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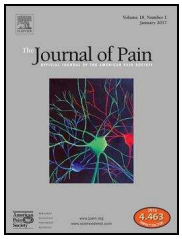

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Disease-related microstructural differences in the brain in females with provoked vestibulodynia

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Highlights

- In PVD microstructural alterations were observed in fibers associated with sensorimotor integration and pain processing.
- Alterations were associated with increased vulvar pain and muscle tenderness in PVD.
- Differences between chronic overlapping pain syndromes may be related to sensorimotor-thalamic-brainstem thalamic connectivity.

ABSTRACT

Provoked vestibulodynia (PVD) is a chronic pelvic pain disorder affecting 16% of the female population. Neuroimaging studies have highlighted central abnormalities in PVD, similar to other chronic pelvic pain disorders, including brain regions involved in sensory processing and modulation of pain. The aim of the study was to determine alterations in the subvoxel, microstructural organization within tissues in PVD compared to healthy controls (HCs) and a disease control group (irritable bowel syndrome, IBS). Diffusion tensor imaging (DTI) magnetic resonance imaging (MRI) was conducted in 87 age-matched premenopausal females (29 PVD, 29 HCs, 29 IBS). Statistical parameter mapping of fractional anisotropy (FA) and mean diffusivity (MD) maps was used to identify microstructural difference in the brain specific to PVD or shared with IBS. PVD alterations in microstructural organization of the brain were predominantly observed in fibers associated with sensorimotor integration and pain processing that relay information between the thalamus, basal ganglia, sensorimotor and insular cortex. PVD, compared to HCs, displayed extensive increases in the FA of somatosensory and basal ganglia regions. In contrast, PVD and IBS subjects did not show any FA-related group differences. PVD subjects displayed greater MD in the basal ganglia compared to HCs (higher MD in the internal capsule and pallidum) and IBS (higher MD in the putamen and pallidum). Increases in MD were associated with increased vaginal muscle tenderness and vulvar pain. The current findings highlight possible shared mechanisms between two different

pelvic pain disorders, but also highlight the wide-spread alterations observed specifically in PVD compared to HCs.

PERSPECTIVE

Alterations in microstructure in provoked vestibulodynia were observed in fibers associated with sensorimotor integration and pain processing, which were also associated with increased vaginal muscle tenderness and vulvar pain. These alterations may be contributing to increased pain sensitivity and tenderness, highlighting the need for new therapies targeting the CNS.

KEY WORDS: Provoked Vestibulodynia (PVD); irritable bowel syndrome (IBS); diffusion tensor imaging (DTI); chronic pain; brain

INTRODUCTION

Provoked vestibulodynia (PVD) is a chronic pelvic pain disorder affecting approximately 16% of women [38], with significant impact on quality of life [3]. Individuals with PVD report localized hypersensitivity and severe pain of the vulvar vestibule upon vaginal penetration (e.g., intercourse, tampon use) in the absence of detectable infection or inflammatory etiology [4, 32]. The underlying pathophysiology of PVD is largely unknown, and treatment options remain unsatisfactory [86]. Available evidence suggests that both peripheral and central alterations in sensory processing appear to be involved [2, 18, 26, 77, 82, 89]. There is evidence that inflammation [31, 56 24, 69] may result in proliferation of peripheral nerve fibers (i.e. altered vestibular innervation) [36] leading to central sensitization and tonic pelvic floor muscle hypercontractility [65, 91]; however, this hypothesis remains controversial.

Non-invasive neuroimaging of the brain permits characterization of functional and structural abnormalities in PVD. Initial studies suggested that vulvar pain hypersensitivity may be associated with central sensitization, dysregulation of endogenous pain modulatory systems, and attentional enhancement of pain perception [5, 35, 37, 70, 76, 79]. Reported alterations in the functional connectivity between sensorimotor and basal ganglia regions are highly correlated with pain sensitivity and muscle tenderness [35]. Gray matter is also altered in basal ganglia regions in PVD[76]. Central abnormalities observed in PVD are similar to those seen in fibromyalgia [37], a wide spread pain disorder, and irritable bowel syndrome (IBS) [35], another chronic pelvic pain disorder. Together, these studies provide evidence that disease specific brain alterations

may play an important role in symptom generation and their identification may be critical for long-term therapeutic control of PVD.

While functional and morphometric alterations in the regions associated with the somatosensory and integrative brain have been observed in PVD, less is known about the microstructural adaptations that occur in dense white matter and dendritic projections. Diffusion tensor imaging (DTI) is a non-invasive magnetic resonance imaging (MRI) technique used to quantify the subvoxel, microstructural organization within tissues by probing the behavior of randomly diffusing water molecules. In particular, measures of the fractional anisotropy (FA) can be used to estimate the degree of directional coherence of the underlying tissue structures within an image voxel, reflecting the strength of axonal or dendritic projections, while mean diffusivity (MD) can be used to estimate relative tissue compactness and degree of myelination [1]. While studies have found differences in both FA and MD in several chronic pain conditions [22, 25, 45, 58, 60, 62], it is unclear whether such differences are also present in PVD. Impairment in functional connectivity and region morphometry are in part due to alterations anatomical connectivity and microstructural properties of white matter tracts[19, 41]. Supporting this notion, morphometric, functional and anatomical neuroimaging studies in other chronic pelvic pain disorder (i.e, IBS and IC/PBS) have demonstrated consistent alterations regions and white matter tracts associated with somatosensory processing and integration regions[16, 22, 23, 43, 45, 47-49, 53, 59]. Approximately 20% of women with PVD also have a comorbid diagnosis of IBS or IC/PBS[55] suggesting the potential for shared central mechanisms between these disorders[61, 67]. Thus, given the morphometric and resting state alterations of sensorimotor processing and integration regions previously reported in PVD, we

hypothesized that women with PVD would exhibit FA and MD alterations in these regions, specifically sensorimotor regions including basal ganglia and thalamus. To test this hypothesis, we compared voxel-wise DTI measurements between PVD and healthy control participants (HCs), and between PVD and IBS patients, to identify alterations specific to PVD compared to another chronic pelvic pain disorder. In addition, we examined the association between microstructural alterations linked with PVD and PVD-specific symptoms and symptom severity.

MATERIALS AND METHODS

Participants

Study subjects were those included in a previously published resting state study; data collection, including symptom variables, and questionnaire data have been described [35]. Women with PVD were recruited through the University of California, Los Angeles (UCLA) Obstetrics and Gynecology clinics. The diagnosis of PVD was confirmed during a clinical examination by an OB/GYN with expertise in this area (AJR) . Inclusion criteria for participants with PVD were ≥6 months of pain in the vulvar vestibule of at least 4 out of 10 in severity (0=no pain to 10=worst pain imaginable) during attempted intercourse or other activities involving vestibular pressure (e.g. tampon use) and findings on exam consistent with vestibulodynia. Examination confirmed PVD if the cotton swab test was positive for pain ≥4 out of 10 (0=no pain to 10=worst pain imaginable) limited to the vulvar vestibule (5, 6, 7, 10 or 2 o'clock) in the absence of other pathology such as infection, dermatitis, dermatoses, atrophy, or peripheral neuropathy. Infections such as candida, bacterial vaginosis or herpes simplex were ruled out by history, visual inspection, vaginal pH, and saline and potassium hydroxide slide prep. Pelvic floor muscles (levator ani and bulbocavernosus) were evaluated for tone and pain. Speculum examination of the vagina and bimanual pelvic examination were preformed to exclude other pathology that could contribute to the pain. Age-matched data for female IBS and female healthy control (HC) subjects was obtained from past subjects enrolled in neuroimaging studies at the Center for Neurobiology of Stress and Resilience and have been used in previous publications[22, 23, 35, 39, 40]. Healthy control subjects (HCs) were recruited by advertisement and screened via history and medical exam for absence of

pain disorders, and were recruited from the UCLA and local Los Angeles community.

Exclusionary criteria for all subjects included pregnancy or lactation, substance abuse, tobacco dependence (smoked half a package of cigarettes or more daily), abdominal surgery other than appendectomy or cholecystectomy, current or past psychiatric illness, extreme strenuous exercise (exercise more than one hour per day), and major medical or neurological conditions. In addition, subjects with current regular use of analgesic drugs (including narcotics, opioids, and α2-δ ligands) were excluded. Use of medications such as antidepressants (low-dose tricyclic anti-depressants, selective serotonin uptake inhibitors, nonselective serotonin reuptake inhibitors) was only allowed if subjects had been on a stable dose for a minimum of 3 months. We did not exclude subjects who used non steroidal anti-inflammatory agents (NSAIDS). Instead we asked that subjects refrain from taking this medication 12 hours prior to their scanning visit. All subjects were female, right handed and premenopausal confirmed by self-report. All subjects were naturally cycling and were excluded if they were on hormonal contraceptives in order to avoid confounds associated with more sensitive vulvar vestibules.

Diagnosis with a comorbid chronic pain disorder was an exclusionary criterion. To assess for the comorbidity between PVD and IBS, presence of IBS symptoms for the PVD subjects and presence of PVD symptoms for the IBS subjects were recorded during the medical history.

The study was approved by the University of California, Los Angeles Institutional Review Board, and was conducted in accordance with the institutional guidelines

regulating research on human subjects. All subjects provided written informed consent to participate and were compensated for participating in the study.

Clinical Assessments and Questionnaires

Clinical Assessment of PVD. Clinical assessment and questionnaires were completed within the week prior to MRI acquisition. During history and clinical examination, detailed information was obtained regarding vulvar pain for the PVD patients. Patients with PVD were asked to report their pain duration, level of pain intensity (scale 0-20, 0=neutral, 20=extremely intense), and level of pain unpleasentness (scale 0-20, 0=neutral, 20=very intolerable) in the past 24 hours using the Gracely Differential Descriptor Pain Scale [33]. Level of pain related to sex and not related to sex was also recorded (scale 0-100, 0=no pain, 100=most intense pain imaginable). A brief neurosensory examination was conducted [55, 91]. Pain testing of the vulva and vestibule was performed using a cotton swab, which is the main diagnostic instrument for PVD [30]. To exclude specific neuropathy, sensory testing of the sensory dermatomes (T12, L1, S2, S3/4, S5) of the mons pubis, vulva and the perineum were examined bilaterally for allodynia (pain with gentle touch with the cotton tip), hyperalgesia (pain with touch with the sharp wooden end of a broken cotton swab), or normal sensation.

Mapping of pain in the vulvar vestibule was then performed by touching the vestibule perpendicularly with the cotton end of 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, and 2 o'clock (peri-urethral, anterior vestibule). Subjects were asked to rate the pain severity (scale=0-10; 0=none, 10=most severe pain imaginable) and describe quality

(verbal descriptor e.g., sharp vs. cotton tip pressure sensation). A vulvar pain total "score" was created by adding the pain scores at each of the 5 vestibule sites (0-50).

The vaginal muscle examination was then performed. Internal muscle tone and tenderness was assessed with a single lubricated digit, applying approximately 2 kg of pressure for 2 seconds. (The examiner's finger pressure was calibrated immediately before the exam with an algometer). The bulbocavernosus muscles at 5 and 7 o'clock, and the levator ani complex were assessed in the midline and laterally at 5 and 7 o'clock. Participants were asked to rate the pain at each site (scale=0-10, 0=no pain, 10=the most severe pain imaginable). A vaginal muscle tenderness total "score" was created by adding the pain scores at each of the 5 muscle sites (0-50).

Clinical Assessment of IBS. Subjects with IBS met Rome III symptom criteria for a diagnosis of IBS [21]. A gastroenterologist or gastrointestinal nurse practitioner obtained histories and conducted physical examinations. Patients with IBS who had all types of predominant bowel habits were included. Questionnaires were completed with in the week prior to MRI acquisition to determine IBS symptom type, severity, duration of symptoms, and abdominal sensation [Bowel Symptom Questionnaire] [15]. Overall GI symptom severity and abdominal pain in the past week were assessed using a 21-point Numerical Rating Scale (scale=0-20, 0=no pain and 20=the most intense symptoms imaginable). Usual symptom severity was assessed on an ordinal scale where 1=None, 2=Mild, 3=Moderate, 4=Severe, and 5=Very Severe.

For all subjects, anxiety and depression were also assessed using a self-report 14-item instrument (Hospital Anxiety and Depression Scale; HADS) [90].

MRI Data Acquisition

Subjects were scanned on a 3.0 Tesla magnetic resonance imaging scanner (Siemens Trio; Siemens, Erlangen, Germany). Subjects underwent DTI using one of two comparable acquisition protocols consisting of either 60 or 64 non-collinear directions with b=1000 s/mm^2, along with either 8 or 1 b=0 s/mm^2 images, respectively. Both protocols had a repetition time (TR)=9400ms, echo time (TE)=83ms, a field of view (FOV)=256mm, an acquisition matrix of 128x128, and a slice thickness of 2mm (no slice gap) to produce 2x2x2mm^3 isotropic voxels. Out of all the subjects, only five HC subjects and one IBS patient underwent scanning with the 64-direction sequence, all other participants were scanned using the 60-direction protocol. All scans were assessed via a quality control pipeline and made part of the Pain and Interoceptive Imaging Network (PAIN) repository (painrepository.org), which is a NIH (NIDA/NCCAM) funded neuroimaging data repository [54]. Briefly, this included visually checking for artifacts and motion on the raw diffusion weighted and b0 images, visual assessment of FA and MD map quality, as well checking for physiologically feasible FA and MD values (FA of 0-0.1 and MD of 3-4 μm2/ms in ventricles, and FA of 0.6-0.9 and MD of 0.6-0.9 μm2/ms in splenium of corpus callosum). Maximum relative motion thresholds for translation and rotation for each direction (x, y, and z) were set at 2mm and 2°, respectively. No subjects presented with serious adverse imaging artifacts and no subjects exceeded motion thresholds: the highest maximum relative translation for any subject was 1.37mm, and the highest maximum relative rotation was 1.59°.

MRI Processing

All raw DTI data was corrected using eddy current correction as part of the FMBRIB Diffusion Toolbox (FDT) [6] in the FMBRIB software library (FSL, Version 5.0.3, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) package [78]. FA and MD maps were created using *MRtrix* (Brain Research Institute, Melbourne, Australia, http://www.brain.org.au/software/). FA images were then registered to the Johns Hopkins University DTI atlas (ICBM-DTI-81 1mm FA atlas) [63] using a linear 12-degree of freedom affine registration using FMRIB's Linear Image Registration Tool (FLIRT) [46]. Nonlinear (elastic deformation) registration was then performed following linear registration using FMRIB's Nonlinear Image Registration Tool (FNIRT) command and default parameters. These transformations (affine matrix and nonlinear warp-fields) were then applied to the MD images so all DTI metrics were aligned to the same atlas space.

Data Analysis

Data analysis of non-imaging data. Clinical and demographic variables were examined using linear contrasts within the framework of a general linear model (GLM) in the Statistical Package for the Social Sciences (SPSS) software (version 19) to examine differences in following contrasts: 1) PVD compared to HC, and 2) PVD compared to IBS. Significance was considered at $p<.05$ [68].

Statistical Parameter Mapping of DTI Data. Voxelwise comparisons were performed using mask consisting of the disjunction between the white matter (defined by an FA >0.3 in the FMRIB FA atlas) and deep gray matter masks of interest (namely the basal ganglia and thalamus, defined by their respective masks from the Harvard-Oxford

subcortical atlas) as outlined in a previous publication [22]. The basal ganglia and thalamus were included in the mask for this analysis because of their importance in pain processing [17, 20] and their role in chronic pain as identified using other neuroimaging techniques [3, 11, 42, 74]. DTI analysis of the thalamus and basal ganglia has been performed in a variety of neurological conditions which have shown altered microstructure compared to healthy controls and correlations with patient symptoms; these conditions include Parkinson's disease [14, 50, 66], multiple sclerosis [13, 81], and recently in chronic pain [22, 87]. Statistical parameters maps (SPMs) were created for both FA and MD indices using a voxelwise general linear model (GLM) design that included patient group (PVD vs. HC; PVD vs. IBS), and covariates, age and body mass index using the Analysis of Functional Neuroimaging (AFNI) *3dTtest++* function (https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dttest++.html). In the presence of group differences in mood scores and group differences in voxel-wise FA and MD, sensitivity analysis were performed to determine the influence of the mood and trauma variables on the significant group differences. This involved specifying the appropriate group contrast (i.e., PVD vs. HC) along with the covariates of interest.

To examine the relationship between FA and MD and symptom variables univariate voxel-wise GLMs were specified for the PVD group only. In these models either FA or MD maps served as the dependent variables and the independent variables included average pain with sex, Vaginal Muscle Tenderness Total score, Vulvar Pain Total score, and Vulvar Pain @ 6 o'clock score. Visual inspection of histograms and Q-Q plots as well as assessment of skewness and kurtosis indicated the covariates in the GLMs were normally distributed. To account for multiple comparisons and spatial

correlations in SPMs, a cluster-based correction approach [12] was used including a level of significance $p < .01$ with a minimum cluster size of 1mL, or approximately 125 contiguous 2x2x2mm^3 voxels, which is more conservative than previous studies using similar methods [22, 23]. In order to ensure that our cluster-size threshold would control for false positives, we performed permutation tests to simulate the cluster-size distribution based on our data. Using the HC data, we estimated the spatial AutoCorrelation Function (ACF) for the maps using the AFNI function 3dFWHMx (https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dFWHMx.html). The ACF parameters were then used to estimate the cluster-size threshold given a p-value threshold of 0.01 and a range of alpha levels (0.05 to 0.0001) for family-wise error rates (FWER) of two-sided tests, using the AFNI function 3dClustSim (https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html). This was done for both FA and MD measures. The results indicated that for a FWER of 0.0001, we would need a cluster-size threshold of 0.55mL for FA, and a cluster-size threshold of 0.57mL for MD. Our chosen cluster-size threshold is nearly twice this size, and thus sufficiently conservative to control for false positives.

RESULTS

Clinical and behavioral characteristics

Mean clinical and behavioral characteristics of PVD, IBS, and HCs are summarized in **Table 1**. The age of subjects ranged from 21 to 52 years (mean=29.71). Although within normal clinical ranges, PVD subjects had significantly higher anxiety and depression symptom scores compared to HCs (Anxiety: $F(1, 57)=18.44$, $p=.00005$,

Cohen's d=1.25; Depression: F(1, 57)=9.82, p=.002, d=.85), but were similar to IBS subjects.

Values for vulvar vestibular pain as assessed by cotton swab and for vaginal muscle tenderness as assessed by finger pressure exam for the PVD subjects are summarized in **Table 2A**. The average duration of PVD pain was about 7.4 years. For the PVD subjects the average level of pain intensity during the past 24 hours was 5.95 (SD=6.17), and average level of pain unpleasantness during the past 24 hours was 5.29 (SD=4.85). The average pain related to sex was 57.48 (SD=26.58). The values for abdominal pain and gastrointestinal symptoms for the IBS subjects are summarized in **Table 2B**. The average symptom duration for IBS subjects was about 11.16 years. Of the 29 PVD subjects, 6 reported IBS symptoms in the past and 4 reported current IBS symptoms within the last six months. None of the 29 IBS patients reported comorbid PVD symptoms.

Disease-specific alterations in fractional anisotropy (FA)

FA was significantly higher in PVD patients compared with HCs in two contiguous regions within the brain (**Figure 1; Table 3**). The first region (**Figure 1; Cluster 1;** 5.808mL) was localized to the right superior longitudinal fasciculus (SLF), which connects regions of the frontal, occipital, parietal, and temporal lobes. The difference in FA along the contiguous cluster neighboring the right SLF appeared highest within white matter projections adjacent to (S1) cortical regions, as well as posterior regions within the right basal ganglia, putamen and pallidum, as well as tissue adjacent to the ventroposterior nucleus of the thalamus and white matter regions projecting into the

temporal lobe. The second region (**Figure 1; Cluster 2;** 1.074mL) was concentrated in deep white matter regions on the left hemisphere localized within intra-abdominal S1 cortical regions. (Note this area was present on the right hemisphere but was part of the larger, contiguous cluster.)

To determine whether the change in FA was due to increased diffusion along the main axis (parallel to axons), decreased diffusion radially (perpendicular to axons) or both, we evaluated the mean axial and radial diffusivity (AD and RD, respectively) in the significantly different FA clusters between PVD and HC. AD and RD maps were calculated and registered to the atlas-space using the same process outlined in the Methods above. Independent t-tests of mean AD and RD in each cluster indicated thatPVD patients presented with significantly increased AD compared to HC, with a mean difference of 0.026 μm2/ms (p=.0004) for Cluster 1, and a mean difference of 0.047 μm2/ms (p=.0007) for Cluster 2 (**Figure 2; A and D**, respectively). PVD patients also presented with significantly decreased RD compared to HC, with a mean difference of 0.030 μm2/ms (p<.0001) for Cluster 1, and a mean difference of 0.026 μm2/ms (p=.002) for Cluster 2 (**Figure 2; B and E**, respectively). Scatter plots of AD vs RD in the significantly different FA clusters indicate that a combination of increased AD and decreased RD is responsible for the difference in FA for both Cluster 1 and Cluster 2 (**Figure 2; C and F**, respectively).

No significant differences in FA were observed between PVD and IBS patients.

Disease-specific alterations in mean diffusivity (MD)

PVD patients had clusters of significantly elevated MD with respect to both the HC and IBS groups (**Figure 3; Table 4**). The region of increased MD with respect to HCs (**Figure 3; Cluster A;** 1.037mL) was located mostly in the left putamen, extending to the inferior portion of the superior longitudinal fasciculus fibers just above the putamen, and appears to present with a uniform (albeit relatively small) difference in MD throughout the region. The cluster with increased MD with respect to IBS (**Figure 3; Cluster B;** 1.512mL) was primarily located within the left posterior limb of the internal capsule, which contains corticospinal and thalamo-cortical white matter fibers heading to and from primary sensory and motor cortex. This region also extended to portions of the left pallidum, and showed some overlap with the left thalamus and putamen, though the highest differences in MD in the cluster were present in the internal capsule.

Sensitivity Analyses

While there were no differences between the PVD and IBS group in terms of depression and anxiety scores, there were significant differences in these scores between the PVD and HC group. To ensure that the reported differences between PVD and HC were not due solely to differences in mood and affect, we created statistical parametric maps for the PVD vs. HC comparison with HAD anxiety and depression scores included as covariates. The resulting clusters were in the same locations as the PVD vs. HC clusters without the mood score covariates, except slightly decreased in size: for FA, cluster 1 decreased in size from 5.808mL to 5.580mL, and cluster 2 decreased from 1.074mL to 1.062mL, while for MD the cluster decreased in size from 1.037mL to

0.990mL. Thus, the group differences between PVD and HC were due mainly to group effects and not differences in anxiety and depression scores.

Correlations between DTI metrics and Clinical and Behavioral Measures

Correlations with FA. No symptom variable showed significant voxel-wise correlations with FA.

Correlations with MD. Significantly associated clusters were found for Vaginal Muscle Tenderness Total score, and Vulvar Pain registered at 6 o'clock (**Figure 4; Table 4**). The correlation of MD with the Vulvar pain recorded at 6 o'clock score produced three positively correlated regions (**Figure 4A; Cluster 1**, 6.866mL) in the right posterior corona radiata, posterior thalamic radiation, and superior longitudinal fasciculus; **Cluster 2** (2.082mL) covering similar regions to the first cluster but on the contralateral (left) side, the superior and posterior corona radiata and some of the superior longitudinal fasciculus; **Cluster 3** (1.013mL) was located mostly in the left superior corona radiata and superior longitudinal fasciculus. Additionally, the correlation of MD with the Vaginal Muscle Tenderness Total score produced one positively correlated region (**Figure 4B; Cluster 1:** 3.026mL) that spanned several brainstem structures, including the cerebral peduncle, which carries the cortico-spinal and spino-thalamic fibers through the brainstem, and the superior and middle cerebellar peduncles.

DISCUSSION

The aim of the study was to identify disease-related differences in deep subcortical gray and white matter microstructure in women with PVD, compared with HCs and another chronic pain group, IBS, using DTI MRI analyses. As hypothesized, in PVD alterations in microstructural organization of the brain were predominantly observed in fibers associated with sensorimotor integration and pain processing that relay information between the thalamus, basal ganglia, sensorimotor cortex, and insula. Women with PVD, compared to HCs, displayed extensive increases in the FA of somatosensory and basal ganglia regions. No differences in FA were observed between PVD and IBS subjects, suggesting similar alterations in these regions for both pain groups as compared to HCs. PVD subjects displayed greater MD in the basal ganglia compared to HCs and IBS. PVD subjects also showed higher MD in the internal capsule in a region adjacent to the pallidum, which contains cortico-spinal and thalamo-cortical fibers, when compared to IBS subjects. In PVD, pain measures including vulvar pain and vaginal muscle tenderness on examination were positively associated with MD in the sensorimotor projection fibers in the posterior corona radiata and association fibers in the superior longitudinal fasciculus in the left and right hemispheres, as well as white matter fibers in the brainstem.

The primary microstructural findings indicated that compared to HCs, PVD subjects have greater white matter fiber directional coherence (higher FA) in pathways associated with sensorimotor integration and processing of pain around the primary sensory cortex [44, 83]: the bilateral superior longitudinal fasciculi [84], posterior and superior corona radiata, the left hemisphere regions of the basal ganglia, thalamus, and internal capsule [7, 88]. PVD subjects on average displayed both increased AD and decreased RD in

these clusters compared to HCs, indicating that the increased FA may reflect an increase in the unidirectionality of white matter fibers in these areas. Compared to HCs, PVD subjects had higher MD in the right putamen and superior longitudinal fibers, while PVD subjects showed increased MD in an adjacent region, the right internal capsule (which contains thalamo-cortical and cortico-spinal fibers) along with medial portions of the pallidum, when compared to IBS subjects.

These results are consistent with previous studies reporting brain alterations in the sensorimotor and integration regions in vulvodynia. For example, Schweinhardt (2008) reported increased gray matter density in the basal ganglia which plays a key role in sensorimotor integration, pain processing and chronic pain[10]. In our previous resting state study, these same subjects exhibited increased connectivity of the supplementary motor area and left primary motor cortex with the sensorimotor network [35]. Functional studies have demonstrated that vulvodynia patients demonstrate greater pressure pain sensitivity and associated increased activations in sensorimotor regions such as bilateral secondary somatosensory cortex, and premotor cortex[37]. Similarly, another study found increased sensorimotor activation in response to painful vestibular stimulation [70].

PVD-associated microstructural alterations are also comparable to the morphometric and functional brain alterations observed in motor and somatosensory regions previously reported in women with painful bladder syndrome/interstitial cystitis [23, 48, 49] and in women with irritable bowel syndrome [35, 53, 85], both of which are often comorbid with PVD. Other studies have also shown that these sensorimotor regions can be activated during pelvic floor muscle contractions in female subjects [51, 52].

A recent study by Ellingson et al. (2013) reported less directional coherence (decrease in FA) and higher MD in regions comprising cortico-thalamic-basal ganglia circuits in IBS compared to HCs [22]. IBS compared to HCs had *lower FA* in thalamic regions, the basal ganglia and sensory/motor association/integration regions. In our study, MD of some of these same regions, namely the basal ganglia, was higher in PVD compared to HCs and IBS. Interestingly, in fibromyalgia subjects another disorder comorbid with PVD, FA has also been reported as decreased in the thalamus, thalamo-cortical, and insular fibers compared to HCs[58]. In the current study, PVD subjects compared to HCs demonstrated increased FA. When viewed together with the current findings, differences between chronic overlapping pain syndromes may be due to differences in sensorimotor-thalamic-brainstem thalamic connectivity.

Elevated FA has been associated with axonal density or branching, myelination, and/or fiber arrangements. Increased AD and decreased RD may suggest an increase in fiber directional coherence in PVD, reflecting a strengthening of specific afferent projections to somatosensory regions as a consequence of constant painful stimuli [23] or tonic muscle contractions. This increase in fiber coherence may underlie the increased pain facilitation along with inefficient pain inhibition observed in PVD [34]. It has also been hypothesized that increased FA may reflect a higher degree of plasticity related to pelvic floor muscle control [25]. Increased MD may reflect a decrease in overall axonal density or an increase in average axonal caliber [27, 28, 75]. This, coupled with increased FA, may signal more highly unidirectional, large caliber fibers or the expense of crossing fibers and otherwise normal complex white matter microstructure. In addition, because microstructural alterations were observed in

regions belonging to the ascending nociceptive pathways, pain sensitive afferents may also be abnormal in PVD, consistent with previous report of hyper-innervation of the vulvar vestibule [9, 82]. Ultimately, longitudinal studies will be required to determine whether pre-existing alterations in the spinal-thalamic-basal ganglia-cortical loops lead to biased sensory perception or if sensitization of afferent and efferent pathways is due to chronically increased sensory input from the vulvar area.

Symptoms are associated with White-Matter alterations

Increased vaginal muscle tenderness on exam was associated with increased MD in several structures in the brainstem, which may reflect lower neuronal density or lower directional cohesion in the organization of axonal fibers. The brainstem is known to have many nuclei relating to pain and sensation, and there is a dire need for more focused MRI structural and functional investigations of this region. Higher vulvar pain reports (at 6 o'clock) were correlated greater MD in pathways associated with sensorimotor integration and pain processing. These pathways were in proximity to the same clusters showing increases in FA in PVD compared to controls but were located slightly more posteriorly, in white matter fibers that are integrating sensory and motor information in the parietal cortex; specifically, this portion of the SLF is involve in the processing of proprioceptive and sensory information, as well motor function that depends on these proprioceptive and sensory feedback [73]. As whole, these findings may suggest that increased fiber integrity in these regions are associated with increasing pain sensitivity and may underlie vulvar pain sensitivity and tonic pelvic floor muscle contractions and tenderness often noted during clinical examination of women with PVD [64, 65].

Limitations

Since psychiatric diagnoses and presence of other comorbid chronic pain conditions were exclusion criteria, generalization of the results are limited as research indicates PVD without comorbidities represent a distinct subgroup associated with shorter duration of the disorder, less severe clinical presentation, and higher quality of life[57, 72]. The failure to assess pelvic floor muscle contractions during the study also limits interpretation of the results. While the 64- and 60-direction DTI sequences are similar and have been used jointly successfully in past studies [87], this slight heterogeneity in DTI sequences is nonetheless still a limitation of this study. We also failed to find a correlation between microstructural organization and pain with intercourse, however many women no longer attempted genital contact. A tampon test[29] may have added a relevant behavioral dimension and should be included in future studies. Finally, the sample size only provided adequate power to detect large effect size differences.

Summary and Clinical implications

PVD subjects compared to HCs and IBS subjects showed microstructural alterations consistent with of increased strength of axonal or dendritic projections and increased myelination in sensorimotor, cortico-thalamic, and basal ganglia circuits involved in sensorimotor integration and pain processing. These neuroplastic alterations may be a defining pathophysiological feature of PVD. It is not clear if these alterations in brain microstructure are a primary (e.g. premorbid) or changes or reflect substantial long term

microstructural reorganization in response to secondary to chronic nociceptive input to the brain from the vulvar vestibule or pelvic floor related to abnormal pelvic motor contractions. It is possible that these brain alterations increase the vulnerability to develop PVD after an inflammatory insult or to maintain or amplify the symptoms once PVD develops. The association between increase vulvar pain and muscle tenderness with increased MD in sensorimotor and pain processing region provides preliminary support for this hypothesis. The symptoms of PVD can undergo spontaneous remission[71], and perhaps those patients with microstructural changes would be less likely to improve spontaneously over time. These questions will have to be clarified through longitudinal studies. It is also unknown if treatments that target the periphery such as physical therapy [6] and vestibulectomy surgery[80]or those that target the CNS including cognitive behavioral [8] or pharmacological management will normalize the alterations observed in PVD subjects. These findings also may have implications for supporting the development and implementation of new therapies such as transcranial stimulation [64], and novel pharmacological therapies that target pain-related neuroplasticity. The current findings highlight possible shared mechanisms between different pain disorders, but more importantly highlight the more wide-spread alterations observed specifically in PVD compared to HCs. Future research is required to determine the extent of the overlap between the various chronic pain disorders.

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FIGURES

Figure 1: Fractional Anisotropy (FA) Differences Between PVD and Healthy Controls
Significant clusters, 1 and 2, showing FA differences between PVD and HC groups. Left, three-dimensional rendering of largest significantly different clusters of FA differences, showing elevated FA values in the PVD group compared to the HC group, with column scatter plots of the mean FA values for the PVD and HCs for each cluster. Right, two-dimensional images of significantly different clusters, sagittal and coronal multi-section images displayed next to probabilistic tractography rendering of the superior longitudinal fasciculus.

Figure 2: Mean Axial Diffusivity (AD) and Radial Diffusivity (RD) in Significantly Different Fractional Anisotropy (FA) Regions for PVD and Healthy Controls
Mean AD and RD for the two significantly different clusters for PVD and HCs. Cluster 1 AD (A), RD (B), and scatter plot of RD vs AD (C), are shown on the left, and the respective quantities (D, E, F) for Cluster 2 are shown on the right.

Figure 3: Mean Diffusivity (MD) Differences Between PVD and Healthy Controls (or IBS)
Significant clusters, A and B, showing MD differences between PVD and HCs and IBS groups. Left, three-dimensional, and right, two-dimensional, renderings of significantly different clusters of MD differences with column scatter plots of the mean MD values for the PVD and HCs for each cluster, showing elevated MD values in the PVD group compared to the HC group in area near the left basal ganglia and internal capsule.

Figure 4: Association Between Mean Diffusivity (MD) and Symptom Scores in PVD
Significantly correlated clusters showing association between MD and symptom variable scores in the PVD group. Color bars represent standardized beta values. Dotted lines (and shaded areas) represent +/- 1 standard deviation
A. Two-dimensional multi-level images of significantly correlated clusters of MD, showing increasing MD with increasing Vulvar Pain registered at 6 o'clock score, with scatter plot and linear regression of MD vs Vulvar Pain in each of the clusters.
B. Two-dimensional multi-level images of significantly correlated clusters of MD, showing increasing MD with increasing Vaginal Muscle Tenderness Total score, with scatter plot and linear regression of MD vs Vaginal Muscle Tenderness for the cluster.

Table 1: Study Demographics and Clinical/Behavioral Measures

Premenopausal Females	PVD (N=29)		IBS (N=29)		HC (N=29)		PVD vs. IBS			PVD vs. HC		
	Mean	SD	Mean	SD	Mean	SD	F(1, 57)	p-value	Cohen's d	F(1, 57)	p-value	Cohen's d
Age (yrs)	30.31	6.79	30.17	7.10	28.66	7.94	.75	.39	.02	.01	.94	.22
HADS Anxiety	7.03	3.95	7.10	4.48	2.72	2.83	.01	.94	.02	18.44	.00005	1.25
HADS Depression	2.84	3.05	3.07	2.52	.86	1.30	.13	.72	.08	9.82	.002	.85

Group differences were tested using contrast analysis within the framework of the general linear model. The F statistic associated with each test is reported along with uncorrected p values and p values corrected for false discovery rate, i.e., q-values.

Abbreviations: HADS, Hospital Anxiety and Depression Scale; HC, Healthy Control; IBS, Irritable Bowel Syndrome; PVD, Provoked Vestibulodynia; N, number of subjects; SD, standard deviation; p-value <.05 uncorrected, y=year, Cohen's d effect size

Table 2: Clinical/Behavioral Characteristics for the A. Subjects with Provoked Vestibulodynia and B. for the Subjects with Irritable Bowel

A.

PVD (N=29)	Range	Mean	SD
Gracely Differential Descriptor Pain Scale			
Pain Duration (months)	15-360	88.66	83.08
Level of Pain Intensity (past 24hrs)	0-18	5.95	6.17
Level of Pain Unpleasantness (past 24hrs)	0-18	5.29	4.85
Pain Related to Sex			
Level of Pain related to Sex	10-100	57.48	26.58
Level of Pain not related to Sex	0-100	23.62	31.75
Vulvar Pain	**Mean**	**SD**	
@ 10 o'clock	3.86	2.76	
@ 7 o'clock	5.00	2.51	
@ 6 o'clock	5.18	2.23	
@ 5 o'clock	5.02	2.48	
@ 2 o'clock	3.61	2.67	
Total Vulvar Pain Score	22.66	11.82	
Vaginal Muscle Tenderness	**Mean**	**SD**	
Bulba @ 5 o'clock	2.04	2.10	
Bulba @ 7 o'clock	1.96	2.13	
Pubococcygeous @ 6 o'clock (levator at midline)	1.29	2.18	
Levator @ 5 o'clock	1.82	1.93	
Levator @ 7 o'clock	2.00	2.16	
Total Vaginal Muscle Tenderness Score	9.11	8.87	

Pain duration and level of pain intensity were assessed on a 21-point numeric rating scale (0=neutral, 20=extremely intense) using the Gracely Differential Descriptor Pain Scale. Level of pain unpleasentness was also assesed using a 21-point numerical rating scale (scale 0-20, 0=neutral, 20=very intolerable) in the past 24 hours using the Gracely Differential Descriptor Pain Scale. Level of pain related to sex and not related to sex were recorded on a scale of 0-100 (0 = no pain, 100 = most intense pain imaginable).
For vulvar pain, pain severity was measured on an 11-point Numerical Rating Scale where 0 = none and 10 = most severe pain imaginable and total vulvar pain was calculated by totaling the scores from the 5 sites (0-50). Vaginal muscle tenderness was measured on an 11-point Numerical Rating Scale, with 0 representing no pain and 10, representing the most severe pain imaginable, almost unconscious and total vaginal muscle tenderness score was calculated by totaling the scores from the 5 sites (0-50).
Abbreviations: PVD, provoked vestibulodynia; N, number of subjects; SD= standard deviation

B.

IBS (N=29)		**Mean**	**SD**
Bowel Symptom Questionnaire			
	Range	**Mean**	**SD**
Overall Symptoms in the past week	0-17	8.86	4.63
Abdominal Pain in the past week	0-19	9.38	4.59
Symptom severity	2-5	3.34	.67
Symptom duration (years)	2-31	11.16	8.45

Questionnaire: Overall symptoms in the past week were measured on a 0-20 scale (0=neutral, 20=extremely intense), Abdominal Pain in the past week was measured on a 0-20 scale (0=neutral, 20=very intolerable).
Abbreviations: IBS, irritable bowel syndrome; N, number of subjects; SD, standard deviation

Table 3: Significant clusters of FA-related differences between groups and correlations with clinical variables in PVD group.

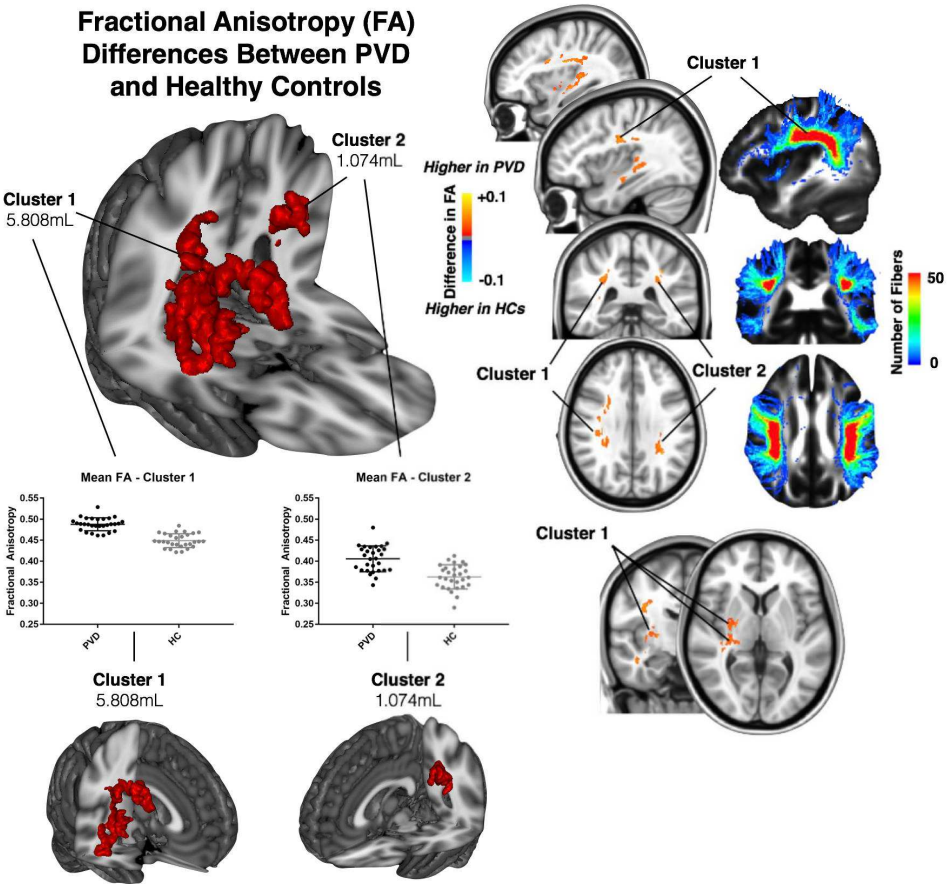
FA Significant Clusters					
Test	**Cluster Number**	**Direction of Change**	**Cluster Size (mL)**	**COM Position (x, y, z)**	**Structures**
PVD vs. HC	1	Increased	5.808	(-32, 17, 11.4)	Right sagittal stratum, cerebral peduncle, retrolenticular and posterior internal capsule, fornix/stria terminalis, external capsule, putamen, pallidum, thalamus, superior and anterior and posterior corona radiata, superior longitudinal fasciculus.
	2	Increased	1.074	(28.7, 41.7, 30.6)	Left posterior corona radiata, superior longitudinal fasciculus.
PVD vs. IBS	None				

Statistical tests (both comparisons between groups and correlations with symptom variables) are listed. Clusters reported from largest to smallest. In 'Direction of Change', 'Increased' and 'Decreased' denote higher or lower values in PVD vs other group, respectively, while 'Positive' and 'Negative' denote increasing or decreasing value with increasing symptom score rating within PVD group only, respectively. Center of Mass (COM) coordinates given for MNI standard space. 'Structures' denote overlap of clusters with white matter ROIs from the JHU white matter atlas or subcortical gray matter ROIs from the Harvard-Oxford subcortical atlas, both available through FMRIB Software Library (FSL, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki).
Abbreviations: HAD, Hospital Anxiety and Depression Scale; HC, Healthy Control; PVD, Provoked Vestibulodynia

Table 4: Significant clusters of MD-related differences between groups and correlations with clinical variables in PVD group.

Test	Cluster Number	Direction of Change	Cluster Size (mL)	COM Position (x, y, z)	Structures
PVD vs. HC	1	Increased	1.037	(27.5, 1, 15.7)	Left putamen, external capsule, superior corona radiata, superior fronto-occipital fasciculus, superior longitudinal fasciculus.
PVD vs. IBS	1	Increased	1.512	(17, 5.9, 0.9)	Left anterior and posterior limb internal capsule, pallidum
Vulvar Pain @ 6 o'clock	1	Positive	6.866	(-32, 53, 13.4)	Right sagittal stratum, posterior thalamic radiation, superior longitudinal fasciculus, splenium corpus callosum, retrolenticular limb internal capsule, tapetum, posterior and superior corona radiata
	2	Positive	2.082	(21.9, 46.9, 47.6)	Posterior and superior corona radiata, superior longitudinal fasciculus Left
	3	Positive	1.013	(29.7, -7.1, 27.4)	Left external capsule, superior corona radiata, superior longitudinal fasciculus
Vaginal Muscle Tenderness Total	1	Positive	3.026	(7.4, 25.2, -8.5)	Left middle and superior cerebellar peduncle, cerebral peduncle Right, cerebral peduncle, thalamus, fornix/stria terminalis, splenium corpus callosum

Statistical tests (both comparisons between groups and correlations with symptom variables) are listed. Clusters reported from largest to smallest. In 'Direction of Change', 'Increased' and 'Decreased' denote higher or lower values in PVD vs other group, respectively, while 'Positive' and 'Negative' denote increasing or decreasing value with increasing symptom score rating within PVD group only, respectively. Center of Mass (COM) coordinates given for MNI standard space. 'Structures' denote overlap of clusters with white matter ROIs from the JHU white matter atlas or subcortical gray matter ROIs from the Harvard-Oxford subcortical atlas, both available through FMRIB Software Library (FSL, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki).
Abbreviations: HC, Healthy Control; IBS, Irritable Bowel Syndrome; PVD, Provoked Vestibulodynia



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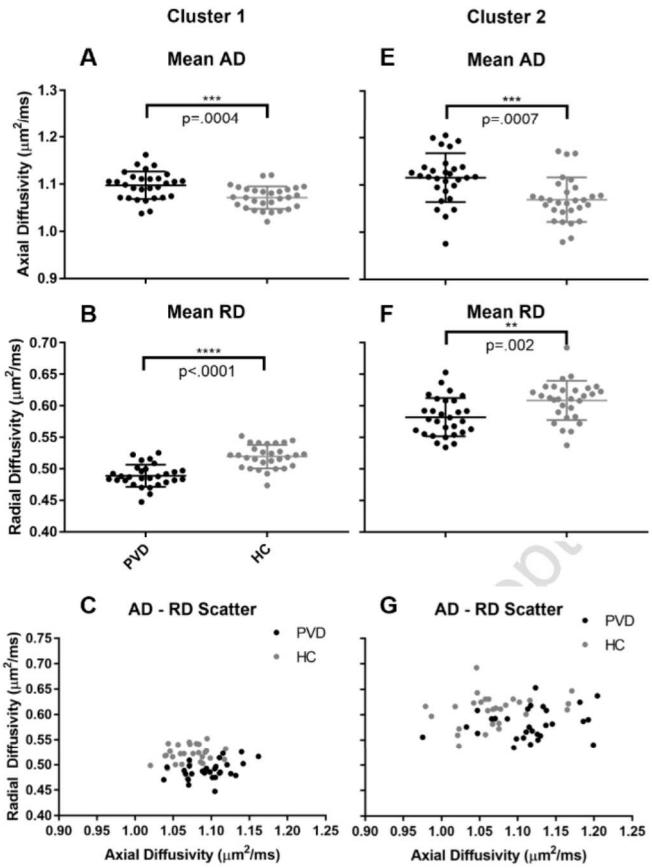

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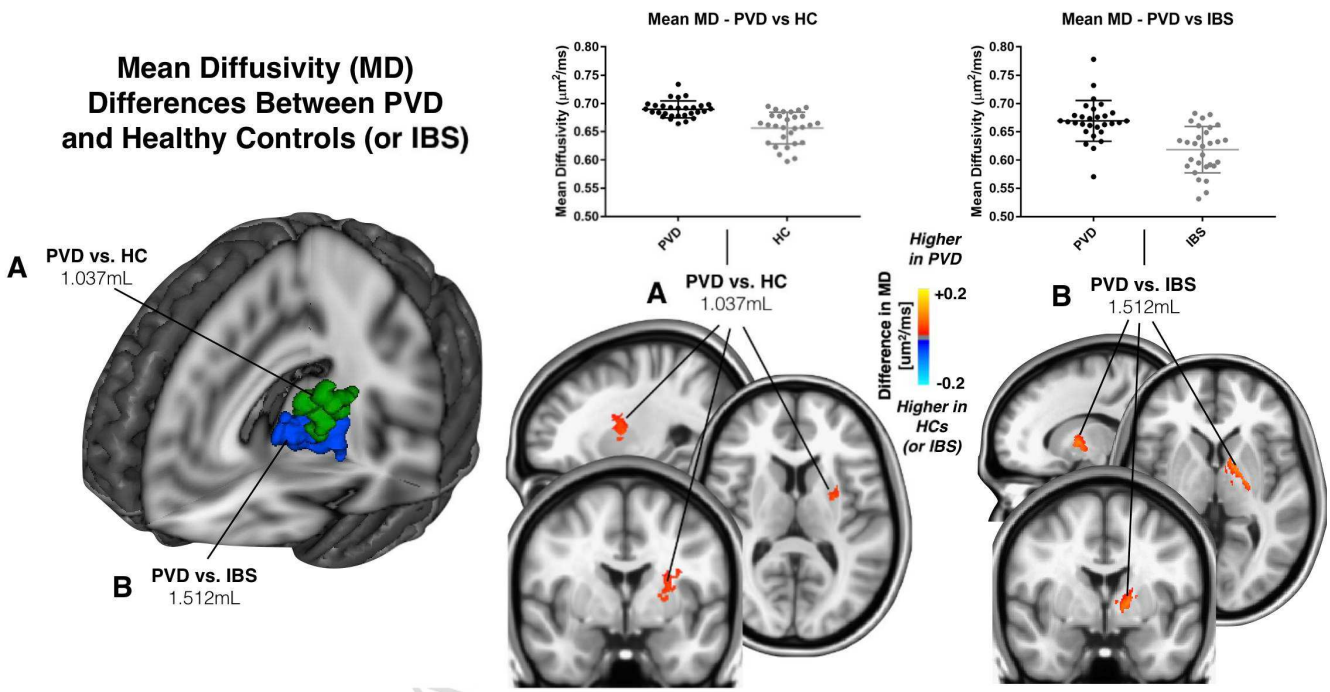

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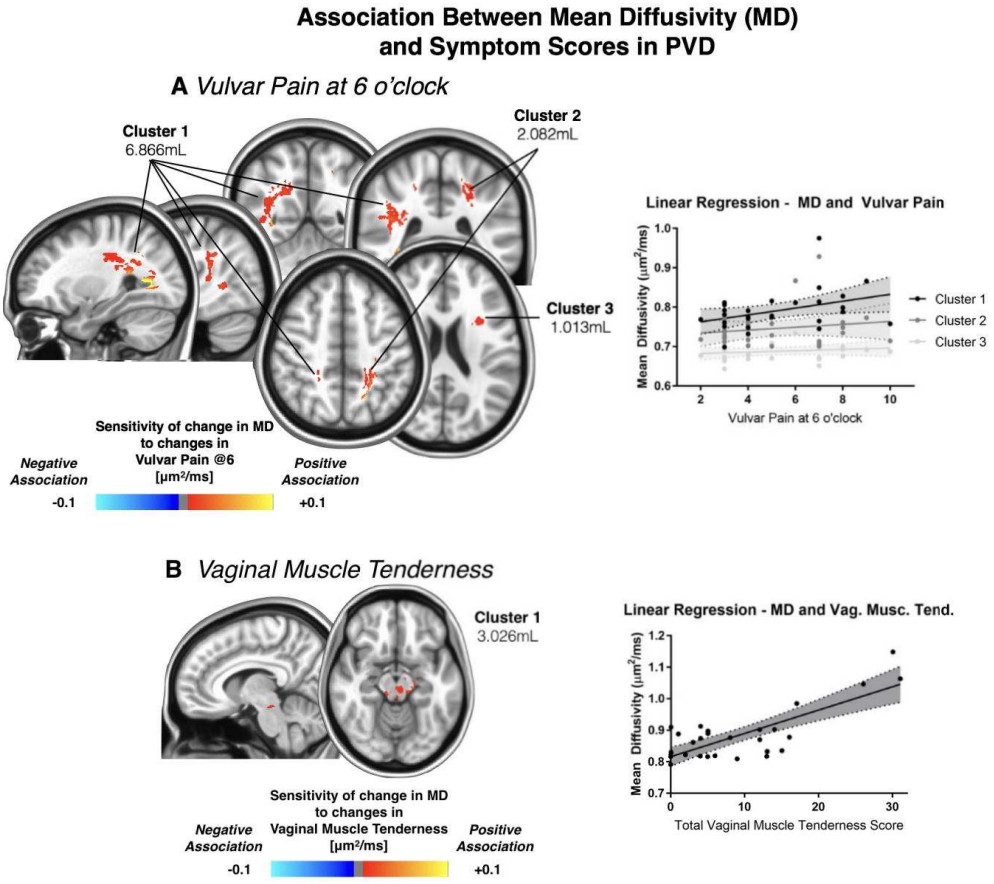

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