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Journal

Pain Forum, 22(12)

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Publication Date

2021-12-01

DOI

10.1016/j.jpain.2021.04.017

Peer reviewed

HHS Public Access

Author manuscript

J Pain. Author manuscript; available in PMC 2023 August 27.

Published in final edited form as:

J Pain. 2021 December ; 22(12): 1586–1605. doi:10.1016/j.jpain.2021.04.017.

Dysregulation in Sphingolipid Signaling Pathways is Associated With Symptoms and Functional Connectivity of Pain Processing Brain Regions in Provoked Vestibulodynia

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Abstract

Provoked vestibulodynia (PVD) is a chronic pain disorder characterized by local hypersensitivity and severe pain with pressure localized to the vulvar vestibule. Despite decades of study, the lack of identified biomarkers has slowed the development of effective therapies. The primary aim of this study was to use metabolomics to identify novel biochemical mechanisms in vagina and blood underlying brain biomarkers and symptoms in PVD, thereby closing this knowledge gap. Using a cross-sectional case-control observational study design, untargeted and unbiased metabolomic profiling of vaginal fluid and plasma was performed in women with PVD compared to healthy controls. In women with PVD, we also obtained assessments of vulvar pain, vestibular and vaginal muscle tenderness, and 24-hour symptom intensity alongside resting-state brain functional connectivity of brain regions involved in pain processing and modulation. Compared to healthy controls, women with PVD demonstrated differences primarily in vaginal (but not plasma) concentrations of metabolites of the sphingolipid signaling pathways, suggesting localized

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Disclosures: The author's report no conflicts of interest.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jpain.2021.04.017.

effects in vagina and vulvar vestibule rather than systemic effects. Our findings reveal that dysregulation of sphingolipid metabolism in PVD is associated with increased vulvar pain and muscle tenderness, sexual dysfunction, and decreased functional connectivity strength in pain processing/modulatory brain regions. This data collectively suggests that alterations in sphingolipid signaling pathways are likely an important molecular biomarker in PVD that could lead to new targets for therapeutic intervention.

Perspective: This manuscript presents the results of a robust, unbiased molecular assessment of plasma and vaginal fluid samples in women with provoked vestibulodynia compared to healthy controls. The findings suggest that alterations in sphingolipid signaling pathways are associated with symptoms and brain biomarkers and may be an important molecular marker that could provide new targets for therapeutic intervention.

Keywords

Ceramides; functional connectivity strength; graph theory; metabolomics; pain; provoked vestibulodynia; vulvodynia; resting-state brain connectivity; sphingolipid metabolism

Advancements in the understanding and treatment of complex chronic pain syndromes using preclinical models and conventional clinical approaches have failed to provide relief for a significant number of affected individuals worldwide. Provoked vestibulodynia (PVD) which affects up to 16% of women is one such disorder, which is characterized by local vestibular hypersensitivity and severe pain with vaginal penetration.[10] Similar to many other chronic pain conditions and providing evidence for central sensitization, PVD has been consistently linked to symptom-associated changes in brain structure and function, particularly in regions involved in pain processing and modulation (e.g., sensorimotor cortex, thalamus, amygdala, hippocampus, basal ganglia, brainstem).[5,32,33,35,60,64,70] Multiple etiologies for PVD have been proposed, including inflammation, mucosal nerve fiber proliferation, hormonal alterations, pelvic floor muscle dysfunction, genetic factors, and altered vestibular microbiota. However, most of these individual factors have been studied largely in isolation and have not yet shown the path toward valuable therapeutic interventions.[48,55,63] We believe that a more comprehensive, discovery driven approach is required to move the field forward.

Metabolomics, which measures the biochemical products of human and microbial molecular events downstream of genomic, transcriptomic, and proteomic systems, reflects current cellular processes.[40] Because these metabolites are small molecular endpoints and intermediates, they serve as important tools for identifying biochemical pathways and metabolites (e.g., small molecules) that may be altered in PVD. In the last decade, metabolomic profiling of biological samples (e.g., vaginal fluid, tissue, stool, plasma, urine) has emerged as an important precision medicine tool for drug and biomarker discovery, determining drug response phenotypes, and identifying new pathophysiological mechanisms underlying chronic disease[40,41,83] including vaginal health,[9,37,81] risk for preterm birth,[62,66] and chronic pain conditions including endometriosis.[52,75,76] As seen in many of these case-control studies, identification of new biological pathways involved in generating and maintaining symptoms in PVD has the potential to improve diagnosis, promote patient

stratification for personal medicine, and lead to the development of new mechanistic-based treatments.

This study utilized a stepwise approach: first using an unbiased, discovery-based approach to assess a large metabolomic dataset, followed by a targeted hypothesis-driven analyses. These targeted analyses were based on integrated central, peripheral, and subjective experimental measurements gathered from a cross-sectional case-control study of women with and without PVD aimed at advancing our understanding of the interactions between the peripheral and central features of this complex disorder. First, to identify key biochemical pathways involved in PVD, an untargeted liquid chromatography-mass spectrometry (LC-MS) metabolomic profiling of vaginal fluid and plasma samples was performed. Pathway analysis indicated that altered sphingolipid metabolism pathways were enriched in the vaginal fluid of women with PVD, meriting further investigation. Local sphingolipid metabolism has been tightly linked to the development of peripheral hyperalgesia and central sensitization associated with pain of diverse etiologies.[38,54,69,74,82] Based on this discovery, we hypothesized that alterations in this pathway may be associated with the functional connectivity strength of pain processing/modulatory regions and PVD symptoms (i.e., vestibular and vaginal pain and muscle tenderness, 24-hour symptom intensity, and reduced sexual functioning). Therefore, we computed the global functional connectivity strength of these hypothesized brain regions of interest using graph-based statistics. As a second step, an integrative multi-omics approach was applied to provide insights into the functional mechanisms linked to the identified alterations in sphingolipid metabolism. Specifically, correlation analysis was applied to identify and visualize the associations of altered vaginal sphingolipid metabolites with brain connectivity and with symptom measures. As a theoretical framework, we posited that altered local sphingolipid pathway metabolites are associated with increased PVD symptoms and alterations in the functional connectivity of pain processing/modulatory brain regions.

Materials and Methods

Subjects

Premenopausal women with PVD and healthy controls (HC), ages 18 to 50, were identified. A diagnosis of PVD was confirmed by clinical examination by a gynecologist or specialized nurse practitioner, both with recognized expertise and examination of women with vulvar pain. Participants were recruited via advertisement on social media, Craigslist, campus-wide emails via the Registrar's office at UCLA, onsite recruiting by the study coordinator at the UCLA Division of Digestive Diseases general GI clinics and OB/GYN clinics, and the UCLA and other local college newspapers. All subjects provided written informed consent to participate and were compensated for participating in the study. The study was approved by the University of California, Los Angeles (UCLA) Institutional Review Board (IRB#13–001113) and was conducted in accordance with the institutional guidelines regulating human subject research. A total of 60 women with PVD and 49 HC were enrolled in the study based on the desire to detect moderate effect size differences (Cohen's d~=.50), if they existed, in metabolite concentrations between PVD and HC.

Inclusion/Exclusion Criteria

Inclusion criteria for women with PVD included: at least 6 months of vulvar vestibular pain, at least 4 out of 10 rating in pain severity during intercourse or other activities involving vestibular pressure (e.g., tampon use), and findings on cotton swab exam consistent with provoked vestibulodynia. Infections (such as candida, bacterial vaginosis or herpes simplex), estrogen deficiency or dermatological disorders were ruled out by history, visual inspection, vaginal pH and saline and potassium hydroxide slide prep. Speculum examination of the vagina and bimanual pelvic examination was performed to exclude other potential etiologies or pathology such as vaginal or pelvic masses or inflammation. We excluded patients who met criteria for generalized vulvodynia and those with only spontaneous but not provoked vestibulodynia.[10] All subjects were asked to abstain from intercourse for 7 days prior to the visit, and to avoid the use of intravaginal products, including douches, sprays, tampons, spermicides, gels, foams, and diaphragms. Women were excluded from the study if they were pregnant, breastfeeding, or postpartum less than 4 months. In addition, subjects were excluded if they have received systemic or vaginal antimicrobial or probiotic therapy within 1 month before the study visit. Inclusion/exclusionary criteria for healthy control subjects were the same as for those with PVD, with the exception of inclusion for pain with vaginal penetration and vestibular allodynia.

Study Design

The subjects' menstrual history was obtained during phone screening so that the first visit could be scheduled around the follicular phase (or day 5–14 after starting first day of the last menstrual period). During Visit 1, all subjects completed study questionnaires and provided biological samples. Only women with PVD participated in a second visit, during which brain imaging was obtained. Time between Visit 1 and Visit 2 for these patients averaged 9 (SD = 9.1) days (median = 7 days).

Visit 1.—During the clinical assessment, PVD subjects were mapped for pain in the vulvar vestibule by touching the vestibule perpendicularly with the cotton end of the swab (enough to indent the mucosa to a depth of less than 1/3 of the cotton end) for 1 second at 5, 6, 7 (posterior vestibule), 10, 12 and 2 o'clock (peri-urethral). Participants were asked to rate the pain at each site from 0 to 10/10. Pain scores across all sites on the cotton swab test were summed for a total vulvar vestibular pain score [0–60, numeric rating scale (NRS)]. Internal muscle pain was assessed in PVD subjects with a single lubricated digit, applying approximately 2 kg of pressure for 2 seconds (The examiner's finger pressure was calibrated before the exam with an algometer). The right and left levator ani muscles (in the vagina) and the perineal complex (at the vaginal entrance) were assessed. The participant was asked to rate the pain severity at each site from 0 to 10/10 (0; none −10; most severe pain imaginable. Scores were summed across all locations to compute a total vaginal muscle tenderness score (0–30, NRS).[2] The study physician or nurse practitioner recorded whether, in addition to provoked pain, pain might also arise spontaneously (unprovoked) and whether the onset of symptoms was primary or secondary (at or after first introital penetration, respectively).[10] Patients also reported the duration of their PVD symptoms.

Female sexual functioning was assessed in women with PVD using the Female Sexual Functioning Index (FSFI). The FSFI is a multi-dimensional self-report instrument consisting of six subscales: desire, arousal, lubrication, orgasm, global satisfaction and pain. Items are summed to indicate a total "sexual function" score, where higher scores indicate greater sexual function, and where women who score below the clinical cut-off of 26 are considered at-risk for sexual dysfunction.[67] For all subjects, we obtained information on ethnicity, race, and subjective social status.[1] Subjective social status is 1-item assessment that captures a subject's sense of social status and has been linked to psychological factors that may predispose individuals to better health trajectories. Participants are asked to place themselves on a drawing of a latter that is anchored at the top by those in society who are best off in terms of income, education, and occupation and at the bottom by those who are worst off (highest score = 9, lowest score = 1). Detailed data on current use of contraceptives or hormones were used to categorize hormone use as "no hormone" (i.e., condoms, abstinence), "local hormones" (e.g., levonorgestrel containing intrauterine device, estrogen or testosterone vaginal cream, or "systemic hormones" (e.g., oral contraceptive pills, vaginal contraceptive ring, transdermal contraceptive patch, etonogestrel sub-dermal contraceptive implant, depo medroxyprogesterone acetate injection).

Diet type was assessed by asking subjects to endorse their usual diet. Choice included Standard American (high consumption of processed, frozen, and packaged foods, pasta and breads, and red meat; infrequent vegetable and fruit consumption), Modified American (high consumption of whole grains including some processed, frozen, and packaged foods; limited intake of red meat; moderate vegetable and fruit consumption), Mediterranean (high consumption of fruits, vegetables, beans, nuts, and seeds; olive oil as the key monounsaturated fat source; low to moderate intake of dairy products, fish, and poultry, infrequent red meat consumption), and other (all other diets that do not fit into the above categories, e.g. vegan, vegetarian, pescatarian, dairy-free).

Biological Sample Collection and Metabolomics

Plasma samples were obtained in 60 women with PVD and 49 HC women. From these women, vaginal swab samples were obtained from 51 of the 60 PVD women providing plasma samples and all 49 HC women. Additionally, 3 women with PVD only provided vaginal swab samples. Vaginal swab and plasma samples were collected during the mid to late follicular phase based on last menstrual period in women not on hormonal contraceptives or in days 5 to 14 of the active pill phase, considering day 1 as the first day of bleeding.

Vaginal Swabs

When swabs arrive from the manufacturer, the study coordinator labeled each sterile tube containing the swabs with two cryo-freezer safe labels. One label was for patient study ID and date of sample, and the second label was for PRE and POST collection weight, in grams. The coordinator weighed each dry swab and container and provided PRE gram weight on the swab label. At Visit 1, the physician/nurse practitioner collected the vaginal sample after gently retracting the outer labia with gloved fingers and inserting a sterile swab approximately 1 inch into the vaginal vault. Swab was rotated 360 degrees, removed, and

inserted into the sterile tube (without liquid medium). This procedure was repeated for the remaining 2 swabs. The swabs were immediately weighed post collection (using the same gram scale), and the POST weight was documented on the cryo-freezer label. The samples were immediately placed into a small lab cooler filled with ice and brought to the lab where they were stored in a −80 degree Celsius freezer within 20 minutes of collection. Samples were shipped on dry ice with a collection log (vaginal sample log) to Metabolon for further processing and analysis on their global metabolomics and bioinformatics platform (Metabolon, 617 Davis Drive, Durham, NC).

Plasma Samples

Using standard venipuncture procedure, blood was collected in EDTA tubes from individuals after an overnight fast. Plasma was centrifuged, aliquoted to 6 (1 mL) sterile polypropylene cryotubes, and immediately stored at −80°C until shipped to Metabolon.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS) was used to perform discovery-based, untargeted global metabolomic profiling to characterize biochemical pathways in vaginal fluid and plasma samples that were altered in PVD compared to HC (Metabolon, 617 Davis Drive, Durham, NC). Specific details on the Metabolomics platform can be found in Supplemental methods I. Datasets provided by Metabolon identified a total of 824 compounds of known identity (named biochemicals) detected in plasma, and 952 in vaginal swabs.

Visit 2

Before the brain imaging scan during Visit 2, PVD patients rated the intensity of their vulvodynia symptoms in the past 24 hours on a verbal descriptor visual analog scale ranging from 0 to 20 (No Sensation to Extremely Intense).

Brain Imaging and Computation of BrainImaging Derived Phenotypes

Using a 3.0T MRI scanner (Siemens Trio; Siemens, Erlangen, Germany), high resolution structural brain images and a ten-minute 6 seconds eyes-closed resting-state scan obtained in women with PVD. Using established computational pipelines, [30,31,57] we applied graph theory to this data to compute the connectivity strength or global influence of each of the pain processing/modulatory brain regions of interest (ROIs) on brain functioning. As defined by the Destrieux (cortical)[19] and Harvard-Oxford Subcortical Atlases,[18] a priori specified individual ROIs included the sensorimotor cortex [precentral gyrus, inferior part of the precentral sulcus, superior part of the precentral sulcus, paracentral lobule and sulcus, subcentral sulci and gyrus (central operculum), central sulcus, postcentral sulcus, postcentral gyrus] including the primary the posterior insula (long insular gyrus and central sulcus of the insula, and the inferior segment of the circular sulcus of the insula) and subcortical regions [amygdala, hippocampus, basal ganglia (nucleus accumbens, caudate nucleus, globus pallidus, putamen), thalamus and brainstem]. Strength represents the weighted sum of connections (i.e., Fisher Z transformed correlations >.30) for a given ROI. High compared to low values for strength indicate greater centrality or influence on the global state of brain functioning. For details on image acquisition, quality control and preprocessing, and computation of connectivity strength, see Supplemental Methods II. Within the framework

of our preprocessing and computational workflows, this subject-specific network metric has been shown to have good and reproducible reliability.[65,85]

Statistical Analysis I, Metabolomics

Pathway enrichment analysis was utilized to identify pathways that differentiated study groups. For each individual pair-wise comparison, this analysis displays the number of statistically significantly different compounds relative to all detected compounds in a subpathway, compared to the total number of statistically significantly different compounds relative to all detected compounds in the study, according to the formula:

Enrichment Value = (k/m)/((n-k)/(N-m)), where m = number of metabolites in the pathway, k = number of significant metabolites in the pathway, n = total number of significant metabolites, and N = total number of metabolites. A pathway enrichment value greater than one indicates that a given pathway contains more significantly changed compounds relative to the number of changes observed in the entire set of metabolites measured in samples. Pathways represented by ≥4 metabolites were analyzed to focus on biochemical processes that showed adequate coverage in the global metabolomic profiling.

Based on the enrichment analysis, we reported the group differences observed in sphingolipid metabolism pathways, specifically 39 validated compounds identified in vaginal fluid and 63 identified in plasma. Following log transformation and imputation of missing values, if any, with the minimum observed value for each compound, Welch's two-sample t-test was used to identify biochemicals that differed significantly between healthy women and women with PVD. Conservative error control for false positives (Type I errors) for discovery-based analysis across all metabolites was realized by calculating the false discovery rate-adjusted p-value (q), based on the total number of compounds identified during global metabolomic profiling in each sample type (952 in vaginal swabs, and 824 in plasma samples).

Due to differences in use of hormone contraceptives in the sample, we performed an additional sensitivity analysis to explore the influence of taking hormonal contraceptives on observed differences in the sphingolipid metabolism pathways. Specifically, we categorized women by hormone usage (no hormones, systemic hormones, local hormones) and performed a contrast analysis within the framework of the general linear model[36,68,74] testing the following contrasts of interest: 1) PVD no hormones – HC no hormones, 2) PVD systemic hormones – HC systemic hormones and 3) PVD systemic hormones – PVD no hormones. We present the significant results from the PVD-HC analysis including fold change (i.e., ratio of the means), uncorrected p values, and FDR corrected p values (q) in Table 4 (see All Subjects). FDR corrected significance levels for the sensitivity analyses are also shown in Table 4 (see Subgroup Analysis). A full list of the biochemical compounds detected in the sphingolipid metabolism pathways in vaginal swabs and plasma is provided in Supplemental Tables 1 and 2, respectively. These tables also contain the fold change estimates and uncorrected p values for the subgroup analyses, as well as a comparison between HC systemic hormones – HC no hormones. Finally, where available, the supplemental tables contain links to the Kyoto Encyclopedia of Genes and Genomes

(KEGG), Human Metabolome Database (HMDB), and PubChem databases containing detailed information about the identified metabolites.

Statistical Analysis II, Integration of Metabolites, Brain Imaging-Derived Phenotypes and Symptom Measures

Correlational analysis was performed to provide insights into the functional mechanisms linked to the identified alterations in sphingolipid metabolism. In women with PVD, Spearman correlations were used to estimate the association between untransformed concentrations of altered metabolites with symptom measures (i.e., pain intensity in the last 24 hours, total vulvar vestibular pain and vaginal muscle tenderness scores, sexual functioning) as well as the global functional connectivity of pain processing/modulatory brain regions. We used Cytoscape v. 3.8.0[24] and implemented multidimensional scaling using a compound spring embedded algorithm[20] to visualize the metabolite-brain, metabolite-symptom, metabolite-brain and brain-symptom correlations as a tripartite association network at $P < .05$.[43,56,58] We considered the exploratory cross-sectional correlation analyses important for generating future hypotheses and reported uncorrected p values. Although we reported correlations as significant at $P < .05$, emphasis should be placed on interpretation of the effect sizes. As a rule of thumb, Cohen (1988) suggested the following guidelines for interpreting effect size, r: small, r = .10 (explain 1% of the variance), medium, r = .30 (explain 9% of the variance), and large, r = .50 (explain 25% of the variance). We used independent t-tests and 2-sided Fisher's exact tests to compare groups on continuous and categorical descriptive variables.

RESULTS

Subject Characteristics

Table 1 presents the demographic characteristics of the PVD and HC subjects. The mean age for PVD (~28 years) was about 3 years older than the mean age for HC (~25 years). HC were more likely to endorse consuming diets other than American or Mediterranean. Body mass index did not differ between the groups.

Clinical Variables

Less than half of participants were taking systemic hormonal contraceptives [PVD (~40%, N_{plasma} = 23; $N_{vaginal}$ = 22), HC (29%, N = 14)]. Few women were using a local hormone [13 % PVD (N_{plasma} = 8, $N_{vaginal}$ = N =7), 13% (HC N = 6)]. About half the sample report no hormone contraceptive use [~47%, PVD (N_{plasma} = 29, $N_{vaginal}$ = 25), 60% HC (N = 29). Table 2 contains the clinical characteristics of the woman with PVD by sample type. There were no differences between the hormone subgroups in pain duration, total vulvar vestibule pain score on exam, total vaginal muscle tenderness, or symptom intensity in the past 24 hours.

Functional Brain Connectivity

The estimated mean and 95% confidence intervals for the functional connectivity strength of each cortical and subcortical region of interest for women with PVD are depicted in the bar

plots in Fig. 1 and 2. All regions of interest showed significant connectivity strength (95% confidence intervals do not include zero).

Metabolite Pathways Alterations Enriched in PVD

Pathway enrichment analysis of metabolomic data was utilized to identify the biochemical processes specifically altered in the PVD group. The results of this approach are summarized in Table 3 and show the top 15 sub-pathways with the highest enrichment score for the PVD to HC no hormone subgroup comparison. Importantly, sphingosines, sphingolipid synthesis, hexosylceramides (HCER) and sphingomyelins showed enrichment scores greater than 1. These sub-pathways are integral to sphingolipid biosynthesis and degradation, suggesting that they may be preferentially altered under conditions of PVD. The enrichment analysis performed for the entire dataset identified the same sub-pathways among the top 15 enriched processes (data not shown). Based on this analysis and the role of sphingolipid metabolism pathways in developing peripheral hyperalgesia and central sensitization, a detailed analysis of this pathway is presented below.

Alterations in Sphingolipid Signaling Pathways in PVD

In the vaginal fluid samples, PVD compared to HC had significantly ($q < .05$) higher levels of metabolites involved in sphingolipid synthesis (e.g., sphinganine, sphingadienine) and sphingosines (e.g., sphingosine, eicosanoylsphingosine (d20:1)*). As shown in Table 4 (All Subjects), in general, there was a decrease in vaginal levels of several sphingomyelins [e.g., sphingomyelin (d18:1/20:0, d16:1/22:0)*, behenoyl sphingomyelin (d18:1/22:0)*, tricosanoyl sphingomyelin (d18:1/23:0)*], ceramides [i.e., ceramide (d18:1/17:0, d17:1/18:0)*, ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)*, ceramide (d18:2/24:1, d18:1/24:2)*] and hexosylceramides (HCER) [e.g., glycosyl ceramide (d18:1/20:0,d16:1/22:0)*]. Fig 3 displays the box plots and raw data for all subjects for each of the altered vaginal sphingolipid metabolites (organized by subpathways shown in Table 4).

In contrast to significant differences in vaginal metabolites, we did not see strong evidence for any significant group differences in plasma samples. Rather, we only observed higher levels ($q < .02$) of a single biochemical in PVD compared to HC, i.e., lactosyl-N-behenoyl-sphingosine (d18:1/22:0).

Sensitivity Analysis Examining the Influence of Hormone Status

As can be seen in Table 4 (Subgroup analysis), many of the differences observed in the vaginal fluid samples of the larger sample were maintained in the subgroup comparison of PVD to HC in women not using hormonal contraceptives. However, no significant differences were observed between PVD and HC using systemic hormones, or between PVD women taking systemic hormones versus no hormones.

In plasma samples, for women not reporting hormone usage, PVD compared to HC only showed trends (q's = .08) for higher levels of two biochemicals in the lactosyl ceramide pathways (i.e., lactosyl-N-behenoyl-sphingosine (d18:1/22:0), lactosyl-N-nervonoyl-sphingosine).

Associations Between Sphingolipid Metabolism, Symptoms and Functional Brain Connectivity in PVD

As shown in Fig 4, moderate effect size associations were observed between many of the PVD-specific alterations in vaginal concentrations in the sphingolipid pathway metabolites and increased severity of PVD symptoms (Table 5). For example, PVD-specific increases in sphingolipid pathway metabolites correlated with increased vaginal muscle tenderness total scores. Further, increases in sphingosines showed moderate effect size correlations with increased vulvar vestibular pain. Finally, many of the alterations in sphingolipid metabolite concentrations observed in PVD compared to HC correlated with greater sexual dysfunction.

Several of the altered metabolites in PVD also showed moderate associations with the connectivity of brain regions involved in pain processing/modulatory brain regions (Table 6). In particular, PVD-specific increases in sphingosines and sphingolipid synthesis pathway metabolites were associated with decreased connectivity strength in the right amygdala and right hippocampus. Increases in sphingosine metabolite concentration observed in PVD versus HC also correlated with decreased connectivity in the bilateral thalamus, and the posterior insula, bilaterally. On the other hand, PVD-specific decreases observed in dihydroceramides and ceramides were associated with increased functional connectivity strength of the superior part of the right precentral sulci and the right caudate nucleus. We also observed decreases in sphingomyelin (d18:0/18:0, d19:0/17:0)* which were associated with decreased connectivity strength in the brainstem.

Several symptom measures showed direct correlations with the connectivity of brain regions (Table 7). For example, decreased connectivity strength in the right hippocampus and left posterior insula were associated with increased vulvar vestibular pain and muscle tenderness scores. Also, decreased connectivity in the right amygdala correlated with increased vulvar vestibular pain and greater sexual dysfunction. Furthermore, increased connectivity in the precentral cortex and paracentral lobule was associated with increased 24-hour symptom intensity.

Discussion

Untargeted and unbiased metabolomics profiling revealed that women with PVD, compared to HC, demonstrated differences primarily in vaginal (but not plasma) concentrations of metabolites from sphingolipid signaling pathways, suggesting localized rather than systemic effects in vagina and vulvar vestibule. Consistent with the clinical importance of these findings, the observed alterations in sphingolipid metabolism were associated with increased vulvar pain, muscle tenderness, and sexual dysfunction as well as primarily decreased functional connectivity strength in pain processing/modulatory brain regions. We observed that while the use of systemic hormonal contraceptives resulted in altered plasma steroid metabolites in PVD and HC, these associations were unlikely to explain the subsequent directionality nor vaginal specificity and link to pain observed in PVD subjects.

Sphingolipid metabolites, including ceramides and sphingosines, have been associated with neuropathic pain and are thought to play a critical role in several inflammatory diseases, attributed to their anti– or pro–inflammatory and pain modulatory effects.[12,26,27,54,59,69,74]

Ceramide is generated from three pathways including de novo biosynthesis (involving sphinganine), hydrolysis of sphingomyelins, and a salvage pathway involving degradation of complex sphingolipids (including sphingomyelins) from ceramides.[7] Once produced, ceramide is converted to sphingosine which is subsequently converted to sphingosine 1-phosphate (S1P). This ceramide to S1P pathway has been implicated in the development of peripheral sensitization and hyperalgesia. [38,54,69,74,82,31]

In this study, there were significantly higher levels of sphinganine but lower levels of sphingomyelins in vaginal swabs in PVD, suggesting alterations in ceramide synthesis. Downstream we observed decreased vaginal fluid levels of ceramide species and an increase of sphingosine. Although S1P, a major bioactive lipid species, was not detected in this study, we observed increases in sphingosine, the precursor to S1P.

The alterations in the ceramide to S1P pathway may reflect alterations in the balance of pro and anti-inflammatory cytokines levels in the vaginal vestibule and/or in circulating blood of women with PVD.[4,10,13,22,23,25,71,77,86] Consistent with an involvement in nociception, animal models of allodynia have demonstrated an association between sphingolipid signal metabolites, increased sensitivity of dorsal root ganglia and overexpression of inflammatory cytokines with peripheral and central pain sensitization[21,54,69] Together, these alterations suggest inflammatory processes which may contribute to both peripheral and mechanical hypersensitivity[59] as well as indicate altered sphingosine kinase activity and hence production of the signaling molecule S1P in PVD.

Metabolomic profiling of biological tissue and fluid has emerged as an important for examining biological processes in chronic diseases including chronic pain [for a review see[52,75,76,84]]. In particular, evidence of altered sphingolipid metabolism has been reported in several chronic pain conditions often found to be comorbid with PVD.[17,50] In fibromyalgia, one study reported an upregulation of plasma levels SP1,[51] and another demonstrated increased plasma sphingosines which correlated with pain and fatigue.[47] Compared to HC, endometriosis patients showed increased concentrations of sphingolipids in both follicular fluid and peritoneal and endometrial tissue samples.[1,46,79,80] Similar to PVD, alterations in pro-inflammatory mediators[8,15,45,53,61,72] have been reported in these chronic pain conditions supporting the hypothesis that observed alterations in sphingolipid metabolism may reflect ongoing inflammatory processes and a shared mechanism.

The use of systemic hormone contraceptives appeared to reduce PVD - HC differences observed in vaginal fluid levels of sphingolipids. Use of hormonal contraceptives did not change the lack of group differences observed between PVD and HCs in circulating levels of sphingolipid metabolites. However, within PVD, use of hormonal contraceptives appeared to lower plasma concentrations of lactosyl ceramides and increase levels of dihydrosphingomyelins and sphingomyelins. This finding is consistent with research demonstrating that steroid hormones are involved in the regulation of sphingolipid metabolism.[49]

Symptom-associated alterations in gray matter density, regional activity during evoked pain studies, and intrinsic resting-state connectivity of primary and secondary sensory and motor

cortices, posterior insula, hippocampus, basal ganglia and thalamus have been reported in women with PVD compared to HC.[5,32,35,70] We have previously shown that women with PVD showed microstructural alterations in cortico-thalamicbasal ganglia white matter tracts associated with ascending nociceptive processing (i.e., primary sensory cortex, thalamus, basal ganglia).[33] The connectivity strength of a brain region is a topological measure reflecting its global influence on brain functioning. In the present study, PVD-specific alterations in metabolites comprising sphingosines, sphingolipid synthesis, HCER and dihydrosphingomyelins sub pathways showed moderate linear associations with decreased connectivity strength for pain processing (thalamus, posterior insula, nucleus accumbens of the basal ganglia, brainstem) and modulatory (amygdala, hippocampus) regions. In turn, the reduced connectivity of many of the regions (posterior insula, amygdala, hippocampus) also showed direct correlation with increased symptomatology. PVD-specific decreases observed in one sphingomyelin and a few ceramide species were associated with increased connectivity strength of the caudate nucleus, hippocampus, and the precentral sulci. The amygdala and hippocampus connections are key features the emotional arousal network. The observed associations with the connectivity strength of these regions may reflect the neurobiological substrates of pain-related anxiety and fear responses secondary to central sensitization that increase the facilitatory descending modulation.[3,29,42] The observed correlations between alterations in vaginal concentration of sphingolipid metabolites and the functional connectivity strength of pain processing/modulatory regions support the hypotheses that sphingolipid species enhance ascending transmission and modulation of pain signals.

The current study has identified sphingolipid metabolites associated with vestibular pain but was not designed to determine the causal nature of these associations nor their directionality. These biochemical alterations could reflect biological markers of persistent inflammatory processes and modulation of peripheral nerve fibers[34] of the vagina and vulvar vestibule of PVD patients leading to long-lasting allodynia and hyperalgesia. The hypothesized nociceptive signaling to the brain may result in structural remodeling of pain processing regions, and alter the balance of facilitatory and inhibitory descending pain modulation contributing to the production and maintenance of vulvar pain.[39] This interpretation is consistent with the moderate to large effect size association observed between vaginal concentrations of sphingolipid metabolites with pain and muscle tenderness, symptom intensity and sexual dysfunction as well as functional connectivity strength of brain regions associated with pain processing and pain modulation.

Together, our findings suggest that sphingolipids may serve as biomarkers for patient stratification in PVD. Emerging evidence identified the S1P receptor 1 subtype (S1PR1), one of the five known G protein-coupled S1P receptor subtypes, as being critical in transducing the effects of S1P in pain.[12,21] Noteworthy, over the last decade, several orally bioavailable and CNS penetrant, S1PR1 functional and competitive antagonists have been developed.[6,14,28,78] Two S1PR1 functional antagonists are now FDA-approved for the treatment of multiple sclerosis: the pro-drug FTY720 (fingolimod; Gilenya, Novartis) approved in 2010[11] and ozanimod (RPC1063, Zeposia, Celgene) approved in 2020.[44,73]

Limitations

We enrolled women with PVD, the most common form of vulvodynia. Therefore, inferences should not be made to women with generalized vulvodynia. The presence of comorbid chronic overlapping pain conditions should be addressed in future studies. Due to the heterogeneity of patient characteristics, it will be important to have larger sample sizes for well-powered subgroup analyses. Larger samples are also important to ensure unbiased parameter estimates and avoiding type II error.

Future studies using targeted sphingolipid metabolomics profiling (i.e., absolute quantification of a pre-defined set of metabolites) and S1P receptor expression studies in vaginal tissue in PVD will be critical to further validate these findings. Research may also benefit from examining the impact of hormone-based therapy which specifically and significantly affects ceramide pathways. Ultimately, longitudinal multi-omics studies comprising concurrent measurement of pro-and anti-inflammatory markers as well as micro-RNA expression associated with inflammation (including gonadotropin-releasing hormone signaling pathways, steroid hormone levels, and sphingolipid metabolite concentrations) are needed to delineate the relationship between these distinct but interacting mechanisms as they relate to peripheral and central sensitization, brain alterations and symptoms in PVD. Finally, metabolomics for chronic overlapping pain syndromes is still in its infancy,[15,16] as a considerable amount of work is required to validate specific metabolites or pathways as candidate biomarkers.

Conclusion

In conclusion, this is the first study to use unbiased, untargeted global metabolomics profiling to identify novel biomarkers in PVD. Although the results from this study are novel and compelling, the cross-sectional and exploratory nature of the study design necessitates validation in independent data sets with thorough patient phenotyping. Women with PVD had significant modulations in sphingolipid signaling pathway metabolites previously linked to peripheral and central sensitization, neuropathic pain and inflammatory pain conditions. These modified metabolites were associated with increased vulvar vestibule pain on exam, vaginal muscle tenderness and sexual dysfunction. Additionally, these modified metabolites were found to be in parallel with modified global connectivity of brain regions critical for pain processing. Ultimately, modified levels of metabolites comprising sphingolipid signaling pathways may be an important molecular marker for understanding the pathophysiology of symptoms in PVD and could lead to new targets for therapeutic intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Dr. Daniela Salvemini for her review of the manuscript and expert consultation regarding the sphingolipid signaling pathways in pain. We thank study participants and staff of the G Oppenheimer Center for Neurobiology of Stress and Resilience (CNSR) for their contributions to this project.

Funding: grants:

R01 NICHD076756 (JSL/AR), R21 NICHD086737 (JSL/AR); NIH R01 DK048351 (EAM); R01-HD091731, R21-ES029462, R01-DK089201 (KMA).

References

1. Adler NE, Epel ES, Castellazzo G, Ickovics JR: Relationship of subjective and objective social status with psychological and physiological functioning: Preliminary data in healthy white women. Health Psychol 19:586–592, 2000 [PubMed: 11129362]
2. Alappattu M, Robinson M, Lamvu G: Vulvodynia is not created equally: Pain-related distress subgroups in vulvodynia. J Pain 17, 2016. S21–S21
3. Bach DR, Hoffmann M, Finke C, Hurlemann R, Ploner CJ: Disentangling hippocampal and amygdala contribution to human anxiety-like behavior. J Neurosci 39:8517–8526, 2019 [PubMed: 31501296]
4. Baker DA, Peresleni T, Kocis C: Inflammatory markers in vestibulodynia [4]. Obstet Gynecol 127(Suppl 1):1s–2s, 2016
5. Bhatt RR, Gupta A, Rapkin A, Kilpatrick LA, Hamadani K, Pazmany E, Van Oudenhove L, Stains J, Aerts L, Enzlin P, Tillisch K, Mayer EA, Labus JS: Altered gray matter volume in sensorimotor and thalamic regions associated with pain in localized provoked vulvodynia: A voxel-based morphometry study. Pain 160:1529–1540, 2019 [PubMed: 30817440]
6. Bigaud M, Guerini D, Billich A, Bassilana F, Brinkmann V: Second generation S1P pathway modulators: Research strategies and clinical developments. Biochim Biophys Acta 1841:745–758, 2014 [PubMed: 24239768]
7. Bikman BT, Summers SA: Ceramides as modulators of cellular and whole-body metabolism. J Clin Invest 121:4222–4230, 2011 [PubMed: 22045572]
8. Bjork E, Vinnars MT, Nagaev I, Nagaeva O, Lundin E, Ottander U, Mincheva-Nilsson L: Enhanced local and systemic inflammatory cytokine mRNA expression in women with endometriosis evokes compensatory adaptive regulatory mRNA response that mediates immune suppression and impairs cytotoxicity. Am J Reprod Immunol e13298, 2020 [PubMed: 32623813]
9. Borgogna JC, Shardell MD, Santori EK, Nelson TM, Rath JM, Glover ED, Ravel J, Gravitt PE, Yeoman CJ, Brotman RM: The vaginal metabolome and microbiota of cervical HPV-positive and HPV-negative women: A cross-sectional analysis. BJOG 127:182–192, 2020 [PubMed: 31749298]
10. Bornstein J, Goldstein AT, Stockdale CK, Bergeron S, Pukall C, Zolnoun D, Coady D: Consensus vulvar pain terminology committee of the International Society for the Study of Vulvovaginal Disease tISftSoWsSH, the International Pelvic Pain S. 2015 ISSVD, ISSWSH and IPPS Consensus Terminology and Classification of Persistent Vulvar Pain and Vulvodynia. Obstet Gynecol 127:745–751, 2016 [PubMed: 27008217]
11. Brinkmann V, Billich A, Baumruker T, Heining P, Schmouder R, Francis G, Aradhye S, Burtin P: Fingolimod (FTY720): Discovery and development of an oral drug to treat multiple sclerosis. Nat Rev Drug Discov 9:883–897, 2010 [PubMed: 21031003]
12. Chen Z, Doyle TM, Luongo L, Largent-Milnes TM, Giancotti LA, Kolar G, Squillace S, Boccella S, Walker JK, Pendleton A, Spiegel S, Neumann WL, Vanderah TW, Salvemini D: Sphingosine-1-phosphate receptor 1 activation in astrocytes contributes to neuropathic pain. Proc Natl Acad Sci USA. 116:10557–10562, 2019 [PubMed: 31068460]
13. Ciszek BP, Khan AA, Dang H, Slade GD, Smith S, Bair E, Maixner W, Zolnoun D, Nackley AG: MicroRNA expression profiles differentiate chronic pain condition subtypes. Transl Res 166:706–720, 2015. e711 [PubMed: 26166255]
14. Cohan S, Lucassen E, Smoot K, Brink J, Chen C: Sphingosine-1-Phosphate: Its Pharmacological Regulation and the Treatment of Multiple Sclerosis: A Review Article. Biomedicines 8, 2020
15. Corbitt M, Eaton-Fitch N, Staines D, Cabanas H, Marshall-Gradisnik S: A systematic review of cytokines in chronic fatigue syndrome/myalgic encephalomyelitis/systemic exertion intolerance disease (CFS/ME/SEID). Bmc Neurol 19, 2019
16. Cordeiro FB, Cataldi TR, Perkel KJ, do Vale, Teixeira da Costa L, Rochetti RC, Stevanato J, Eberlin MN, Zylbersztejn DS, Cedenho AP, Turco EG: Lipidomics analysis of follicular fluid by

ESI-MS reveals potential biomarkers for ovarian endometriosis. J Assist Reprod Genet 32:1817–1825, 2015 [PubMed: 26497128]

17. Chronic Pain Research Alliance. Impact of chronic overlapping pain conditions on public health and the urgent need for safe and effective treatment: 2015 analysis and policy recommendations. 2015
18. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ: An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31:968–980, 2006 [PubMed: 16530430]
19. Destrieux C, Fischl B, Dale A, Halgren E: Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. Neuroimage 53:1–15, 2010 [PubMed: 20547229]
20. Dogrusoz U, Giral E, Cetintas A, Civril A, Demir E: A layout algorithm for undirected compound graphs. Inform Sci 179:980–994, 2009
21. Doyle TM, Chen Z, Durante M, Salvemini D: Activation of Sphingosine-1-Phosphate Receptor 1 in the Spinal Cord Produces Mechanohypersensitivity Through the Activation of Inflammasome and IL-1beta Pathway. J Pain, 2019
22. Foster DC, Hasday JD: Elevated tissue levels of interleukin-1 beta and tumor necrosis factor-alpha in vulvar vestibulitis. Obstet Gynecol 89:291–296, 1997 [PubMed: 9015038]
23. Foster DC, Piekarz KH, Murant TI, LaPoint R, Haidaris CG, Phipps RP: Enhanced synthesis of proinflammatory cytokines by vulvar vestibular fibroblasts: Implications for vulvar vestibulitis. Am J Obstet Gynecol 196(346):e341–e348, 2007
24. Franz M, Lopes CT, Huck G, Dong Y, Sumer O, Bader GD: Cytoscape.js: A graph theory library for visualisation and analysis. Bioinformatics 32:309–311, 2016 [PubMed: 26415722]
25. Gerber S, Bongiovanni AM, Ledger WJ, Witkin SS: Defective regulation of the proinflammatory immune response in women with vulvar vestibulitis syndrome. Am J Obstet Gynecol 186:696–700, 2002 [PubMed: 11967493]
26. Gerdle B, Larsson B, Forsberg F, Ghafouri N, Karlsson L, Stensson N, Ghafouri B: Chronic widespread pain: Increased glutamate and lactate concentrations in the trapezius muscle and plasma. Clin J Pain 30:409–420, 2014 [PubMed: 23887335]
27. Gomez-Munoz A, Presa N, Gomez-Larrauri A, Rivera IG, Trueba M, Ordonez M: Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate. Prog Lipid Res 61:51–62, 2016 [PubMed: 26703189]
28. Grassi S, Mauri L, Prioni S, Cabitta L, Sonnino S, Prinetti A, Giussani P: Sphingosine 1-Phosphate receptors and metabolic enzymes as druggable targets for brain diseases. Front Pharmacol 10:1–20, 2019 [PubMed: 30728774]
29. Greenwald JD, Shafritz KM: An integrative neuroscience framework for the treatment of chronic pain: From cellular alterations to behavior. Front Integr Neurosci 12 (18), 2018
30. Gupta A, Bhatt RR, Naliboff BD, Kutch JJ, Labus JS, Vora PP, Alaverdyan M, Schrepf A, Lutgendorf S, Mayer EA, Network MR: Impact of early adverse life events and sex on functional brain networks in patients with urological chronic pelvic pain syndrome (UCPPS): A MAPP research network study. Plos One 14:e0217610, 2019 [PubMed: 31220089]
31. Gupta A, Mayer EA, Acosta JR, Hamadani K, Torgerson C, van Horn JD, Chang L, Naliboff B, Tillisch K, Labus JS: Early adverse life events are associated with altered brain network architecture in a sex- dependent manner. Neurobiol Stress 7:16–26, 2017 [PubMed: 28239631]
32. Gupta A, Rapkin AJ, Gill Z, Kilpatrick L, Fling C, Stains J, Masghati S, Tillisch K, Mayer EA, Labus JS: Disease-related differences in resting-state networks: A comparison between localized provoked vulvodynia, irritable bowel syndrome, and healthy control subjects. Pain 156:809–819, 2015 [PubMed: 25735001]
33. Gupta A, Woodworth DC, Ellingson BM, Rapkin AJ, Naliboff B, Kilpatrick LA, Stains J, Masghati S, Tillisch K, Mayer EA, Labus JS: Disease-related microstructural differences in the brain in women with provoked vestibulodynia. J Pain 19, 2018. 528 e521–528 e515
34. Halperin R, Zehavi S, Vaknin Z, Ben-Ami I, Pansky M, Schneider D: The major histopathologic characteristics in the vulvar vestibulitis syndrome. Gynecol Obstet Invest 59:75–79, 2005 [PubMed: 15542936]

35. Hampson JP, Reed BD, Clauw DJ, Bhavsar R, Gracely RH, Haefner HK, Harris RE: Augmented central pain processing in vulvodynia. J Pain 14:579–589, 2013 [PubMed: 23578957]
36. Hancock GR, Klockars AJ: The quest for alpha: Developments in multiple comparison procedures in the quarter century since Games (1971). Rev Educ Res 66:269–306, 1996
37. Handelman SK, Romero R, Tarca AL, Pacora P, Ingram B, Maymon E, Chaiworapongsa T, Hassan SS, Erez O: The plasma metabolome of women in early pregnancy differs from that of non-pregnant women. Plos One 14, 2019
38. Hannun YA, Obeid LM: Principles of bioactive lipid signalling: Lessons from sphingolipids. Nat Rev Mol Cell Bio 9:139–150, 2008 [PubMed: 18216770]
39. Heinricher MM: Pain modulation and the transition from acute to chronic pain. Adv Exp Med Biol 904:105–115, 2016 [PubMed: 26900066]
40. Ivanisevic J, Thomas A: Metabolomics as a Tool to Understand Pathophysiological Processes. Methods Mol Biol 1730:3–28, 2018
41. Kaddurah-Daouk R, Weinshilboum R, Pharmacometabolomics Research N: Metabolomic signatures for drug response phenotypes: Pharmacometabolomics enables precision medicine. Clin Pharmacol Ther 98:71–75, 2015 [PubMed: 25871646]
42. Kim WB, Cho JH: Encoding of contextual fear memory in hippocampal-amygdala circuit. Nat Commun 11(1382), 2020
43. Labus JS, Osadchiy V, Hsiao EY, Tap J, Derrien M, Gupta A, Tillisch K, Le Neve B, Grinsvall C, Ljungberg M, Ohman L, Tornblom H, Simren M, Mayer EA: Evidence for an association of gut microbial Clostridia with brain functional connectivity and gastrointestinal sensorimotor function in patients with irritable bowel syndrome, based on tripartite network analysis. Microbiome 7:45, 2019 [PubMed: 30898151]
44. Lamb YN: Ozanimod: First approval. Drugs 80:841–848, 2020 [PubMed: 32385738]
45. Le NXH, Loret de Mola JR, Bremer P, Groesch K, Wilson T, Diaz-Sylvester P, Braundmeier-Fleming AG: Alteration of systemic and uterine endometrial immune populations in patients with endometriosis. Am J Reprod Immunol e13362, 2020 [PubMed: 33070438]
46. Lee YH, Tan CW, Venkatratnam A, Tan CS, Cui L, Loh SF, Griffith L, Tannenbaum SR, Chan JK: Dysregulated sphingolipid metabolism in endometriosis. J Clin Endocrinol Metab 99:E1913–E1921, 2014 [PubMed: 24960545]
47. Lesnak J, Merriwether E, Taylor E, Dailey D, Vance C, Zimmerman M, Crofford L, Law LF, Sluka K: Select metabolomics reveal potential biomarkers of fibromyalgia that correlate with pain and fatigue. J Pain 20, 2019. S57–S57
48. Lev-Sagie A, Witkin SS: Recent advances in understanding provoked vestibulodynia. F1000Res 5:2581, 2016 [PubMed: 27853523]
49. Lucki NC, Sewer MB: Multiple roles for sphingolipids in steroid hormone biosynthesis. Subcell Biochem 49:387–412, 2008 [PubMed: 18751920]
50. Maixner W, Fillingim RB, Williams DA, Smith SB, Slade GD: Overlapping chronic pain conditions: Implications for diagnosis and classification. J Pain 17:T93–T107, 2016 [PubMed: 27586833]
51. Menzies V, Starkweather A, Yao YW, Thacker LR, Garrett TJ, Swift-Scanlan T, Kelly DL, Patel P, Lyon DE: Metabolomic differentials in women with and without fibromyalgia. Cts-Clin Transl Sci 13:67–77, 2020
52. Miller JS, Rodriguez-Saona L, Hackshaw KV: Metabolomics in central sensitivity syndromes. Metabolites 10:1–30, 2020
53. Montoya JG, Holmes TH, Anderson JN, Maecker HT, Rosenberg-Hasson Y, Valencia IJ, Chu L, Younger JW, Tato CM, Davis MM: Cytokine signature associated with disease severity in chronic fatigue syndrome patients. Proc Natl Acad Sci U S A 114:E7150–E7158, 2017 [PubMed: 28760971]
54. Muhle C, Reichel M, Gulbins E, Kornhuber J: Sphingolipids in psychiatric disorders and pain syndromes. Handb Exp Pharmacol 431–456, 2013 [PubMed: 23563670]
55. Murina F, Caimi C, Di Pierro F, Di Francesco S, Cetin I: Features of the vaginal and vestibular microbioma in patients with vestibulodynia: A case-control study. J Low Genit Tract Dis 24:290–294, 2020 [PubMed: 32205761]

56. Osadchiy V, Labus JS, Gupta A, Jacobs J, Ashe-McNalley C, Hsiao EY, Mayer EA: Correlation of tryptophan metabolites with connectivity of extended central reward network in healthy subjects. Plos One 13:e0201772, 2018 [PubMed: 30080865]
57. Osadchiy V, Mayer EA, Bhatt R, Labus JS, Gao L, Kilpatrick LA, Liu C, Tillisch K, Naliboff B, Chang L, Gupta A: History of early life adversity is associated with increased food addiction and sex-specific alterations in reward network connectivity in obesity. Obes Sci Pract 5:416–436, 2019 [PubMed: 31687167]
58. Osadchiy V, Mayer EA, Gao K, Labus JS, Naliboff B, Tillisch K, Chang L, Jacobs JP, Hsiao EY, Gupta A: Analysis of brain networks and fecal metabolites reveals brain-gut alterations in premenopausal females with irritable bowel syndrome. Transl Psychiatry 10:367, 2020 [PubMed: 33139708]
59. Patti GJ, Yanes O, Shriver LP, Courade JP, Tautenhahn R, Manchester M, Siuzdak G: Metabolomics implicates altered sphingolipids in chronic pain of neuropathic origin. Nat Chem Biol 8:232–234, 2012 [PubMed: 22267119]
60. Pazmany E, Ly HG, Aerts L, Kano M, Bergeron S, Verhaeghe J, Peeters R, Tack J, Dupont P, Enzlin P, Van Oudenhove L: Brain responses to vestibular pain and its anticipation in women with genito-pelvic pain/penetration disorder. Neuroimage Clin 16:477–490, 2017 [PubMed: 28932680]
61. Peck MM, Maram R, Mohamed A, Ochoa Crespo D, Kaur G, Ashraf I, Malik BH: The influence of pro-inflammatory cytokines and genetic variants in the development of fibromyalgia: A traditional review. Cureus 12:e10276, 2020 [PubMed: 33042712]
62. Polat IH, Marin S, Rios J, Larroya M, Sanchez-Garcia AB, Murillo C, Rueda C, Cascante M, Gratacos E, Cobo T: Exploratory and confirmatory analysis to investigate the presence of vaginal metabolome expression of microbial invasion of the amniotic cavity in women with preterm labor using high-performance liquid chromatography. Am J Obstet Gynecol, 2020
63. Pukall CF, Goldstein AT, Bergeron S, Foster D, Stein A, Kellogg-Spadt S, Bachmann G: Vulvodynia: Definition, prevalence, impact, and pathophysiological factors. J Sex Med 13:291–304, 2016 [PubMed: 26944461]
64. Pukall CF, Strigo IA, Binik YM, Amsel R, Khalife S, Bushnell MC: Neural correlates of painful genital touch in women with vulvar vestibulitis syndrome. Pain 115:118–127, 2005 [PubMed: 15836975]
65. Ran Q, Jamoulle T, Schaeverbeke J, Meersmans K, Vandenberghe R, Dupont P: Reproducibility of graph measures at the subject level using resting-state fMRI. Brain Behav 10, 2020
66. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, Nien JK, Yoon BH, Mazor M, Luo J, Banks D, Ryals J, Beecher C: Metabolomics in premature labor: A novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med 23:1344–1359, 2010 [PubMed: 20504069]
67. Rosen R, Brown C, Heiman J, Leiblum S, Meston C, Shabsigh R, Ferguson D, D'Agostino R Jr: The female sexual function index (FSFI): A multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther 26:191–208, 2000 [PubMed: 10782451]
68. Rosenthal R, Rosnow RL, Rubin DB: Contrasts and effect sizes in behavioral research: A correlational approach. Cambridge, U.K.; New York, Cambridge University Press, 2000
69. Salvemini D, Doyle T, Kress M, Nicol G: Therapeutic targeting of the ceramide-to-sphingosine 1-phosphate pathway in pain. Trends Pharmacol Sci 34:110–118, 2013 [PubMed: 23318139]
70. Schweinhardt P, Kuchinad A, Pukall CF, Bushnell MC: Increased gray matter density in young women with chronic vulvar pain. Pain 140:411–419, 2008 [PubMed: 18930351]
71. Seckin-Alac E, Akhant SE, Bastu E, Tuzlalik S, Yavuz E: Elevated tissue levels of tumor necrosis factor-alpha in vulvar vestibulitis syndrome. Clin Exp Obstet Gynecol 41:691–693, 2014 [PubMed: 25551965]
72. Singh L, Kaur A, Bhatti MS, Bhatti R: Possible molecular mediators involved and mechanistic insight into fibromyalgia and associated co-morbidities. Neurochem Res 44:1517–1532, 2019 [PubMed: 31004261]
73. Squibb BM: U.S. Food and Drug administration approves bristol myers squibb's ZEPOSIA® (ozanimod), a new oral treatment for relapsing forms of multiple sclerosis. Businesswire. A Berkshire Hathaway Company, 2020

74. Stockstill K, Doyle TM, Yan X, Chen Z, Janes K, Little JW, Braden K, Lauro F, Giancotti LA, Harada CM, Yadav R, Xiao WH, Lionberger JM, Neumann WL, Bennett GJ, Weng HR, Spiegel S, Salvemini D: Dysregulation of sphingolipid metabolism contributes to bortezomib-induced neuropathic pain. J Exp Med 215:1301–1313, 2018 [PubMed: 29703731]

75. Teckchandani S, Nagana Gowda GA, Raftery D, Curatolo M: Metabolomics in chronic pain research. Eur J Pain, 2020

76. Tokarz J, Haid M, Cecil A, Prehn C, Artati A, Moller G, Adamski J: Endocrinology meets metabolomics: Achievements, pitfalls, and challenges. Trends Endocrin Met 28:705–721, 2017

77. Ventolini G, Gygax SE, Adelson ME, Cool DR: Vulvodynia and fungal association: A preliminary report. Med Hypotheses 81:228–230, 2013 [PubMed: 23707510]

78. Vestri A, Pierucci F, Frati A, Monaco L, Meacci E: Sphingosine 1-Phosphate receptors: Do they have a therapeutic potential in cardiac fibrosis? Front Pharmacol 8:296, 2017 [PubMed: 28626422]

79. Vouk K, Hevir N, Ribic-Pucelj M, Haarpaintner G, Scherb H, Osredkar J, Moller G, Prehn C, Rižner TL, Adamski J: Discovery of phosphatidylcholines and sphingomyelins as biomarkers for ovarian endometriosis. Hum Reprod 27:2955–2965, 2012 [PubMed: 22859507]

80. Vouk K, Ribic-Pucelj M, Adamski J, Rizner TL: Altered levels of acylcarnitines, phosphatidylcholines, and sphingomyelins in peritoneal fluid from ovarian endometriosis patients. J Steroid Biochem Mol Biol 159:60–69, 2016 [PubMed: 26921767]

81. Watson E, Reid G: Metabolomics as a clinical testing method for the diagnosis of vaginal dysbiosis. Am J Reprod Immunol 80, 2018

82. Welch SP, Sim-Selley LJ, Selley DE: Sphingosine-1-phosphate receptors as emerging targets for treatment of pain. Biochem Pharmacol 84:1551–1562, 2012 [PubMed: 22971335]

83. Wishart DS: Emerging applications of metabolomics in drug discovery and precision medicine. Nat Rev Drug Discov 15:473–484, 2016 [PubMed: 26965202]

84. Wishart DS: Metabolomics for investigating physiological and pathophysiological processes. Physiol Rev 99:1819–1875, 2019 [PubMed: 31434538]

85. Xiang J, Xue JY, Guo H, Li DD, Cui XH, Niu Y, Yan T, Cao R, Ma Y, Yang YL, Wang B: Graph-based network analysis of resting-state fMRI: Test-retest reliability of binarized and weighted networks. Brain Imaging Behav 14:1361–1372, 2020 [PubMed: 30734917]

86. Zanotta N, Campisciano G, Scrimin F, Ura B, Marcuzzi A, Vincenti E, Crovella S, Comar M: Cytokine profiles of women with vulvodynia: Identification of a panel of proinflammatory molecular targets. Eur J Obstet Gynecol Reprod Biol 226:66–70, 2018 [PubMed: 29852336]

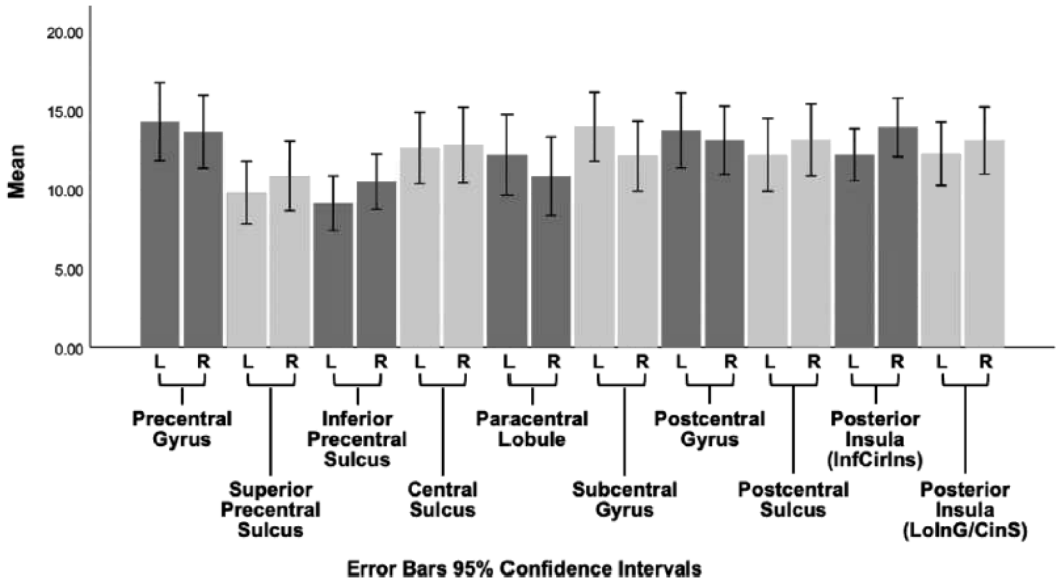


Figure 1.

Mean functional connectivity strength for cortical regions of interest. Bar plots representing the mean functional connectivity strength with 95% confidence intervals. *Abbreviations*: L = Left, R = right.

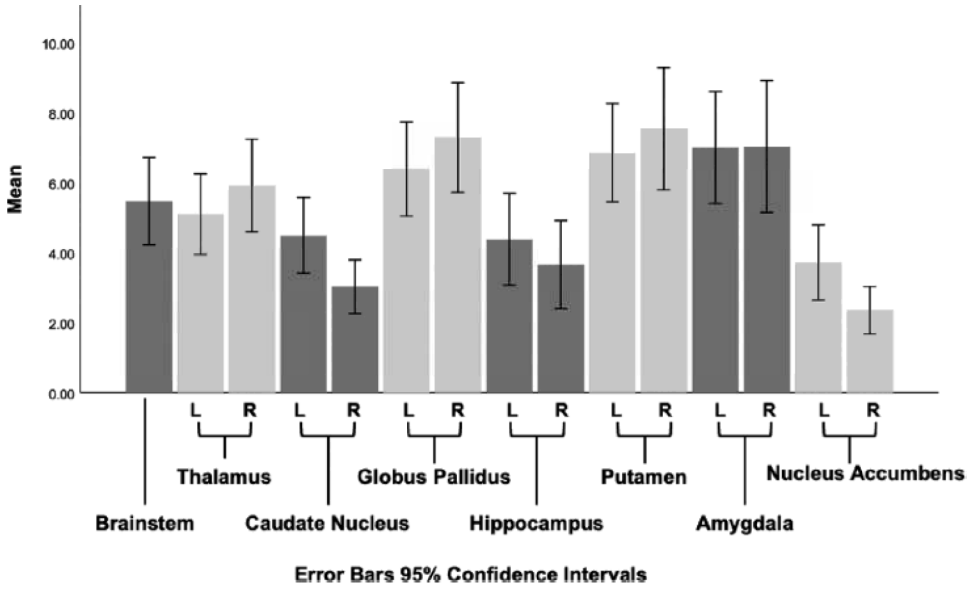
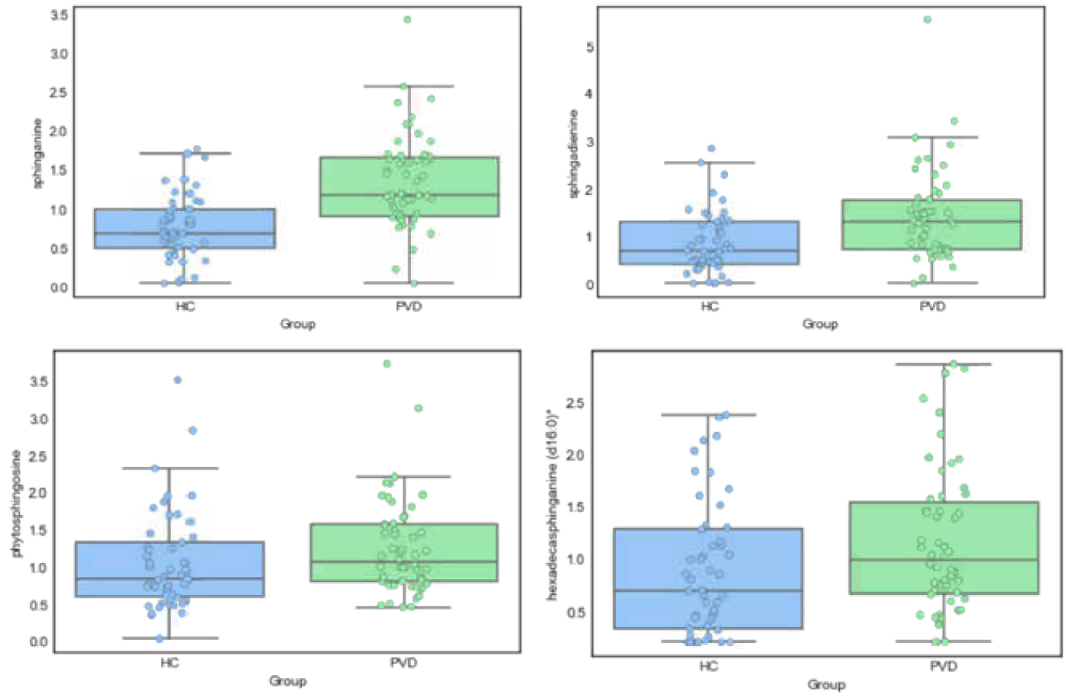


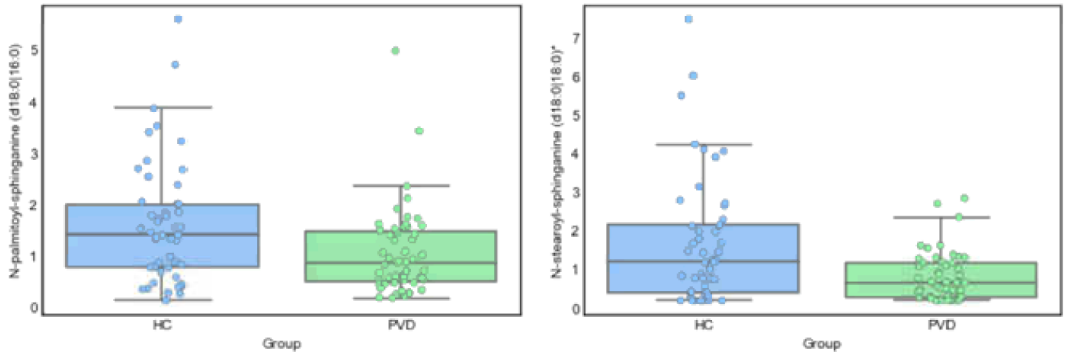
Figure 2.

Mean functional connectivity strength for subcortical regions of interest. Bar plots representing the mean functional connectivity strength with 95% confidence intervals. *Abbreviations*: L = Left, R = right.

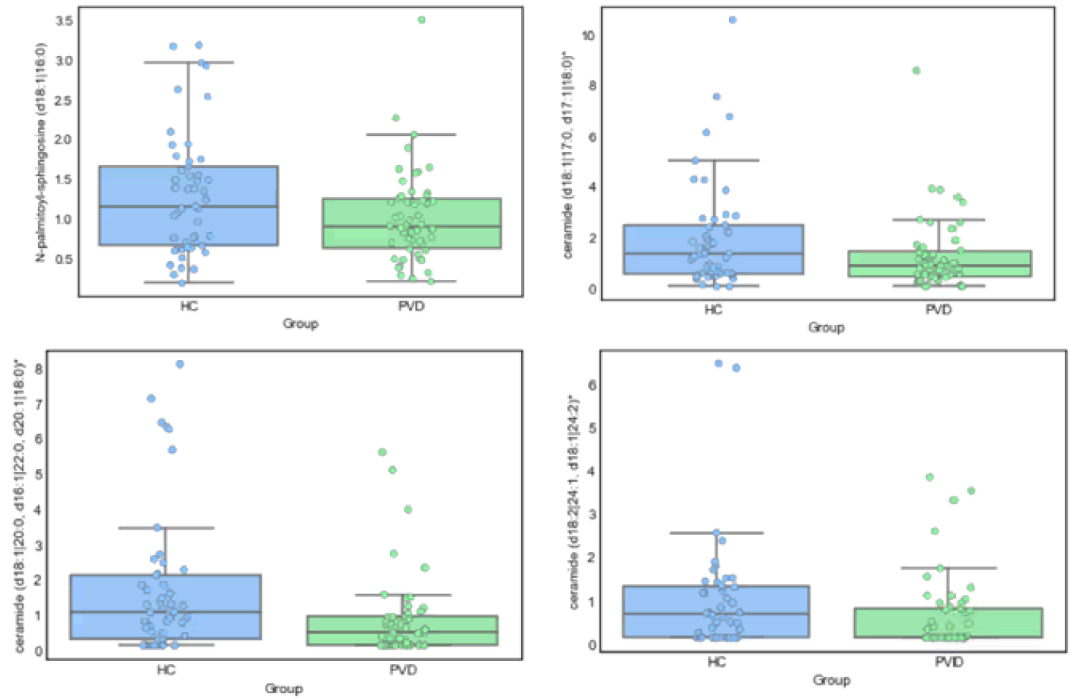
Sphingolipid Synthesis



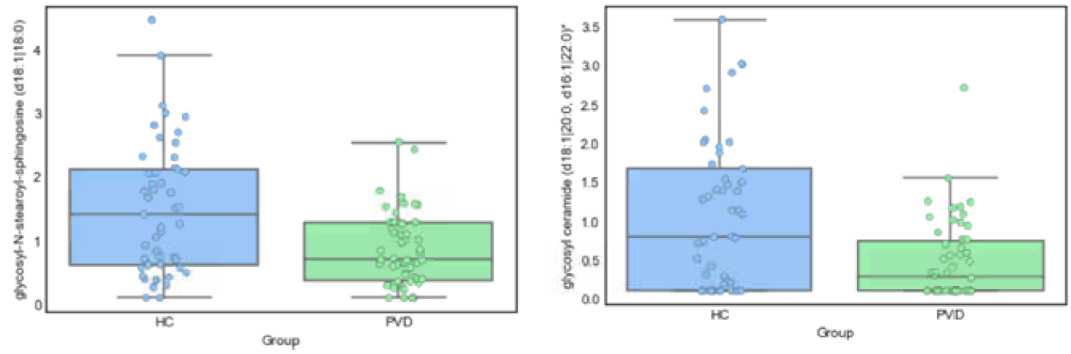
Dihydroceramides



Ceramides



Hexosylceramides (HCER)



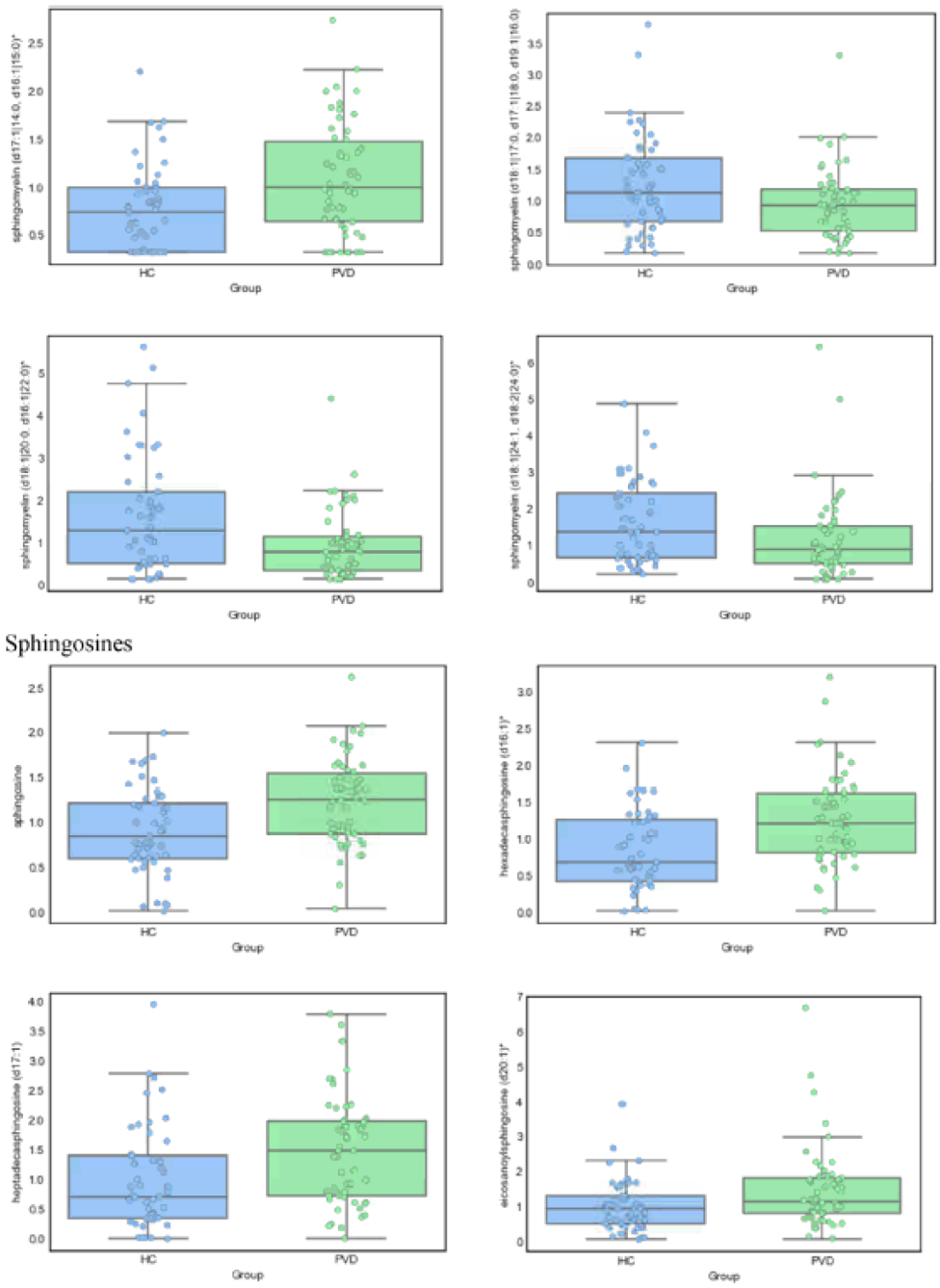


Figure 3.

Box plots of concentrations (untransformed) of sphingolipid signaling pathway metabolites in vaginal fluid showing alterations in women with PVD compared healthy controls. The box was drawn from the first quartile to the third quartile. The horizonal line through the box represents the median untransformed concentration. Individual subject data points are represented as circles. *Abbreviations*: HC = Healthy Controls, PVD = provoked vestibulodynia.

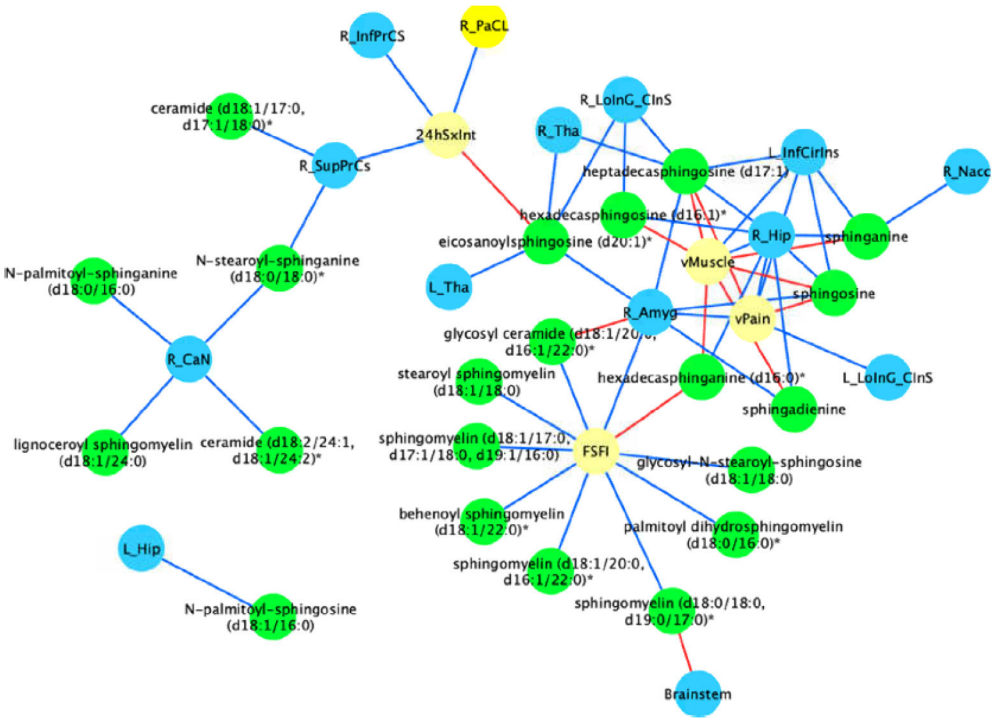


Figure 4.

Metabolite-brain-symptom association network in provoked vestibulodynia. This figure depicts the significant correlations ($P < .05$) between altered metabolites, PVD symptoms, and brain functional connectivity as a tri-partite association network. The nodes of the network (i.e., circles) are color coded and include sphingolipid signaling pathway metabolites (green) altered in PVD, symptom measures (yellow) and function connectivity strength of pain processing and pain modulatory brain regions (blue). The edges (i.e., lines) reflect correlations. Positive correlations are depicted by red lines and negative correlations by blue lines. A compound spring embedding algorithm was used to organize the nodes of the network based on similar patterns of correlations. Abbreviations: Symptoms: FSFI, female Sexual Functioning Index Full Scale Score; Vmuscle, Vaginal muscle tenderness total score, vPain = Vulvar vestibular pain total score, 24hSxINt, 24-hour symptom intensity score. Brain: L, left; R, Right. Amyg, Amygdala; CaN, Caudate Nucleus; Hip, Hippocampus; InfCirIns, inferior segment of the circular sulcus of the insula; LoInG_CInS, long insular gyrus and central sulcus of the insula; Nacc, Nucleus Accumbens; PaCL, paracentral lobule, SupPrCS, superior part of the precentral sulcus; Tha, Thalamus (color version of figure is available online.).

J Pain. Author manuscript; available in PMC 2023 August 27.

Table 1.

Characteristics of the Participants Providing Plasma and Vaginal Swab Samples

		VAGINAL SWAB (V) **PVD**	**PLASMA SAMPLE (P)** **PVD**	**V/P** **HC**	**PVD_v v. HC**	**PVD_P v HC**
					Fisher's Exact test	Fisher's Exact test
Hormone type		N(%)	N(%)	N(%)	*P* = .11	*P* = .37
	None	25 (46.3%)	29(48.3%)	29 (59.2%)		
	Local	7 (13.0%)	8 (13.3%)	6 (12.2%)		
	Systemic	22 (40.7%)	23 (38.3%)	14 (28.6%)		
Diet Type					*P* = .039	*P* = .032
	Standard American	6(14)	9(18.4)	4(8.3)		
	Modified American	23(53.5)	25(51)	26(54.2)		
	Mediterranean	9(20.9)	12(18.4)	3(6.3)		
	Other	5(11.6)	6(12.2)	15(31.3)		
Ethnicity	Hispanic	6(11.1)	6(10)	8(16.3)		
Race	Amer Ind	0	0	2(4.1)		
	Asian	10(18.5)	12(20)	25(51)		
	Black	3(5.6)	2(3.3)	4(5.6)		
	Hawaiian	0	1(2)	1(2)		
	White	46(85.2)	51(85)	25(51)		
	Multiracial	5(9.3)	5(8.3)	4(8.1)		
		Mean(SD) range	Mean(SD) range	Mean(SD) range		
Age		27.5 (4.9) 20–42	27.6(6.0) 18–50	24.6(6.1) 18–46	t(101) = 2.62, *P* = .01	t(107) = 2.55, *P* = .012
Body mass index (kg/m^2)		22.9(3.6) 14.9–31.5	22.8(3.5) 14.9–31.5	23.0(3.7) 16.1–32.4	t(101) = −0.14, *P* = .89	t(107) = −0.27, *P* = .79
Subjective social status		6.33(1.4) 3–10	6.25(1.3) 3–9	6.59(1.6) 3–9	t(96) = −0.86 P = .39	t(103) = −1.17 P = .25

HAC, healthy controls; N, sample size; PVD, provoked vestibulodynia; SD, standard deviation; t, independent t-test.

Table 2.

Provoked Vestibulodynia-Specific Sample Characteristics

	VAGINAL SWAB (V) N = 54 N(%)	**PLASMA SAMPLE (P) N = 60 N(%)**
Unprovoked *	8(14.8)	9 (15)
Onset		
Primary	27 (50)	28 (47)
Secondary	27 (50)	32 (53)
	Mean(SD),range	Mean(SD), range
Pain Duration †	81.5(47.8), 36–144	78(47.0), 26–144
Total Vulvar vestibular pain on exam #	44.6(15.4), 17–77	44.2 (14.5),17–77
Total vaginal muscle tenderness score ‡	13.87(6.9), 0–25	13.2 (6.9), 0–25
24-hour symptom intensity reports §	2.15 (3.93), 0–16	2.6 (4.3), 0–16

N, sample size, SD, standard deviation

* 8 patients with both provoked and unprovoked vestibulodynia

† pain duration in months

2 missing datapoints in plasma

‡ 1 missing data point in vaginal swab samples, 4 missing data points in plasma samples

§ 2 missing data points in vaginal swab samples, 1 missing data point in plasma samples

Table 3.

Pathway Enrichment Scores in PVD vs HC (No hormones)

SUB-PATHWAY	K	M	N	N	ENRICHMENT
Sphingosines	4	4	242	952	3.98
Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	4	5	242	952	3.18
Sphingolipid Synthesis	3	4	242	952	2.97
Tocopherol Metabolism	8	11	242	952	2.92
Plasmalogen	3	5	242	952	2.38
Partially Characterized Molecules	8	15	242	952	2.14
Benzoate Metabolism	12	23	242	952	2.11
Hexosylceramides (HCER)	2	4	242	952	1.97
Phenylalanine Metabolism	4	9	242	952	1.76
Tyrosine Metabolism	10	23	242	952	1.74
Poly a mi ne Metabolism	6	14	242	952	1.7
Androgenic Steroids	6	14	242	952	1.7
Fatty Acid Metabolism (Acyl Carnitine, Hydroxy)	2	5	242	952	1.58
Sphingomyelins	6	16	242	952	1.49
Glycine, Serine and Threonine Metabolism	4	11	242	952	1.44

Enrichment Value, (k/m)/((n-k)/(N-m))m Where m, number of metabolites in the pathway; k, number of significant metabolites in the pathway; n, total number of significant metabolites; N, total number of metabolites

Table 4.

Group Differences in Sphingolipid Signaling Pathway Metabolites in Vaginal Fluid

		ALL SUBJECTS (PVD(N= 54)/HC(N= 49)			SUBGROUPANALYSES NO HORMONES PVD(N= 25)/HC(N= 29)	SYSTEMIC HORMONES PVD (N = 22)/HC(N= 14)	PVD SYSTEMIC/ PVD NO HORMONE
Sub Pathway	Biochemical Name	Fold Change	*P*	q	q	q	q
Sphingolipid Synthesis	sphinganine	1.79	.00002	0.001	.036	.31	0.95
	sphingadienine	1.63	.002	0.011	.082	.32	0.94
	phytosphingosine	1.19	.030	0.044	.230	.79	0.71
	hexadecasphinganine (d16:0)*	1.30	.016	0.030	.112	.64	0.73
Dihydroceramides	N-palmitoyl-sphinganine (d18:0/16:0)	0.65	.010	0.024	.201	.44	0.98
	N-stearoyl-sphinganine (d18:0/18:0)*	0.47	.005	0.017	.233	.33	0.98
Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)	0.78	.073	0.073	.414	.61	0.98
	ceramide (d18:1/17:0, d17:1/18:0)*	0.64	.052	0.059	.433	.55	0.94
	ceramide (d18:1/20:0, d16:1/22:0, d20:1/18:0)*	0.49	.008	0.022	.307	.40	0.94
	ceramide (d18:2/24:1, d18:1/24:2)*	0.63	.021	0.036	.152	.63	0.71
Hexosylceramides (HCER)	glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	0.57	.002	0.011	.071	.52	0.88
	glycosyl ceramide (d18:1/20:0, d16:1/22:0)*	0.44	.001	0.011	.066	.50	0.88
Dihydrosphingomyelins	palmitoyl dihydrosphingomyelin (d18:0/16:0)*	0.74	.032	0.046	.076	.64	0.65
	sphingomyelin (d18:0/18:0, d19:0/17:0)*	0.56	.004	0.015	.063	.51	0.86
Sphingomyelins	stearoyl sphingomyelin (d18:1/18:0)	0.77	.039	0.051	.181	.60	0.81
	behenoyl sphingomyelin (d18:1/22:0)*	0.57	.008	0.022	.063	.62	0.70
	tricosanoyl sphingomyelin (d18:1/23:0)*	0.44	.002	0.011	.073	.55	0.84
	lignoceroyl sphingomyelin (d18:1/24:0)	0.48	.002	0.011	.045	.65	0.63
	sphingomyelin (d17:1/14:0, d16:1/15:0)*	1.41	.004	0.015	.061	.61	0.93
	sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	0.77	.101	0.091	.240	.62	0.90
	sphingomyelin (d18:1/20:0, d16:1/22:0)*	0.58	.015	0.029	.072	.62	0.70
	sphingomyelin (d18:1/24:1, d18:2/24:0)*	0.74	.016	0.030	.043	.64	0.22
Sphingosines	sphingosine	1.37	.01	0.02	.128	.38	0.93
	hexadecasphingosine (d16:1)*	1.50	.002	0.01	.083	.33	0.97
	heptadecasphingosine (d17:1)	1.48	.01	0.02	.072	.38	0.88

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	ALL SUBJECTS			SUBGROUPANALYSES		
	(PVD(N= 54)/HC(N= 49)			NO HORMONES PVD(N= 25)/HC(N= 29)	SYSTEMIC HORMONES PVD (N = 22)/HC(N= 14)	PVD SYSTEMIC/ PVD NO HORMONE
eicosanoylsphingosine (d20:1)*	1.49	.01	0.03	.049	.51	0.61

Abbreviations: Fold Change: ratio of means, *P*, probability; q, corrected *P* value

The table shows the main analysis results from comparing provoked vestibulodynia (PVD) and healthy controls (HC) subjects in all subjects (Fold Change, *P*, q) as well as the false discovery rate corrected *P* values (q) associated with the hormone subgroup sensitivity analyses.

Table 5.

Association Between Vaginal Concentrations of Altered Sphingolipid Pathway Metabolites and PVD Symptom Measures

SUB PATHWAY	BIOCHEMICAL NAME	SYMPTOMS	CORR	P	DIRECTION OF BIOCHEMICAL CHANGE IN PVD COMPARED TO HC
Sphingolipid Synthesis	sphingadienine	vMuscle	0.44	.001	increased
	sphinganine	vMuscle	0.41	.003	
HCER	glycosyl ceramide (d18:1/20:0, d16:1/22:0)*	FSFI	−0.4	.004	decreased
	glycosyl-N-stearoyl-sphingosine (d18:1/18:0)	FSFI	−0.42	.002	
Dihydrosphingomyelins	palmitoyl dihydrosphingomyelin (d18:0/16:0)*	FSFI	−0.32	.022	decreased
	sphingomyelin (d18:0/18:0, d19:0/17:0)*	FSFI	−0.32	.024	
Sphingomyelins	behenoyl sphingomyelin (d18:1/22:0)*	FSFI	−0.33	.018	decreased
	Sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	FSFI	−0.36	.011	
	sphingomyelin (d18:1/20:0, d16:1/22:0)*	FSFI	−0.36	.009	
	stearoyl sphingomyelin (d18:1/18:0)	FSFI	−0.45	.001	
	behenoyl sphingomyelin (d18:1/22:0)*	FSFI	−0.33	.018	
Sphingosines	eicosanoylsphingosine (d20:1)*	4hSxInt	0.29	.032	increased
	heptadecasphingosine (d17:1)	vPain	0.31	.021	
	hexadecasphinganine (d16:0)*	FSFI	0.29	.044	
		vPain	0.42	.002	
	hexadecasphingosine (d16:1)*	vPain	0.46	.001	
	sphingosine	FSFI	0.5	.0001	
		vPain	0.28	.044	

Corr, Spearman's correlation coefficient; *P*, probability, HC, healthy controls; HCER, Hexosylceramides; PVD, provoked vestibulodynia

Symptoms: FSFI, female Sexual Functioning Index Full Scale Score; Vmuscle, Vaginal muscle tenderness total score, vPain, vulvar vestibular pain total score; 24hSxINt, 24-hour symptom intensity score

Table 6.

Association Between Vaginal Concentrations of Altered Sphingolipid Pathway Metabolites and Functional Connectivity of Pain Processing and Pain Modulatory Brain Regions

SUB PATHWAY	BIOCHEMICAL NAME	BRAIN REGIONS	CORR	P	DIRECTION OF BIOCHEMICAL CHANGE PVD COMPARED TO HC
Sphingolipid Synthesis	sphingadienine	R Hippocampus	−0.36	.012	increased
		R Amygdala	−0.32	.027	
	sphinganine	R Nucleus Accumbens	−0.35	.016	increased
		R Hippocampus	−0.33	.024	
		L_InfCirIns	−0.29	.049	
Dihydroceramides	N-palmitoyl-sphinganine (d18:0/16:0)	R Caudate Nucleus	−0.29	.05	decreased
	N-stearoyl-sphinganine (d18:0/18:0)*	R Caudate Nucleus	−0.33	.026	decreased
		R SupPrCs	−0.31	.036	
Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)	L Hippocampus	−0.34	.021	decreased
	ceramide (d18:1/17:0, d17:1/18:0)*	R SupPrCs	−0.32	.028	decreased
	ceramide (d18:2/24:1, d18:1/24:2)*	R Caudate Nucleus	−0.34	.019	decreased
HCER	glycosyl ceramide (d18:1/20:0, d16:1/22:0)*	R Amygdala	0.32	.031	decreased
Dihydrosphingomyelins	sphingomyelin (d18:0/18:0, d19:0/17:0)*	Brainstem	0.31	.034	decreased
Sphingomyelins	lignoceroyl sphingomyelin (d18:1/24:0)	R Caudate Nucleus	−0.31	.034	decreased
Sphingosines	sphingosine	R Hippocampus	−0.35	.017	increased
		R Amygdala	−0.3	.042	
		L InfCirIns	−0.29	.05	
	eicosanoylsphingosine (d20:1)*	R Amygdala	−0.41	.004	increased
		R Thalamus	−0.33	.024	
		R LoInG_CInS	−0.32	.027	
		L Thalamus	−0.29	.048	
	heptadecasphingosine (d17:1)	R Amygdala	−0.41	.004	increased
		R Hippocampus	−0.39	.007	
		R LoInG_CInS	−0.37	.01	
		R Tha	−0.34	.021	
		L InfCirIns	−0.33	.025	
	hexadecasphinganine (d16:0)*	R Hippocampus	−0.33	.025	increased
	hexadecasphingosine (d16:1)*	R Hippocampus	−0.32	.027	increased
		R LoInG_CInS	−0.29	.045	

Abbreviations: Corr, Spearman's correlation coefficient; HC, healthy controls; HCER, Hexosylceramides; *P*, probability PVD, provoked vestibulodynia. Brain regions: InfCirIns, inferior segment of the circular sulcus of the insula; L, Left; LoInG_CInS, long insular gyrus and central sulcus of the insula; PVD, R, right; SupPrCS, superior part of the precentral sulcus.

Table 7.

Association of PVD Symptom Measures with the Functional Connectivity of Pain Processing and Pain Modulatory Brain Regions

SYMPTOMS	BRAIN REGION	CORR	P
FSFI	R Amygdala	−0.38	.010
24hSxInt	R SupPrCs	−0.31	.035
24hSxInt	R PaCL_S	−0.30	.044
24hSxInt	R InfPrCS	−0.29	.048
vMuscle	L InfCirIns	−0.30	.040
vMuscle	R Hippocampus	−0.29	.050
vPain	L InfCirIns	−0.38	.009
vPain	R Hippocampus	−0.34	.020
vPain	L LoInG_CInS	−0.32	.029
vPain	R Amygdala	−0.31	.035

Abbreviations: Corr, Spearman's correlation coefficient, p=probability, PVD, provoked vestibulodynia. Symptoms: FSFI, female sexual functioning index full scale score; Vmuscle, vaginal muscle tenderness total score, vPain, vulvar vestibular pain total score, 24hSxInt, 24-hour symptom intensity score. Brain regions: InfCirIns, inferior segment of the circular sulcus of the insula; L, Left; LoInG_CInS, long insular gyrus and central sulcus of the insula; PaCL, paracentral lobule; R, right; SupPrCS, superior part of the precentral sulcus.