Medical Research Archives, Volume 4, Issue 7.

RNA-Seq on patients with chronic inflammatory response syndrome (CIRS) treated with vasoactive intestinal peptide (VIP) shows a shift in metabolic state and innate immune functions that coincide with healing

Authors:

James C Ryan1*

Ritchie C Shoemaker^{1,2}

Authors Note:

¹ ProgeneDx, LLC, Deerfield Beach, FL 33442 USA

² Center for Research on Biotoxin-Associated Illnesses, Pocomoke, MD, USA

* Corresponding author

jryan@progenedx.com,

Deerfield Beach, FL 33442 USA

Key words: Gene expression, HLA, Immune, Chronic inflammation, Ribosomal inactivating proteins, Transcriptomics, Vasoactive Intestinal Peptide

Abbreviations used:

C4a split product of activation of complement component 4.

CIRS-WDB chronic inflammatory response syndrome caused by exposure to the interior environment of water-damaged buildings

ERMI Environmental Relative Moldiness Index HERTSMI-2 Health Effects Roster of Type Specific Formers of Mycotoxins and Inflammagens, version -2

MMP9 matrix metalloproteinase 9

TGFB1 transforming growth factor beta-1

VEGF vascular endothelial growth factor

VIP vasoactive intestinal polypeptide

Abstract

Exposure to the microbial growth in Water Damaged Buildings (WDB) can result in a chronic, multi-symptom, multisystem illness, last years, termed Chronic which can Inflammatory Response Syndrome (CIRS). Symptoms of CIRS include but are not limited to fatigue, respiratory problems, including cough, interstitial lung disease and asthma-like executive cognitive conditions; deficits. neurologic deficits, pain and gastrointestinal distress among others. There are published treatment options for CIRS patients but relatively little is known about the genomic basis of the illness. One of the most striking features of CIRS is the absence of the anti-inflammatory neuropeptides vasoactive intestinal peptide (VIP) and melanocyte stimulating hormone (MSH). Additionally, the master immune regulator, $TGF \square 1$ is abnormally high in most patients. This study surveyed 14 patients coming to a specialized clinic for CIRS with refractory symptoms, despite use of a published treatment protocol. who agreed to have their transcriptomes monitored while receiving off label, exogenous VIP nasal spray treatments. Several key immune regulators were found to be differentially expressed over the course of treatment with VIP, such as CD244, CD3D, CD48 and CD52, granzymes, defensins, and the Ikaros family of lymphoid transcription factors. In addition to down regulated innate immune functions, there appears to be an overall metabolic shift, with a down regulation of ribosomal and mitochondrial gene expression, possibly indicating a calming of components of immune responses. Understanding the need for accurate diagnosis of CIRS in order to effectively treat patients, this transcriptomic study sheds important light on new aspects of pathophysiology.

1. Background

Chronic inflammatory response syndrome (CIRS) is a chronic, progressive, multi-system, multi-symptom syndrome characterized by biotoxins. exposure to HLA genetic predisposition, altered innate and adaptive immunity, peripheral hypoperfusion at multiple sites and multiple hypothalamic-pituitary-end organ dysregulations (R. C. Shoemaker, Ryan, J.C., 2013). This inflammatory dysregulation can affect virtually any organ system of the body and if left untreated can become debilitating. Diagnosis of CIRS begins with a lengthy symptom registry followed by exposure risk analysis. CIRS diagnosis is completed using objective blood labs, with the most commonly seen abnormalities including key regulators of inflammation such as elevated transforming growth factor beta 1 (TGF \Box -1) and depressed vasoactive intestinal peptide (VIP) (Gonzalez-Rey & Delgado, 2005) and melanocyte stimulating hormone (MSH) (Catania, Airaghi, Colombo, & Lipton, 2000; R. C. Shoemaker, Ryan, J.C., 2013).

There are several known triggers for CIRS; more are likely to be discovered. Ciguatera fish poisoning results from eating fish contaminated with the marine toxin ciguatoxin. A single exposure to this toxin may result in CIRS-Ciguatera (Ryan, Wu, & Shoemaker, 2015). Many who suffer chronic illness seen after antibiotic therapy of acute Lyme disease, Post Lyme disease syndrome, present with CIRS-Lyme (R. C. Shoemaker et al., 2006). CIRS-WDB is CIRS developed after chronic exposure to the interior of water-damaged buildings typified by resident microbial growth, including bacteria. filamentous fungi (molds), mycobacteria and actinomycetes; together with resultant biologically produced toxins and inflammagens, including mannans, beta glucans, hemolysins, and proteinases (R. C. Shoemaker & House, 2006).

Regardless of initial trigger, all CIRS show similar characteristics in their final manifestations. Patients with CIRS are often

misdiagnosed as having depression, anxiety, post-traumatic stress disorder and somatization; as well as Alzheimer's, Parkinsonism, allergy, fibromvalgia and Chronic Fatigue Syndrome. among others. Treating patients for these seemingly diverse conditions does not improve their symptoms of CIRS, although effective therapies for CIRS exist. VIP administration has been shown to reduce symptoms and corresponds with normalization of blood labs for patients refractory to standard therapies. To gain an understanding of the effects of VIP on CIRS at the cellular level, this study used a top down globally at approach, looking immune perturbations through gene expression, seeking elements implicated in pathophysiology. By focusing on global gene expression in the blood, we hope to broaden our understanding of dysfunctional systems, encourage a focus on the proteomic discovery of biomarkers and establish molecular guideposts for treatment protocols in these patients.

2. Methods

2.1. Subjects

Participation in this study was voluntary; informed consent was obtained from patients to use their data for research purposes. Institutional Review Board (IRB) approval for this study was granted by Copernicus Group IRB, LLC, Research Triangle Park, NC. Medical history and symptoms registries were recorded on all patients, as well as physical exam and blood labs before and after VIP use. Patients (9 females and 5 males) all met a case definition established in 2008 by the US GAO (Stephenson JB, 2008) with satisfaction of (a) the potential for exposure to a water-damaged building with microbial growth following water intrusion combined with (i) visible microbial growth or; (ii) identification of fungi to species level using MSQPCR or; (iii) presence of musty smells; (b) presence of symptoms seen in published peer-reviewed literature; presence of laboratory (c) abnormalities seen in published, peer-viewed literature and (d) response to treatment. Patients

were treated with a published, sequential intervention protocol, with improvement confirmed by reduction of symptoms, correction of visual contrast sensitivity testing (R. C. Shoemaker, 2005) and resolution of many proteomic abnormalities (R. C. Shoemaker, Rvan, J.C., 2013). For patients still functioning at less than pre-morbid state, use of intra-nasal VIP (Hopkinton Drug, MA) was offered with PAXgene tubes drawn before beginning VIP and at final endpoint. Patients had no confounding illnesses (e.g., untreated diabetes, acute hepatitis or presence of any illness requiring acute intervention).

2.2. Serum protein labs

Laboratory blood tests were performed by CLIA-licensed facilities, LabCorp, Quest Diagnostics, National Jewish Center (Denver) and Cambridge Biomedical. Testing included alpha melanocyte stimulating hormone (MSH), VIP, matrix metalloproteinase 9 (MMP9), split products of complement component 3 (C3a) and C4a, TGF β -1, vascular endothelial growth factor (VEGF), lipid profile, complete blood count (CBC), comprehensive metabolic panel (CMP), gamma-glutamyl transpeptidase (GGTP), thyroid stimulating hormone (TSH), lipid profile, and von Willebrand's profile. Patients with levels of Factor VIII, von Willebrand's antigen or ristocetin associated cofactor either < 50 or > 150 IU were classified as abnormal for von Willebrand's antigen. Dysregulation of simultaneously measured adrenocorticotropic hormone (ACTH) /cortisol and antidiuretic hormone (ADH)/osmolality was determined by (i) absolute high (ACTH > 45 or cortisol > 21; ADH > 13 or osmolality > 300) or low (ACTH <5 or cortisol < 4; ADH < 1.3 or osmolality <275) values; or (ii) ACTH was below 10 when cortisol was below 7; or ADH was below 2.2 when osmolality was 292-300 for the twopaired tests; or (iii) ACTH was > 15 when cortisol was > 16; and ADH > 4.0 when osmolality was 275–278 for the two-paired tests. The diagnosis of CIRS was made using the standard process of differential diagnosis,

including the assessment of exposure risks, symptoms and blood lab results that included abnormalities in serum protein markers as described previously (R. C. Shoemaker, Ryan, J.C., 2013). Briefly, patients must have presented with abnormal results in at least four of the eight objective serum markers found most informative for CIRS, which are TGF β 1, VIP, MSH, MMP9, C4a, VEGF, ACTH/cortisol and ADH/osmolality; and have completed 10 of the 11 steps of sequential therapy.

2.3. Administration of VIP

For patients with normal VCS, normal MSQPCR (ERMI or HERTSMI-2), negative nasal culture, and with normal lipase who wanted to enroll in the trial, a single test dose of VIP, one spray consisting of 50 mcg/0.1 ml, was given in one nostril while being monitored in the medical office after baseline lab testing was drawn. There were no adverse events noted. Repeat labs were drawn 15 minutes after the test dose, understanding that a rise of TGF beta-1 was correlated with ongoing exposure to WDB (unpublished). Titration of VIP dosing began with one spray given four times a day for one month, increasing to two sprays four times a day each month if symptoms were not resolved by the end of the first month of VIP treatment. Patients were allowed to stay on VIP as long as they felt improvement or maintained stability. There were no dropouts for adverse events during the clinical trial.

2.4. RNA collection, extraction and labeling

Venous blood was drawn from the arms of subjects into PAXgene RNA blood collection tubes (http://www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/), incubated for four hours at room temperature, then frozen at -80 °C until RNA extractions were performed. Total RNA was extracted with the Qiagen PAXgene Blood miRNA System kit according to manufacturer's protocol. The total RNA was analyzed using an Agilent 2100 bioanalyzer (Agilent Technologies,

USA) for RNA integrity and was then quantified using a NanoDrop ND-2000 (Wilmington, DE). Only samples with Agilent RIN scores ≥ 8 were used for sequencing.

2.5. Sequencing

Total RNA samples were submitted to the North Carolina State Genomic Sciences Laboratory (Raleigh, NC, USA) for Illumina RNA library construction and sequencing. Prior to library construction, RNA integrity was assessed using an Agilent 2100 Bioanalyzer. Purification of messenger RNA (mRNA) was performed using the oligo-dT beads provided in the NEBNExt Poly(A) mRNA Magnetic Isolation Module (New England Biolabs, USA). Complementary DNA (cDNA) libraries for Illumina sequencing were constructed using the NEBNext Ultra Directional RNA Library Prep Kit (NEB) and NEBNext Mulitplex Oligos for Illumina (NEB) using the manufacturer-specified protocol. The amplified library fragments were purified and checked for quality and final concentration using an Agilent 2100 Bioanalyzer with a High Sensitivity DNA chip (Agilent Technologies, USA). The final quantified libraries were pooled in equimolar amounts for clustering and sequencing on an Illumina NextSeq 500 DNA sequencer, utilizing a 75 bp single end sequencing reagent kit (Illumina, USA) and a target depth of 50 million reads per sample. The software package Real Time Analysis (RTA), was used to generate raw bcl, or base call files, which were then de-multiplexed by sample into fastq files for data submission.

2.6. Sequencing analysis

Fastq sequencing data were imported into CLC Biomedical Genomics Workbench (BGW) analysis software version 2.5.1 and mapped to gene regions of the human genome. For each sample, hemoglobin alpha and beta reads were removed and then data were scaled in transcripts per million. Data were then filtered by using genes that were present in either the before or after treatment points (even if only a single transcript per 50 million reads was found). This typically reduced genes for analysis from a 57,000 known population to 22,000. Genes were normalized again using quantile normalization and the mean was calculated for pre-VIP and post-VIP classes. Using the mean, sequences with counts greater than or equal to 1 per million in both conditions (13,574) were selected for analysis. A two tailed, paired t test was run and genes with p < 0.01 were selected for gene ontology (GO) and molecular pathway analysis to identify the possible enrichment of genes with specific biological themes using Elsevier's Pathway Studio.

3. Results

Nine female and five male patients (mean age 41.9, std 14.27, range 14-61) self-administered VIP as a last stage in CIRS therapy. Duration of use was self-determined and based on patient response (mean duration 6 months, std 4.61, range 1-12 months). Blood samples were drawn before first dose and after last dose of VIP. Comparison of before and after treatments groups was performed by paired t test.

3.1. Symptoms and Blood labs

Patients were interviewed by a physician using a symptoms roster and TGFB1, MMP9, VEGF and C4a blood labs were run both before and after use of VIP. Both symptoms and blood labs normalized over the course of VIP use (table 1). Patient reporting of CIRS symptoms decreased from a mean of 12.9 to 3.3 over the duration of therapy and was shown to be statistically significant using a paired t test. TGF β 1 and C4a were also significantly lower after VIP therapy. MMP9 was lower post VIP, but not significant and VEGF was virtually unchanged.

3.2. Transcriptomics

Because the frequency and duration of VIP use varied from subject to subject, first pass molecular pathway analysis of the t test results was performed with only a minor multiple test

healing

correction in order to capture more genes of potential interest. As a result, the paired t test of Pre-VIP and Post-VIP data resulted in 80 genes with a p < 0.001 (table 2) and 728 genes of interest with p < 0.01. These 728 genes were subjected to functional annotation and cluster analysis using the web tool DAVID v6.7 (The Database for Annotation, Visualization and Integrated Discovery) (Huang da, Sherman, & Lempicki, 2009). The functional annotation tool organizes genes with related functions into clusters and scores their enrichment based on expected incidence from random sampling. Functional annotation of the genes found by t test showed changes in cellular metabolism with hits on mRNA splicing, ribosomal activity and electron transport in mitochondria (table 3). Additionally, all gene expression values were input into Elsevier Pathway Studio for pathway analysis. This program analyzes pathway perturbation based on both amplitude of signal as well as overall change between conditions using Fishers exact test. The output showed ribosomal and mitochondrial molecular pathways and ontologies as the most down regulated while identifying a group of transcription factors, the Ikaros family, as the most importantly up regulated (table 4).

4. Discussion

This study identified inflammation abatement through the use of VIP. Rather than setting a fixed endpoint to the trial, we allowed patients to continue the treatment until they felt they had reached maximum benefit. This could be considered a pitfall to the work, but in fact we consider this to be a strength. Patients entered the trial with varied levels of both VIP and VIP receptor densities, as well as illness intensity and response to the treatment, thusly patient therapy was delivered according to safety and tolerance. This warranted a different study model, one that allowed patients to work through the therapy at their own pace to an endpoint configured around health benefits rather than an arbitrary time point. The following genes and pathways are a few that we found particularly interesting in light of known pathophysiology in CIRS.

4.1. Ribosomes and Mitochondria

Ribosomes are cellular complexes of proteins and RNA that are responsible for protein synthesis while mitochondria produce the energy needed for cellular metabolism. Both have critical roles in the initiation of an immune response as more protein production and energy is quickly required for naïve immune cells to proliferate and differentiate in the face of an inflammatory or infectious threat. Microbes found in water damaged buildings can secrete a wide variety of toxins and possibly chemical compounds that impact ribosomal function. Over 50 years ago a compound secreted from Aspergillus was found to have anti-tumor activity (Olson & Goerner, 1965) by inhibiting protein biosynthesis. It has since been determined that this compound, termed a ribotoxin, is widely produced by filamentous fungi and results in cleavage of the 28S ribosomal RNA at the highly conserved sarcinricin loop (Herrero-Galan et al., 2013). Over 100 similar acting toxins, called ribosomal inactivating proteins (RIPs) have been identified, mainly in plants, with the most wellknown of the group probably being ricin (for review see (Walsh, Dodd, & Hautbergue, 2013). Ricin and other ribotoxins are known to induce an inflammatory response through selective up regulation of cytokine transcription even while depressing overall protein synthesis (Moon, 2014). Trichothecenes are toxic metabolites of various fungal genera, including Fusarium, Stachybotrys, Myrothecium, Spicellum. Cephalosporium. Trichoderma. and Trichothecium. Deoxynivalenol (DON) and T-2 toxin are representatives of type A and B trichothecenes and known to induce rRNA cleavage through indirect methods(Li & Pestka, 2008). Substantial daily exposure to trichothecenes may accrue from consumption of contaminated foods. Additionally, bacterial toxins have also been identified that inhibit protein synthesis, such as shigatoxin, which

similarly acts at the 28S rRNA sarcin-ricin loop and is known to cause hemolytic uremic syndrome (Keir, Marks, & Kim, 2012). More and more ribosomal toxins are being discovered in microbes, such as in the case of Mycobacterium tuberculosis which was recently found to have over 40 toxin-antitoxin pairs of ribotoxin genes (Winther, Brodersen, Brown, & Gerdes, 2013), as well as non-protein ribosome disabling toxins and stress such as palytoxin, anisomysin and UV light (Moon, 2014).

Mitochondria are also dysregulated as a consequence of CIRS, as well as in Chronic Fatigue Syndrome, with these two syndromes showing near complete overlap in symptoms. In mammals, the mitochondrion has transferred most of its genome to the nucleus but still possess their own ribosomes, mitoribosomes. Although different from eukaryotic and prokaryotic isoforms mitoribosome large subunits possess a 16S rRNA containing the highly conserved sarcin-ricin loop (Mears et al., 2006), making them vulnerable to ribotoxins as well. Although the full effects of mitochondrial dysfunction in CIRS is not understood, dysfunction of mitoribosomes may play an important role.

With the diminution of ribosomal and mitochondrial functions, and full immune system engagement in response to an inflammagenic, microbial insult, definitive pathology is hard to predict in patient exposures. One of the biggest problems with identifying illness from exposure to WDBs is the scientific certainty that different materials, humidity, temperature, etc., favor the growth of certain microbes over others. That is to say, no two WDBs are the same, so no two exposures are the same. Additionally, no two people are the same (even identical twins), so host response will also be different. What we do know is that many microbes have evolved survival strategies that target rudimentary, highly conserved cellular processes, capable of debilitating any form of life. Ribosomal genes as well as nuclear encoded mitochondria genes were shown to be down

regulated after treatment with VIP, which coincided with abatement of symptoms. As discussed, when elements of immune response respond to a threat, there is systemic need for rapid and robust expansion. High energy demands are placed on immune cells. Ribosomal biogenesis is known to be an energy intense endeavor for the cell and more genes are expressed to both build protein biosynthesis machinery as well as increase energy output through the mitochondria. In rapidly growing yeast it was reported that ribosome production consumes 60% of total cellular transcription, as well as the majority of RNA splicing events (Warner, 1999). In a negative feedback model, a lack of production from ribosomes and mitochondria would argue the up-regulation of those genes. The down regulation of these genes after VIP, along with abatement of symptoms, argues return of normal function. This type of dysregulation of mitochondrial and ribosomal gene expression has also been reported in metabolic syndrome (Tangen et al., 2013)

4.2. Genes of the innate immune system

Two families of genes that are shown to be upregulated in microarray studies of CIRS are the α -defensions and the granzymes (unpublished data). Granzymes are serine proteases secreted by cytotoxic lymphocytes, including natural killer (NK) cells and cytotoxic T lymphocytes (Wensink, Hack, & Bovenschen, 2015). All five human isoforms we detected in blood were down regulated after VIP treatment (A;-1.39 fold, B;-1.11, H;-1.16, K;-1.28 and M;-1.26). Although substrates for these proteases may partially overlap, they have different cleavage sites and produce different degradation products. It was long thought that granzymes functioned exclusively to kill damaged or infected cells by apoptosis. However, these proteases have demonstrated roles in extracellular matrix remodeling as well as the production, release, and/or processing of pro-inflammatory cytokines (Wensink et al., 2015), processes important to the pathology of CIRS.

Defensins, consisting of alpha and beta subgroups, are cationic, antimicrobial peptides (AMP). The α -defensions can make up more than 5% of all neutrophil protein (Kagan, Ganz, & Lehrer, 1994). All four isoforms of α -defensions found in blood were down-regulated in patients using VIP (A1; -1.44 fold, A1b; -1.48, A3; -1.39 and A4; -1.40). These AMPs act to repel microbial infection by both direct killing of microbes but also by acting as immunomodulators. α Defensins 1–3 are chemotactic for monocytes, immature DCs and CD4+ and CD8+ T-cells, as well as induce the release of pro-inflammatory cytokines such as IFN- λ and IL-6 from T-cells as well as TNF- α and IL-1ß from monocytes (Martin, van Meegern, Doemming, & Schuerholz, 2015).

4.3. Cluster of Differentiation (CD)

CD is a nomenclature system used to classify immune cell surface markers, commonly identifying stages of cell activity or differentiation. Of the 78 CD marker genes remaining after filtering based on signal intensity, the patients in this cohort showed statistical (p<.01) differential expression of 4 CD markers, 3D, 48, 52 and 244, all were down regulated after treatment with VIP and worth a brief mention. CD48 is an immunoglobulin and interestingly its highest affinity partner is CD244, which binds in both a cis and trans manner, meaning both together on the same cell or each on different cells (Claus, Wingert, & Watzl, 2016). The exact immunomodulatory effect of this binding is complex, but among other effects, has been shown to regulate killing activity in natural killer and cytotoxic T cells (McArdel, Terhorst, & Sharpe, 2016). CD52 is a small glycoprotein expressed at high density on lymphoid cells and a highly prized therapeutic target. Lymphocyte depletion techniques using the FDA approved, engineered monoclonal antibody against CD52, alemtuzumab, has been shown to be an effective strategy for treatment of multiple sclerosis, lymphoproliferative diseases such as chronic lymphocytic leukemia, rheumatoid arthritis and organ transplants (for

review see (Holgate, Weldon, Jones, & Baker, 2015). CD3D is part of the T cell receptor (TCR) complex and appears to couple the TCR and CD8 (Doucey et al., 2003). The CD8-TCR interaction is critical for T cell receptor surveillance of peptides presented by Class 1 MHC molecules and determination of self vs non-self-antigens.

4.4. Ikaros Transcription Factors

The Ikaros family of five different zinc finger transcription factors (Ikz1-5) is expressed preferentially in the hematopoietic system and are crucial at two developmental points, first at initial hematopoietic stem cell differentiation between lymphoid and myeloid pathways; and later in maturation and non-proliferative stages of T and B cells (for review see, (Yoshida & Georgopoulos, 2014). The Ikaros factors associate with nucleosome remodeling histone deacetylase (NuRD) chromatin remodeling complex and reduced expression of Ikaros in both humans and mice results in expression of genes associated with cell growth and proliferation, even to the point of T and B cell leukemia. The up-regulation of Ikaros here may indicate a decline in lymphocyte proliferation after treatment with VIP. Interestingly, Ikaros has been shown to up-regulate VIP receptor expression through the 12 putative binding sites in the promotor regions in each of the two VIP receptor genes (Dorsam, Benton, Failing, & Batra, 2011). Patients in the study showed an up-regulation in all 5 Ikaros isoforms (Ikz1; +1.24 fold, Ikz2; +1.41, Ikz3; +1.41, Ikz4; +1.38, and Ikz5; +1.18). Although all five were expressed, Ikz1 had anywhere from 30-300 times the highest expression. However, VIP receptors 1 and 2 showed no change in this study, and of note, VIP receptor 1 (known as constitutive) had 35 times higher expression than VIP receptor 2 (known as inducible).

5. Conclusions

With proper detection and diagnosis, CIRS may be treated effectively. The use of VIP was

Medical Research Archives, Volume 4, Issue 7.

RNA-Seq on patients with chronic inflammatory response syndrome (CIRS) treated with vasoactive intestinal peptide (VIP) shows a shift in metabolic state and innate immune functions that coincide with healing

shown to quell gene expression of several inflammatory features of CIRS and greatly reduce symptoms in patients. Key information regarding the role of ribosomal and mitochondrial transcriptional response, coupled with Ikaros transcription factor activity controlling lymphocyte proliferation gives us a much better understanding of CIRS pathology. This new understanding will be the basis for additional studies going forward, including mitochondrial functional assays. Additionally, with several innate and adaptive inflammatory genes to investigate further, we feel that genomic testing will serve an expanded clinical role as work proceeds on defining the parameters of CIRS.

Funding and Competing interests

This study was funded by ProGene DX, LLC. Drs. Ryan and Shoemaker are principals in ProGene DX, LLC, a private company that researches proteomic and genomic biomarkers of inflammatory illness for use in clinical diagnostics. ProGene DX, LLC has applied for a patent using genes and proteins from whole blood to diagnose chronic inflammatory syndromes.

Table Legend

Table 1. Symptoms and blood labs. Mean values and standard deviation for symptoms and blood labs are reported for patient classes before VIP therapy began (pre) and after VIP therapy concluded (post). T test p values are reported and statistical significance is indicated by shading.

Table 2. Significant genes. Gene list and fold change resulting from paired t test of Pre-VIP and Post-VIP data classes with a p value < 0.001.

Table 3. Functional annotation and cluster analysis. Analysis of 728 genes with p < 0.01using the web tool DAVID v6.7 for functional annotation and cluster analysis. Count = number of genes from category found in dataset, % = percentage of total number of genes in cluster, enrichment = score for magnitude of overrepresentation, Benjamini = p value after Benjamini multiple test correction.

Table 4. Pathway Analysis results. The seven most up and down regulated processes as determined by Gene Set Enrichment Analysis (GSEA) on all data using Elsevier Pathway Studio. # of entities = number of genes in category, # measured = number of genes measured from dataset, change = mean change of genes measured.

References

- Catania, A., Airaghi, L., Colombo, G., & Lipton, J. M. (2000). Alphamelanocyte-stimulating hormone in normal human physiology and disease states. *Trends Endocrinol Metab*, 11(8), 304-308.
- Claus, M., Wingert, S., & Watzl, C. (2016). Modulation of natural killer cell functions by interactions between 2B4 and CD48 in cis and in trans. *Open Biol*, 6(5). doi:10.1098/rsob.160010
- Dorsam, G. P., Benton, K., Failing, J., & Batra, S. (2011). Vasoactive intestinal peptide signaling axis in human leukemia. *World J Biol Chem*, 2(6), 146-160. doi:10.4331/wjbc.v2.i6.146
- Doucey, M. A., Goffin, L., Naeher, D., Michielin, O., Baumgartner, P., Guillaume, P., . . . Luescher, I. F. (2003). CD3 delta establishes a functional link between the T cell receptor and CD8. *J Biol Chem*, 278(5), 3257-3264.

doi:10.1074/jbc.M208119200

- Gonzalez-Rey, E., & Delgado, M. (2005). Role of vasoactive intestinal peptide in inflammation and autoimmunity. *Curr Opin Investig Drugs*, 6(11), 1116-1123.
- Herrero-Galan, E., Garcia-Ortega, L., Olombrada, M., Lacadena, J., Del Pozo, A. M., Gavilanes, J. G., & Onaderra, M. (2013). Hirsutellin A: A Paradigmatic Example of the Insecticidal Function of Fungal Ribotoxins. *Insects*, 4(3), 339-356. doi:10.3390/insects4030339
- Holgate, R. G., Weldon, R., Jones, T. D., & Baker, M. P. (2015). Characterisation of a Novel Anti-CD52 Antibody with Improved Efficacy and Reduced Immunogenicity. *PLoS ONE* [*Electronic Resource*], 10(9), e0138123. doi:10.1371/journal.pone.0138123
- 8. Huang da, W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists

using DAVID bioinformatics resources. *Nat Protoc*, *4*(1), 44-57. doi:10.1038/nprot.2008.211

- Kagan, B. L., Ganz, T., & Lehrer, R. I. (1994). Defensins: a family of antimicrobial and cytotoxic peptides. *Toxicology*, 87(1-3), 131-149.
- Keir, L. S., Marks, S. D., & Kim, J. J. (2012). Shigatoxin-associated hemolytic uremic syndrome: current molecular mechanisms and future therapies. *Drug Des Devel Ther*, 6, 195-208. doi:10.2147/DDDT.S25757
- Li, M., & Pestka, J. J. (2008). Comparative induction of 28S ribosomal RNA cleavage by ricin and the trichothecenes deoxynivalenol and T-2 toxin in the macrophage. *Toxicol Sci*, 105(1), 67-78.

doi:10.1093/toxsci/kfn111

- Martin, L., van Meegern, A., Doemming, S., & Schuerholz, T. (2015). Antimicrobial Peptides in Human Sepsis. *Front Immunol*, 6, 404. doi:10.3389/fimmu.2015.00404
- McArdel, S. L., Terhorst, C., & Sharpe, A. H. (2016). Roles of CD48 in regulating immunity and tolerance. *Clin Immunol*, *164*, 10-20. doi:10.1016/j.clim.2016.01.008
- Mears, J. A., Sharma, M. R., Gutell, R. R., McCook, A. S., Richardson, P. E., Caulfield, T. R., . . . Harvey, S. C. (2006). A structural model for the large subunit of the mammalian mitochondrial ribosome. *J Mol Biol*, 358(1), 193-212. doi:10.1016/j.jmb.2006.01.094
- Moon, Y. (2014). Ribosomal alterationderived signals for cytokine induction in mucosal and systemic inflammation: noncanonical pathways by ribosomal inactivation. *Mediators Inflamm, 2014*, 708193. doi:10.1155/2014/708193
- 16. Olson, B. H., & Goerner, G. L. (1965).Alpha Sarcin, a New Antitumor Agent.I. Isolation, Purification, Chemical Composition, and the Identity of a New

Copyright 2016 KEI Journals. All Rights Reserved.

Medical Research Archives, Volume 4, Issue 7.

RNA-Seq on patients with chronic inflammatory response syndrome (CIRS) treated with vasoactive intestinal peptide (VIP) shows a shift in metabolic state and innate immune functions that coincide with healing

Amino Acid. Appl Microbiol, 13, 314-321.

- 17. Ryan, J. C., Wu, Q., & Shoemaker, R. C. (2015). Transcriptomic signatures in whole blood of patients who acquire a chronic inflammatory response syndrome (CIRS) following an exposure to the marine toxin ciguatoxin. *BMC Medical Genomics*, 8(1). doi:10.1186/s12920-015-0089-x
- Shoemaker, R. C. (2005). Sick Building Syndrome in water-damaged buildings: Generalization of the chronic biotoxinassociated illness paradigm to indoor toxigenic fungi. In E. Johanning (Ed.), *Bioaerosols, Fungi, Bacteria, ,Mycotoxins and Human Health.* (pp. 66-77). Albany, NY: Fungal Research Group Foundation
- 19. Shoemaker, R. C., & House, D. E. (2006). Sick building syndrome (SBS) and exposure to water-damaged buildings: time series study, clinical trial and mechanisms. *Neurotoxicology & Teratology*, 28(5), 573-588.
- Shoemaker, R. C., Hudnell, H. K., House, D. E., Van Kempen, A., Pakes, G. E., & Team, C. O. L. S. (2006). Atovaquone plus cholestyramine in patients coinfected with Babesia microti and Borrelia burgdorferi refractory to other treatment. *Advances in Therapy*, 23(1), 1-11.
- 21. Shoemaker, R. C., Ryan, J.C. (2013). Vasoactive intestinal polypeptide (VIP) corrects chronic inflammatory response syndrome (CIRS) acquired following exposure to water-damaged buildings. *Health*, 5(3), 396-401.
- 22. Stephenson JB, F. C., Anderson KB, Crothers N, Howe B, Johnson RP, Sloss N, Solomon R, Choy L, Derr M,

Feldesman A, Horner T, Liles A, Moy L, Rhodes-Kline A. . (2008). GAO-08-980. United States Government Accountability Office: Indoor Mold: Better Coordination of Research on Health Effects and More Consistent Guidance Would Improve Federal Efforts (GAO-08-980). Retrieved from Washington, DC:

 Tangen, S. E., Tsinajinnie, D., Nunez, M., Shaibi, G. Q., Mandarino, L. J., & Coletta, D. K. (2013). Whole blood gene expression profiles in insulin resistant Latinos with the metabolic syndrome. *PLoS ONE [Electronic Resource]*, 8(12), e84002. doi:10.1371/journal.pone.0084002

24. Walsh, M. J., Dodd, J. E., &

- 24. Walsh, M. J., Dodd, J. E., & Hautbergue, G. M. (2013). Ribosomeinactivating proteins: potent poisons and molecular tools. *Virulence*, 4(8), 774-784. doi:10.4161/viru.26399
- 25. Warner, J. R. (1999). The economics of ribosome biosynthesis in yeast. *Trends Biochem Sci*, 24(11), 437-440.
- 26. Wensink, A. C., Hack, C. E., & Bovenschen, N. (2015). Granzymes regulate proinflammatory cytokine responses. *J Immunol*, 194(2), 491-497. doi:10.4049/jimmunol.1401214
- Winther, K. S., Brodersen, D. E., Brown, A. K., & Gerdes, K. (2013). VapC20 of Mycobacterium tuberculosis cleaves the sarcin-ricin loop of 23S rRNA. *Nat Commun*, 4, 2796. doi:10.1038/ncomms3796
- Yoshida, T., & Georgopoulos, K. (2014). Ikaros fingers on lymphocyte differentiation. *Int J Hematol*, 100(3), 220-229. doi:10.1007/s12185-014-1644-5