hepatic insufficiency that decreases with the severity of hepatic dysfunction or portosystemic shunting.[33] Further studies evaluating the BCAA/AAA ratio over the course of chronic liver disease in dogs and in dogs with acquired portosystemic shunting are warranted. Ours is the first study to examine the serum metabolome of dogs with chronic liver disease using an untargeted approach. Additional

TABLE 3 Serum biochemistry variables pertinent to hepatic inflammation and cholestasis and hepatic copper concentrations in dogs with chronic hepatitis (n = 6)

Clinical pathological variable	Median	Range	Number (%) of dogs with value outside reference interval	Reference interval
Alkaline phosphatase (IU/L)	283	98–481	4/6 (66.7)	24–147
Alanine transaminase (IU/L)	519	231–989	6/6 (100)	10–130
Gamma-glutamyl transferase (IU/L)	12	<10–32	1/6 (16.7)	0–25
Total Bilirubin (mg/dL)	0.3	<0.1–0.4	0/6 (0)	0–0.8
Tissue copper (μg/g dry weight)	494.5	170–1080	3/6 (50)	120–400

TABLE 4 Serum metabolites that differed significantly between healthy control dogs (n=12), dogs with a congenital portosystemic shunt (n=10), and dogs with chronic hepatitis (n=6). KW – Kruskal – Wallis Test

Compound name	*p*-value_KW	*q*-value_KW
xylitol	4.70E-05	3.62E-03
phenylalanine	5.75E-05	3.62E-03
valine	1.84E-04	6.30E-03
tyrosine	2.35E-04	6.30E-03
phenylethylamine	3.00E-04	6.30E-03
cysteine	2.67E-04	6.30E-03
serine	4.24E-04	7.05E-03
leucine	5.04E-04	7.05E-03
2-deoxypentitol	4.75E-04	7.05E-03
methionine	6.85E-04	8.48E-03
ethanolamine	7.40E-04	8.48E-03
threonine	9.03E-04	8.86E-03
3-phenyllactic acid	9.14E-04	8.86E-03
arachidic acid	1.06E-03	9.54E-03
lactamide	1.70E-03	1.43E-02
methionine sulfoxide	1.94E-03	1.43E-02
hypoxanthine	1.93E-03	1.43E-02
isoleucine	2.19E-03	1.53E-02
trans-4-hydroxyproline	2.57E-03	1.70E-02
succinic acid	3.23E-03	1.85E-02
mannose	3.20E-03	1.85E-02
creatinine	3.20E-03	1.85E-02
uric acid	3.92E-03	2.12E-02
inosine	4.04E-03	2.12E-02
pyruvic acid	4.60E-03	2.32E-02
pseudo uridine	5.36E-03	2.60E-02
stearic acid	6.11E-03	2.75E-02
aspartic acid	5.93E-03	2.75E-02
glycolic acid	7.27E-03	3.16E-02
2-deoxytetronic acid	7.85E-03	3.30E-02
myristic acid	1.03E-02	4.17E-02
hexuronic acid	1.13E-02	4.44E-02
thymidine	1.28E-02	4.88E-02
4-hydroxyphenylacetic acid	1.33E-02	4.92E-02
ribonic acid	1.52E-02	5.44E-02
proline	1.55E-02	5.44E-02
sucrose	1.75E-02	5.94E-02
myo-inositol	1.79E-02	5.94E-02
urea	1.95E-02	6.29E-02
uracil	2.01E-02	6.33E-02
ribose	2.20E-02	6.61E-02
cytosin	2.17E-02	6.61E-02
2-ketoisocaproic acid	2.51E-02	7.36E-02
conduritol-beta-epoxide	2.85E-02	8.16E-02
citric acid	3.38E-02	9.06E-02
allantoic acid	3.30E-02	9.06E-02
4-hydroxymandelic acid	3.31E-02	9.06E-02
pipecolinic acid	3.51E-02	9.21E-02
5-methoxytryptamine	3.84E-02	9.87E-02

(Continues)

TABLE 4 (Continued)

research to characterize the role of these metabolic changes in disease diagnosis, stratification, and treatment is warranted.

4.2 | Potential biomarkers for dogs with congenital portosystemic shunts and dogs with chronic hepatitis

A number of compounds in serum were individually identified for their value in distinguishing among healthy dogs, dogs with chronic hepatitis, and dogs with congenital portosystemic shunts. Random forest analysis identified 15 metabolites that were able to differentiate dogs with congenital portosystemic shunts (100% accuracy) and dogs with chronic hepatitis (83% accuracy) from apparently healthy dogs (Figure 3). Significant decreases in serum proline and hydroxyproline were found in the dogs with congenital portosystemic shunts and in dogs with chronic hepatitis compared to healthy control dogs (Figure 5). Hydroxyproline is a non-proteinogenic amino acid that is fairly specific to collagen in mammals, and its serum concentration reflects collagen metabolism in tissues with a high metabolic turnover of this protein.[35] Additional research is warranted to determine the relevance of this finding for

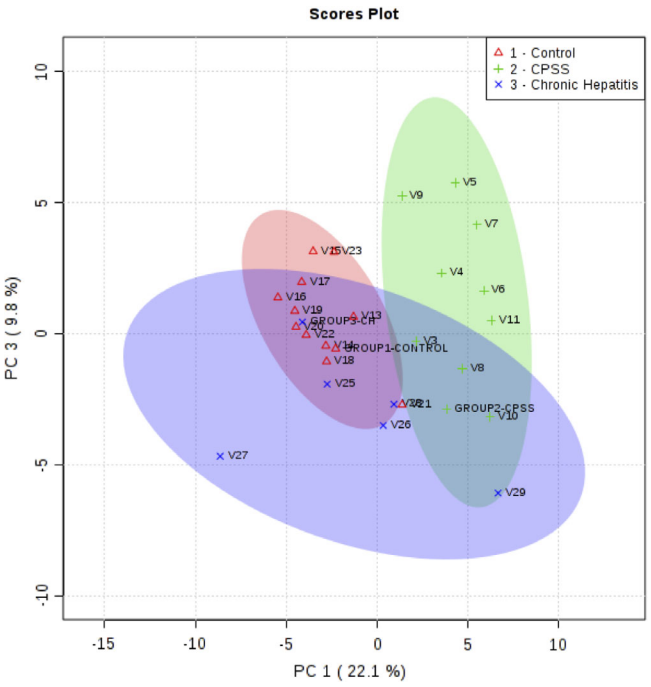

FIGURE 1 Principal component analysis of the serum metabolome showing clustering of samples based on group. The scores plot shows a clustering of groups

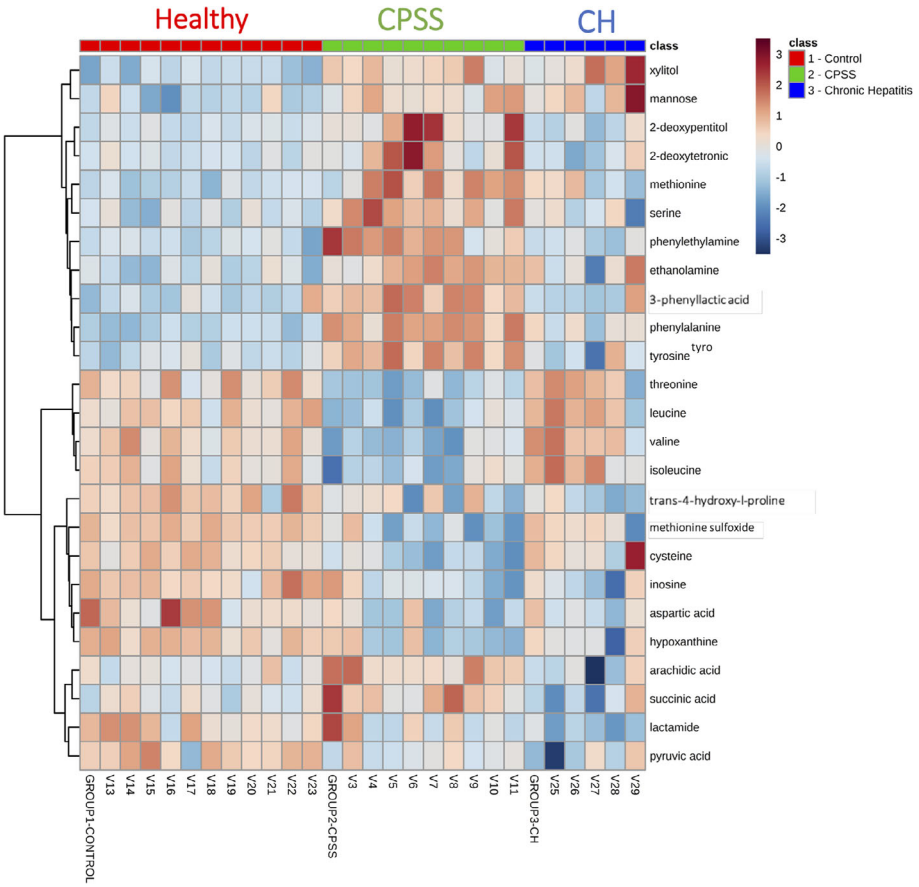

FIGURE 2 Heat map of the most abundant metabolites in all groups, as identified by VIP scores in PLS-DA. Each sample is represented by a single column. The higher the intensity of the red color, the higher the abundance of the metabolite. CH, chronic hepatitis; CPSS, congenital portosystemic shunt; PLS-DA, partial least-squares discriminant analysis; VIP, variable importance in projection

collagen metabolism in dogs with hepatic fibrosis and the role of this molecule as a marker of disease progression. A significant increase in xylitol also was found in the serum of dogs with chronic hepatitis and dogs with congenital portosystemic shunts compared to healthy control dogs (Figure 5). Xylitol, a 5-carbon polyalcohol, is widely distributed in nature with trace amounts of xylitol produced by animals.[36] Although xylitol can enter almost all cells of an organism, hepatocytes are especially permeable.[36] This molecule, which is part of xylose metabolism,

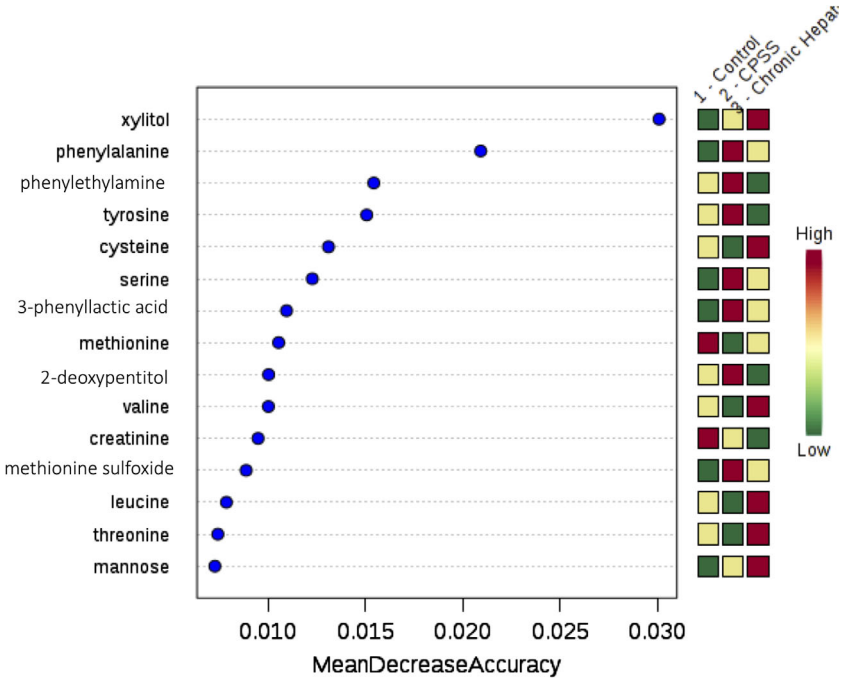

FIGURE 3 Top 15 metabolites in serum (left panel) in rank order of importance for differentiating among control dogs, congenital portosystemic shunt dogs, and chronic hepatitis dogs. Data were generated by random forest analysis. Compounds contributing the most to the accuracy of disease classification were identified as those having the highest mean decrease accuracy (MDA) of the predicted classification when permuted within the data set

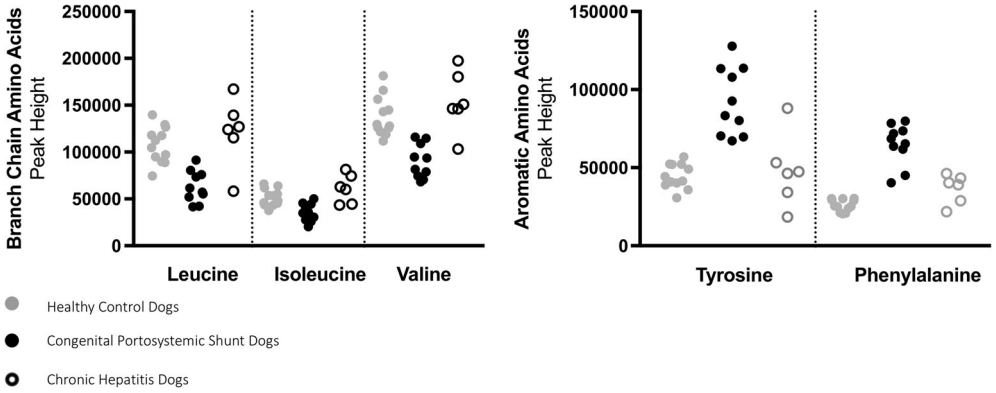

FIGURE 4 Branch chain amino acids and aromatic amino acids identified as potential biomarkers of congenital portosystemic shunting and chronic hepatitis in dogs. Each metabolite was identified by random forest or univariate analysis as important for differentiation of dogs into control, congenital portosystemic shunt, or chronic hepatitis groups. The abundance of phenylalanine was significantly increased in dogs with a congenital portosystemic shunt compared to control dogs ($q < 0.0001$), and the abundance of tyrosine was significantly increased in dogs with a congenital portosystemic shunt compared to control dogs ($q = 0.0003$) and chronic hepatitis dogs ($q = 0.01$). The abundance of leucine, isoleucine, and valine was significantly decreased in dogs with a congenital portosystemic shunt compared to control dogs ($q = 0.003$, 0.01, 0.002, respectively) and compared to chronic hepatitis dogs ($q = 0.002$, 0.005, 0.001, respectively). Peak height is a unit less and represents relative intensity

has been found to be increased in the serum and urine of humans with liver disease.[17,37,38] The role of this metabolite in liver disease has not been fully characterized.

4.3 | Future potential medical strategies

Limited evidence-based strategies are available for the medical management of dogs with chronic hepatitis or congenital portosystemic shunting. A number of compounds identified in our study have a potential therapeutic role in the management of patients with liver disease. Studies in humans have shown that IV or PO supplementation of BCAAs improves not only nutritional status but also prognosis and quality of life in patients with chronic liver disease.[14] The presence and impact of a clinically relevant deficiency or overabundance of any of these compounds will require validation in a targeted quantitative analysis.

4.4 | Study limitations

Limitations of our study include the influence of concurrent drug administration on our findings and the lack of inclusion of a group of dogs with other forms of hepatic disease such as acute hepatitis. Some of our findings also may be observed in dogs with extrahepatic disease, and the effect of diet on circulating metabolites was not considered. Control dogs were not assessed for portosystemic shunting and therefore the possibility of subclinical portosystemic shunting in this group exists. These findings will need to be validated in a prospective targeted study in healthy control dogs, chronic hepatitis dogs, congenital portosystemic shunt dogs, and dogs with other forms of hepatic or gastrointestinal disease. Another limitation of our study is the discrepancy in the ages of dogs with chronic hepatitis compared to healthy control dogs and dogs with congenital portosystemic shunting. The random forest analysis may have identified some compounds

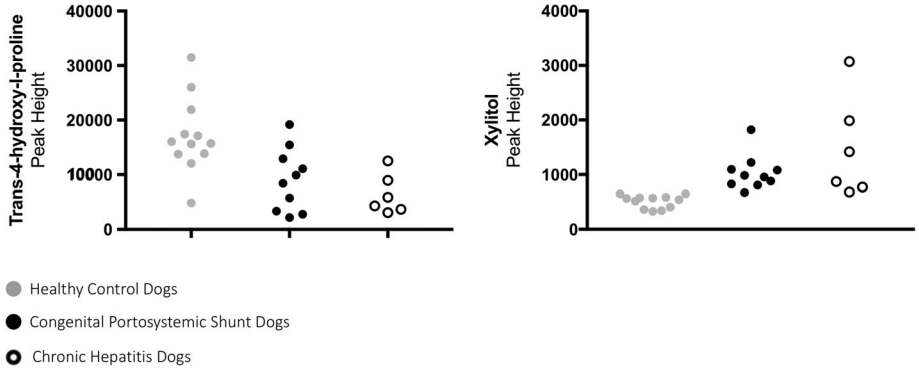

FIGURE 5 Selected serum metabolites with potential value as biomarkers of a congenital portosystemic shunt or chronic hepatitis dogs. Each metabolite was identified by random forest, univariate analysis, or both as important for the differentiation of dogs into control, congenital portosystemic shunt, or chronic hepatitis groups. The serum abundance of trans-4-hydroxy-l-proline was significantly increased in control dogs compared to dogs with a congenital portosystemic shunt or chronic hepatitis ($q = 0.02$, 0.01, respectively). The serum abundance of xylitol was significantly decreased in control dogs compared to dogs with a congenital portosystemic shunt or chronic hepatitis ($q = 0.0003$, 0.001, respectively). Peak height is unit less and represents relative intensity

simply on the basis of a difference in age among these groups of dogs. Because metabolites in serum are affected by the cumulative effect of complex physiological processes across all tissues in the body, it is not possible to definitively determine the organ, cell type, or intracellular compartment from which identified compounds originated. Furthermore, the impact of renal excretion versus reabsorption and intestinal microbial metabolism on the types and quantity of compounds detected in the serum is unknown. Metabolomic investigation of the urine of dogs with chronic hepatitis and congenital portosystemic shunting compared to healthy control dogs would provide additional insight into the metabolic abnormalities identified in our study.

5 | CONCLUSION

The untargeted metabolomic profiling of serum from dogs with chronic hepatitis or dogs with congenital portosystemic shunts identified significant semi-quantitative differences in 50 of 126 named serum metabolites, including BCAAs, AAAs, xylitol, and hydroxyproline. Further validation of these results in a targeted study and determination of their utility as clinical biomarkers are warranted and under way.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The study was approved by the Texas A&M University IACUC (Animal Use Protocol #2014 0142, 2014-0320, and 2015-0043).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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