The Journal of Sexual Medicine, 2025, 1–8
https://doi.org/10.1093/jsxmed/qdae201
Original Research

Evaluation of polygenic risk scores for hormones and receptors levels in patients with vestibulodynia: a case–control study

Filippo Murina, MD[1,*], Cecilia Fochesato, MD[1], Chiara Leo, PhD[2], Giuseppe E. Condorelli, PhD[3], Anna Rocchi, PhD[2], Sara Amitrano, MMedSci[2], Valerio Napolioni, PhD[4], Valeria Savasi, MD[5]

[1]Lower Genital Tract Disease Unit, V. Buzzi Hospital—University of the Study of Milan, Via Castelvetro 24-20124, Milan, Italy
[2]Laboratory of Genomics—Polo d'Innovazione di Genomica, Genetica e Biologia, Strada del Petriccio e Belriguardo 35-53100, Siena, Italy
[3]Genetic and Ecology Research Centre—Polo d'Innovazione di Genomica, Genetica e Biologia, Via Fiorentina 1-05100, Terni, Italy
[4]Genomic and Molecular Epidemiology (GAME) Lab, School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Via Gentile III Da Varano 62032 Camerino (MC), Italy
[5]Clinical Obstetric and Gynecological V Buzzi, ASST-FBF-Sacco, Via Castelvetro 24-20124-University of the Study of Milan, Milan, Italy
*Corresponding author: Lower Genital Tract Disease Unit, V. Buzzi Hospital—University of the Study of Milan, Via Castelvetro 24-20124, Milan, Italy. Email: filippo.murina@unimi.it

Abstract

Background: Vulvodynia is a multifactorial disease affecting 7%–16% of reproductive-aged women in general population; however, little is still known about the genetics underlying this complex disease.
Aim: To compare polygenic risk scores for hormones and receptors levels in a case–control study to investigate their role in vulvodynia and their correlation with clinical phenotypes.
Methods: Our case–control study included patients with vestibulodynia (VBD) and healthy women. All participants underwent a vestibular cotton swab test and the assessment of their: pelvic floor, vestibular trophism, ultrasound vestibular mucosa thickness, and current perception threshold levels (Neurometer CPT device). Shallow whole genome sequencing and polygenic risk score calculations were performed. Linear regression models were applied to predict whether genomic predisposition varied significantly between cases and controls, and to investigate the relationship of polygenic risk scores with clinical endophenotypes.
Outcomes: The genomic predisposition to hormones and receptors levels, together with clinical endophenotypes, can support VBD diagnosis and personalized treatment of related pain condition.
Results: Thirty women with VBD and 30 controls were recruited. Significant differences between cases and controls were observed for body mass index, vestibular mucosa thickness, vestibular trophic health, pelvic floor hypertone and pain sensitivity ($P < .05$). Cases showed a genomic predisposition to higher levels of membrane-associated progesterone receptor component 1 compared to controls ($P < .05$). When considering the clinical endophenotypes, cases showed significant correlations between their polygenic risk scores with several clinical measures: predicted genomic levels of testosterone and estrogen receptor and the vestibular mucosa thickness values (estimates: 9.74E-09 and 9.16E-08, respectively; $P < .05$); predicted genomic levels of prolactin and Neurometer data at 250 Hz (-2.15E-07; $P < .05$); predicted genomic levels of prolactin, membrane-associated progesterone receptor component 2 and mineralocorticoid receptor and Neurometer data at 5 Hz (-3.75E-07, -3.43E-07 and -3.06E-07, respectively; $P < .05$).
Clinical Implications: Introduction of polygenic risk scores evaluation in clinical practice can assist early diagnosis and personalized therapeutic treatment of VBD.
Strengths and Limitations: Polygenic risk scores and clinical data allowed the identification of disease endophenotypes and highlighted the possibility of a personalized therapeutic approach. As limitations, these data should be confirmed on a larger cohort and polygenic risk score calculation should be adapted to ancestries other than European.
Conclusion: Cases showed significant differences compared to controls on both clinical and genetic data and specific endophenotypes necessary to classify disease development and treatment were identified.

Keywords: vestibulodynia; vulvar mucosa; dyspareunia; vulvodynia; polygenic risk score; personalized medicine.

Introduction

In 2015, a committee constituted by the International Society for the Study of Vulvovaginal Disease (ISSVD), the Board of Directors of the international Society for the Study of Women's Sexual Health and the International Pelvic Pain Society established a new nomenclature to define vulvodynia as vulvar pain lasting at least three months, with no clearly identifiable cause, but with potentially associated factors including genetic, inflammatory or neuro-proliferative variables.[1] The prevalence of vulvodynia in the general population ranges from 7% to 16% of women of reproductive age, and its' incidence is believed to be on the rise.[2-4] Most studies focused on localized provoked vulvodynia at the vestibule, known as vestibulodynia (VBD), which is the most common subtype (about 80%).[4]

The discomfort associated with VBD is commonly characterized as a burning sensation and is often intensified by restrictive clothing or extended periods of sitting or sexual activity, resulting in a relevant negative impact regarding relationships and quality of life. The pattern of VBD responses indicates sensory disorders, such as hyperalgesia or allodynia. This aligns with biopsy findings indicating heightened

Received: July 27, 2024. **Revised:** December 13, 2024. **Accepted:** December 25, 2024
© The Author(s) 2025. Published by Oxford University Press on behalf of The International Society for Sexual Medicine. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/jsm/advance-article/doi/10.1093/jsxmed/qdae201/7951995 by ISSM Member Access user on 16 January 2025

innervation of the vulvar vestibule, accompanied by elevated subepithelial heparinase activity and cytokines associated with neuroinflammatory processes.[5]

Musculoskeletal factors contributing to pelvic floor hypertonic dysfunction/overactivity have been recognized in vulvodynia. Women with VBD exhibited diminished strength, coordination, endurance, and contraction speed of the pelvic-floor muscles relative to healthy controls. Alongside pathogenesis, pelvic-floor hypertonicity can exacerbate pain perception, since pain may induce a reflex contraction and heightened tension in the pelvic-floor muscles of VBD patients.[6] Numerous factors have been identified in various research, revealing pathways that contribute to a genetic susceptibility for vulvodynia.[7] Indeed, genetic polymorphisms affecting the immune system's response to infection or trauma by increasing the susceptibility to pain after exposure to a trigger (for example infection) and/or by decreasing the ability to terminate the inflammatory process, were found to associate with the condition.[8] The interleukin-1beta (IL-1β) gene, the interleukin-1 receptor antagonist gene, and the mannose binding lectin (MBL) gene, at least, have been studied in association with VBD.[9] While vestibular pain is likely caused by multiple factors, there is a possibility that alteration in hormonal pathways may play a role in vestibular dysfunction.

In hypoestrogenic postmenopausal patients with dyspareunia and vulvar pain related to the genitourinary syndrome of menopause (GSM),[10] the most exquisitely tender area is the vulvar vestibule, and the non-keratinized squamous epithelium here exhibits atrophic features like pallor, petechiae, erosions and thinning. Using topical estrogen directly on the vestibule resulted in a statistically significant reduction in symptoms for postmenopausal women with vulvar or vestibular complaints.[11] It is increasingly evident that VBD is not likely a singular disease, but rather a spectrum of conditions, all culminating in vestibular hypersensitivity and pelvic floor hypertonic dysfunction.

The aim of this study was to apply publicly available genome-wide association study (GWAS) findings concerning quantitative trait loci for sexual hormones and their receptors to elucidate their role in VBD. This will be achieved through a comparative analysis of their respective polygenic risk scores (PRS) in a small case–control group study, where all subjects underwent shallow whole-genome sequencing. We also aimed to investigate the associations between these PRS and pain sensitivity, as well as vestibular thickness, among women with VBD compared to healthy controls.

Materials and methods

Subjects

The present case–control study compared patients with VBD to control women without vulvar pain. All cases aged ≥18 years and premenopausal (cessation of menstruation for 12 months) with VBD who presented to our unit of lower genital tract disease for a fractional CO_2 laser treatment to the vestibule. Cases with VBD met the criteria of the International Society for the Study of Vulvovaginal Disease,[1] including vulvar pain, burning, irritation localized to the vestibule during vaginal intercourse and activities exerting pressure on the vestibule (eg, inserting a tampon insertion, wearing tight jeans or pants) that were present for at least 3 months, with no other demonstrable cause for their symptoms. The control group included healthy asymptomatic women of reproductive age without any vulvovaginal conditions that attended to our hospital (Buzzi Hospital—University of Milan, Milan, Italy) for cervical cancer screening programs.

Subjects meeting any of the following criteria were excluded: active vulvo-vaginal infections at the time of their gynecological examination, genital bleeding of unknown origin, concomitant vulvar dermatosis or other vulvar disorders and symptoms or signs in the past related to VBD.

A written informed consent was obtained by all subjects. The study protocol was reviewed and approved by the Institutional Review Board (approval no. 2023-02-034) and it was registered at http://www.isrctn.com (ISRCTN12345678).

Clinical assessment

All enrolled participants completed a detailed questionnaire to assess demographics including age, ethnic origin, body mass index (BMI), age for onset of disease, use of contraception, and recurrent vulvovaginal infections history. A vestibular cotton swab test was also performed to confirm the presence of VBD. It consisted of a small cotton tipped applicator which was lightly rolled over the surfaces of the vestibule by asking the subject to report pain feeling instead of physiological pressure sensation (ie, allodynia). Subsequently, the investigator assessed the vestibular trophism using the vestibular trophic health (VeTH), a validated score which evaluated five criteria: petechiae, pallor, thinning, dryness and redness, rating each of the 5 parameters on a 4-point scale, with 0 being none,1 mild, 2 moderate, and 3 severe.[12] Vestibular trophism was also objectively assessed by ultrasound vestibular mucosa thickness measurement (B-scan ultrasonography) with a 20-MHz validated system (DermaScan C, Cortex Technology, Denmark).[13] The thickness of the vestibular mucosa (expressed in micrometers) was determined by the B-mode, established to include both the epidermis and dermis, using automated edge detection software from the DermaScan C device. The evaluation of hypertonic pelvic floor was established by a physical exam documenting hypertonus of the levator ani complex using an empirical score that allows reproduction of pelvic floor hypertonus with acceptable reliability (grade 0 = no hypertonicity; grade 1 = mild hypertonicity; grade 2 = moderate hypertonicity and grade 3 = severe hypertonicity).[6]

Finally, participants underwent the current perception threshold testing (CPT) evaluation, in a standard mode using the Neurometer CPT device (Neurotron Inc., Baltimore, MD, USA), that applies a constant current stimulator which depolarizes afferent neurons of different sizes based on the frequency of the stimulating waves. This allows for neuroselectivity of the three major types of afferent nerve fibers—2000 Hz (specific for large, myelinated A-β fibers), 250 Hz (specifically for A-δ), and 5 Hz (specific for C fibers).[14] A G-trode Vaginal/Rectal Electrode from Neurotron Inc. (Baltimore, MD) was used to determine vulvar vestibule CPT values (1 = 0.01 mA). The examiner instructed the participants to inform them when they first perceived the stimulus while gradually increasing its intensity. The examiner gradually turned off the stimulus once the subject perceived it, until it was no longer perceptible. We averaged these two measurements to obtain a CPT value.

The lower the value of the CPT measure, the more hypersensitive the nerve ending.

Downloaded from https://academic.oup.com/jsm/advance-article/doi/10.1093/jsxmed/qdae201/7951995 by ISSM Member Access user on 16 January 2025

Deoxyribonucleic acid extraction, library preparation and sequencing

Total genomic Deoxyribonucleic acid (DNA) was extracted from 3 ml of whole blood sample using the automated DNA/ribonucleic acid extractor MagCore Super (RBC-Bioscience, New Taipei City, Taiwan), using the MagCore Genomic DNA Tissue Kit (RBC-Bioscience, New Taipei City, Taiwan). The DNA libraries were prepared according to the Illumina DNA PCR-Free Library Prep kit, and after successful quality control, sequenced onto Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) platform (with 2×150 bp paired-end run). The sequence data were submitted to the EMBL database under accession number: PRJEB75751.

Pre-processing of shallow whole genome sequencing data

The quality of sequenced raw data was checked using the program FastQC v0.11.9[15] and the reads were pre-processed using the tool Trimmomatic 0.39.[16] Each sequenced sample was then aligned to the human reference genome (v GRCh37/hg19) using the tool bwa-mem (Burrows-wheeler Aligner) v0.7.17-r1188.[17]

Genotype imputation, sample relatedness, and individual ancestry

The genotypes of each sample were phased and imputed by applying the tool GLIMPSE2.[18,19] The program Plink 1.9[20] was used to filter the variants' list through Linkage Disequilibrium pruning (window-size = 1500, a step size = 150 and r2 threshold = 0.1) and based on Minor Allele Frequency threshold of $\leq$5%. Sample relatedness was assessed using the program Plink 1.9,[20] defining the identical by descent (IBD) threshold as 0.0625 (eg, equivalent to 1st cousin degree). Finally, individual ancestry was investigated using the 1000 Genome Project populations as a reference (21) and applying the tool Plink 1.9[20] to perform the principal component analysis (PCA).

Statistical analysis

Based on the normality test (Shapiro–Wilk test), parametric (T) and non-parametric (Mann162 Whitney U) statistical tests were used to compare the cases and the controls for anamnestic (BMI) and clinical data (vestibular mucosa thickness, Neurometer at 2000 Hz, 250 and 5 Hz, basal VeTH and pelvic floor hypertone). Statistical analyses were carried out using R package stats v4.3.0.[22]

To calculate the PRS, reference summary statistics on hormones and their respective receptors levels were downloaded from the IEU open GWAS project (IEU Open GWAS project, https://gwas.mrcieu.ac.uk/, accessed in November 2023).[23-26] The following keywords were applied to the search: androgen, progesterone, anti-Mullerian hormone, estrone, estriol, estradiol, estrogen, follicle stimulating hormone, luteinizing hormone, prolactin, human chorionic gonadotropin, oxytocin, corticosteroids, corticotropin, thyrotropin releasing hormone, adrenocorticotropic hormone, and corticoid. The available summary statistics were downloaded, and further filtered based on the sample size (1000 participants as cutoff) and the number of single nucleotide polymorphisms (SNPs) included (over 1 000 000 as cutoff).

To finally select the summary statistics, the PRS of the European (EUR) individuals from the 1000 Genomes Project[21] was calculated using the tool Plink 1.9.[20] The normality of PRS distribution was evaluated by means of the Shapiro–Wilk test using the R package stats v4.3.0.[22] Only summary statistics with normal distribution ($P > .05$) were applied to downstream analyses.

The genetic predisposition to different hormones and respective receptors' levels was calculated for each subject within the case and control groups. In order to achieve this, Plink 1.9 program[20] was used to merge the samples from the present study with the EUR population from the 1000 Genomes Project.[21] Linear regression models were applied to predict: (1) whether PRS significantly varied between cases and controls; and (2) to investigate linear relationships of PRS with clinical endophenotypes within cases and controls, separately. The clinical endophenotypes considered were the vestibular mucosa thickness and Neurometer data (2000 Hz, 250 and 5 Hz).

Individual BMI, contraceptive use (C), recurrent vaginitis (RV), as well as the first and second PCA (PCA1 and PCA2) were added as covariates to improve the predictive ability of the model tested. The duration of the disease (DD) was ulteriorly inserted to such covariates exclusively for the model applied within the cases. All the statistical analyses were carried out using "stats" and "olsrr" R packages.[22] Given the preliminary and exploratory nature of this study, a nominal P-value of 0.05 was chosen as the threshold for statistical significance, without applying any adjustment for multiple testing correction. This choice is also supported by the absence of information regarding the independence between the tested PRS.

Results

Sequencing data pre-processing

The shallow whole genome sequencing yielded a total of 3.7 billion reads, and an average per sample of 61 million sequences. After the initial quality control and filtering, the average number of reads per sample was 54 million with a mean coverage of 3.0X. Following the genotype imputation, a total of 43 534 016 SNPs were imputed for each sample. Based on samples' relatedness analysis, two controls and one case showed a resulting proportion of IBD of 0.49 (ie, equivalent to first-degree relatives). Therefore, the two controls were excluded from downstream analyses.

The remaining 58 samples were merged with the 1000 Genomes Project populations[21] to investigate their individual ancestry, so that no bias is introduced when calculating the polygenic risk score. The application of GWAS summary statistics obtained from a European cohort may lead to inaccurate PRS when used as reference on individuals with a different ancestry. In this respect, one control and one case were further excluded from the downstream analysis due to their clustering with South Asian and African populations, respectively (Supplementary Fig. 1).

Thus, the final data set included 56 samples (29 cases and 27 controls), and a PCA was performed to summarize information on the total genomic variation of our data set and to extract the first and second principal components for further analyses (Supplementary Fig. 2).

Anamnestic and clinical data

Statistically significant differences ($P < .05$) between cases and controls emerged from the investigation of BMI and the following clinical data: ultrasound vestibular mucosa thickness,

Downloaded from https://academic.oup.com/jsm/advance-article/doi/10.1093/jsxmed/qdae201/7951995 by ISSM Member Access user on 16 January 2025

Table 1. Overview of the anamnestic and clinical data of the two groups analyzed in the present study.

Anamnestic and clinical data			Cases ($n = 29$)	Controls ($n = 27$)	
Age			28.3 ± 5.5	30 ± 5.0	
BMI			19.2 ± 1.9	20.7 ± 1.5	$P < .01$
Recurring vaginitis (RV)			11	0	
Contraceptive use (C)			21	20	
Disease duration (DD)	3-12 months		5	NA	
	24-48 months	15			
	60-120 months	9			
Neurometer (N)	2000 HZz		361.0 ± 124.4	475.2 ± 137.4	$P < .01$
	250 Hz	148 ± 85.8	268.5 ± 85.8	$P < .001$	
	5 Hz	53.7 ± 36.8	115.7 ± 51.2	$P < .001$	
Vestibular Mucosa Thickness (Eco)			994.6 ± 171.9	1180.4 ± 169.8	$P < .05$
Basal VeTH			6.1 ± 1.2	3.0 ± 0.85	$P < .001$
Pelvic floor hypertone	Absent		5	24	$P < .001$
	Mild	19	3		
	Moderate	5	NA		
	Severe	NA	NA		

NA: not available data.

Table 2. List of GWAS summary statistics applied to the PRS calculation on our data set constituted by 27 controls and 29 cases.

Phenotypic trait	GWAS summary statistics ID	# SNPs	Cohort	Distribution of polygenic risk scores
Corticotropin	H001	10.534.735	3.301	$P > .05$
Thyrotropin-releasing hormone	H002	10.534.735	3.301	$P > .05$
Mineralocorticoid receptor	HR001	10.534.735	3.301	$P > .05$
Glucocorticoid receptor	HR002	10.534.735	3.301	$P > .05$
Corticosteroid-binding globulin transport of glucocorticoids and progesterone	H003	10.534.735	3.301	$P > .05$
Oxytocin-neurophysin 1	H004	10.534.735	3.301	$P > .05$
Total testosterone	H005	10.534.735	3.301	$P > .05$
Estrogen receptor	HR003	10.534.735	3.301	$P > .05$
Membrane-associated progesterone receptor component 1	HR004	10.534.735	3.301	$P > .05$
Membrane-associated progesterone receptor component 2	HR005	10.534.735	3.301	$P > .05$
Follicle stimulating hormone	H006	10.534.735	3.301	$P > .05$
Prolactin	H007	10.534.735	3.301	$P > .05$
Prolactine receptor	HR006	10.534.735	3.301	$P > .05$
Luteinizing hormone	H008	10.534.735	3.301	$P > .05$
Oestradiol	H009	12.321.875	17.134	$P > .05$

The number of SNPs in the summary statistics, the sample size of the GWAS study, and the Shapiro–Wilk's test results are shown. The reported summary statistics refer to European ancestry population.

Neurometer at 2000, 250 and 5 Hz, basal VeTH and pelvic floor hypertone (Table 1).

GWAS summary statistics selection

At the time when we accessed the IEU Open GWAS project database (November 2023), no GWAS summary statistics were available for the following keywords: "androgen", "progesterone", "anti-mullerian hormone", "estrone", and "estriol". All the summary statistics found using the remaining keywords were successfully downloaded and filtered based on GWAS cohort size and total number of SNPs. The remaining summary statistics were applied to calculate the PRS of the 1000 Genomes Project EUR population[21] and to assess whether the PRSs showed a normal distribution.

The PRS was calculated for each case and control applying the GWAS summary statistics that passed the filtering process (Table 2). The genomic predisposition to different hormone and respective receptor levels of cases and controls was then investigated to predict whether PRSs significantly varied between the two groups.

The analyses indicated that controls have a genomic predisposition towards lower levels of membrane-associated progesterone receptor component 1 (HR004) compared to cases ($P \leq .01$; Figure 1). The fitted model explained 38.0% of the variation and produced normally distributed regression residuals. The covariates were weakly correlated to each other and three of them, BMI, RV, and PCA02, significantly varied between cases and controls. No further meaningful relationship emerged from the investigation of the remaining hormone and respective receptor levels.

Within cases, we applied the PRSs to test any correlation between their genomic predisposition to hormones and receptors levels to Neurometer data and ultrasound vestibular mucosa thickness. Instrumental measurements, which are more objective, led us to choose these two factors. The analysis of linear relationships between PRSs and clinical phenotypes highlighted that the cases evidenced a genomic predisposition to higher prolactin (PRL) levels (H007) in response to unit decrease of the Neurometer values at 250 and 5 Hz ($P \leq .05$; Table 3). Cases also showed genomic predisposition to reduced levels of mineralocorticoid receptor (HR001) and progesterone receptor component 2 (HR005) in response to unit increase of the Neurometer values at 5 Hz ($P \leq .05$; Table 3). In addition, the genomic predisposition to low levels

Downloaded from https://academic.oup.com/jsm/advance-article/doi/10.1093/jsxmed/qdae201/7951995 by ISSM Member Access user on 16 January 2025

Table 3. Results of linear regression analysis on PRS and clinical phenotypes.

Clinical variable: Neurometer at 250 Hz	GWAS summary statistics ID	Estimate	*P*-value	R^2 adjusted
Prolactin	H007	-2.15E-07	0.02*	0.24
Clinical variable: Neurometer at 5 Hz				
Mineralocorticoid receptor	HR001	-3.06E-07	0.03*	0.15
Prolactin	H007	-3.75E-07	0.04*	0.19
Progesterone receptor component 2	HR005	-3.43E-07	0.04*	0.18
Clinical variable: vestibular mucosa thickness (μm)				
Estrogen Receptor	HR003	9.16E-08	0.002**	0.48
Total Testosterone	H005	9.74E-09	0.04*	0.10

Significant correlations are shown for each comparison; *: $P \leq .05$; **: $P \leq .01$.

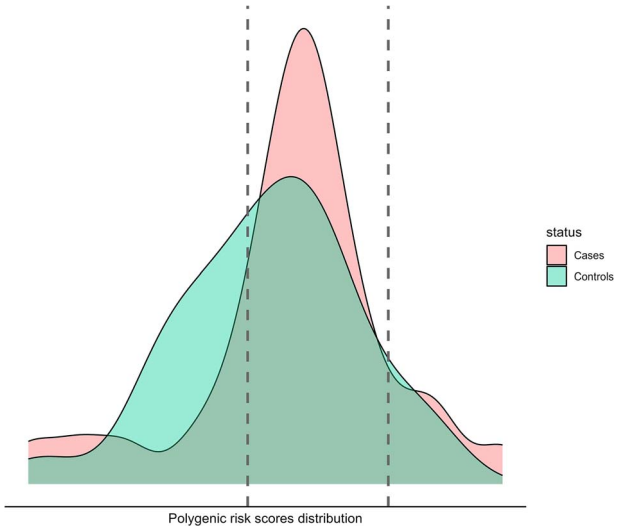

Figure 1. Distribution of PRSs calculated for each case (red distribution) and control (green distribution), using the GWAS summary statistics on HR004 levels. Dashed bars indicate the 25th and 75th quantiles of the PRSs' distribution in EUR population of 1000 genomes project.[21] Individuals with PRSs lower than the 25th quantile show a genomic predisposition to low levels of HR004, while individuals with PRS higher than the 75th quantile show a genomic predisposition to high levels of HR004.

of estrogen receptor (HR003) and total testosterone (H005) respectively decreased for any unit rise of vestibular mucosa thickness ($P \leq .05$; Table 3). In contrast, the above-mentioned meaningful relationships were not observed within control group.

Discussion

The aim of our case–control genomic trial was to investigate a possible polygenic risk to develop VBD-related phenotypic traits, such as altered levels of hormones and respective receptors. For each case and control (total 56 individuals) within our study, the imputed SNPs were used to calculate the genomic predisposition to levels of sex hormones and their respective receptors. Patients with VBD resulted to have a genomic predisposition to higher levels of membrane-associated progesterone receptor component 1 compared to controls. Genetic predisposition to high levels of progesterone receptors may be an expression of low hormone susceptibility. In fact, receptors' density can be influenced by their ligand levels, and low levels of ligand are associated with higher levels of receptor. A genetic predisposition to high levels of progesterone component 2 receptor in response to unit decreases of the Neurometer at 5 Hz, correlated to nociceptive C fibers, was also pointed out within cases.

Our analysis also reported within the cases a significant positive correlation between genetic predisposition to low levels of estrogen receptors and total testosterone with a higher probability of reduced ultrasound vestibular mucosa thickness. The vulvar vestibule exhibits high density of estrogen and androgen receptors, which plays a crucial role in providing lubrication and comfort during sexual intercourse.[26] The predisposition to a sex hormone "disturbance" in our patients with VBD may induce a thinning of the vestibular mucosa because of impaired trophism. This disorder makes the tissue more prone to damage (eg, infections, microtrauma, allergy), and more sensitive to nociception as suggested by the Neurometer data. Our data are consistent with the finding of Labus et al.[27] who observed that women with VBD compared to healthy controls had reduced concentrations of pregnenolone, progestin and androgen metabolites involved in the steroid hormone biosynthesis. In addition, the vaginal fluid was the primary source of reductions observed, suggesting that the effects were localized in the vagina and vestibule rather than systemic.

The role of combined hormonal contraceptive (CHC) in the development of VBD was investigated in previous studies with conflicting results. In fact, a systematic review revealed that VBD and CHC use have a significant association, with an odds ratio of 2.10.[28] In contrast, some studies do not demonstrate any association between CHC and VBD, although these trials were mostly based on telephone surveys that relied on self-reported vulvodynia and did not involve vestibular evaluation.[29,30] Our data suggest that CHC does not induce VBD, but it can help to trigger it in women with an increased polygenic risk for a hormonal alteration, whose phenotypic expression occurs at the vestibular level. This assumption is supported by the case–control study of Goldstein et al.[31] who reported that a group of women developed VBD after using antiandrogenic CHC containing ethinyl estradiol with drospirenone. The researchers identified a genetic polymorphism on the androgen receptor gene with a significantly higher frequency in the group with provoked vulvodynia (PVD) compared to control subjects. Furthermore, in our case studies, only about 25% of patients were currently using contraception, suggesting that other possible triggers may be involved.

Downloaded from https://academic.oup.com/jsm/advance-article/doi/10.1093/jsxmed/qdae201/7951995 by ISSM Member Access user on 16 January 2025

Steroid hormones were found to directly stimulate pain perception, according to research largely based on experimental animal models.[32,33] In our study, the analysis of linear relationships between PRSs and clinical phenotypes highlighted that cases had a genomic predisposition to high prolactin (PRL) and mineralocorticoid (MR) levels in response to lower values of the Neurometer at 250 and 5 Hz (Table 3). To our knowledge, this is the first trial highlighting a link between the genetic predisposition to PRL and MR levels and VBD.

Despite its primary function of promoting lactation, PRL has also been linked to several physiological functions, such as reproduction, regulating the endocrine and immune systems, and modulating the nervous system.[34] PRL receptors (PRLR), which are found everywhere in the nervous system, including the brain and nerve endings, mediate its actions.[35] The nociceptive effect caused by PRL is due to its ability to simulate the transient receptor potential vanilloid (TRVP), that are highly expressed in sensory neurons and contribute to nociception.[32] In addition, PRL has an activating effect on lymphocytes, macrophages, and mast cells to increase the production of proinflammatory and pain-inciting cytokines.[36] In a chronic inflammatory pain mouse model, no differences in PRL concentrations were found in serum of rats, while increased PRL levels were detected in inflamed hind paw skin, suggesting that local endogenous PRL/PRLR contributes selectively to inflammatory and pain.[37]

Similarly, MR have been detected on peripheral immunoreactive nociceptive neurons. In addition to their role in mediating homeostasis, it has been suggested that MR may upregulate the transcription of pain signaling molecules, such as TRVP1, Nav1.8 (sodium voltage-gated channel alpha subunit 10) and trkA (neurotrophic receptor tyrosine kinase 1).[33] In particular, the application of MR antagonist has shown not only the translocation of MR from cytosol to nucleus in peripheral neurons, but also a transcriptional downregulation of pain signaling molecules, that, in turn, attenuates mechanical hypersensitivity.[33] Considering these results, we can hypothesize to associate the genomic predisposition to an increased pain sensitivity related to PRL and MR levels in a "vestibular setting".

In addition, the possible genetic predisposition to low progesterone susceptibility, expressed as cases' trend to higher levels of the respective component 1 receptor, was also a significant finding of our study. Progesterone is commonly recognized as a sex hormone, but it has also been shown to be synthesized and metabolized by both the peripheral and central nervous systems. According to some studies, progesterone is also involved in the modulation of both nociceptive and neuropathic pain.[38] Some studies suggest that progesterone produces antinociceptive effects against trigeminal neuropathic pain,[38,39] and it prevents mechanical allodynia to cold stimulation in animals subjected to a spinal cord hemi section.[40]

Regarding study limitations, the sample groups were too small to proceed with a GWAS, and the results can only be applied to a European population because the reference data were collected from previous GWAS on European ancestry cohorts. At this stage, the accuracy of polygenic risk scores applied to the present study may only be valid and useful for European ancestry populations, and more research is needed to derive the data for making polygenic risk scores useful for other populations. Also, we highlighted that patients with VBD have a genetic predisposition to high levels of progesterone receptor with a higher frequency compared to controls, but the lack of summary statistics in the reference database for our study has prevented us from calculating the predisposition to low or high levels of progesterone. Finally, we included patients with VBD eligible for a fractional CO_2 laser treatment to the vestibule, in which a criterion of inclusion was an altered vestibular trophism, as reported in previous studies.[41,42] It is possible that this is a selection bias that is not representative of all VBD cases.

An increase in local nerve density in the vulvar vestibule from those with PVD has been identified in comparison with controls, to define some kind of "neuro-proliferative dyspareunia" as a quantitative increase in nerve fibers at an anatomic structure that is contacted with sexual activity.[43,44] Our hypothesis is that genome-wide polymorphisms significantly associated to hormones and their receptors levels could define a specific "cluster" of patients. The expression of genetic polymorphism in a specific anatomical body site refers to how genetic variations influence traits, functions, or disease susceptibility in a localized area of the body. This concept highlights the relationship between genotype and phenotype at the molecular and cellular levels within a distinct anatomical region. Gene polymorphisms may directly alter protein expression or function in the target tissue (eg, melanin synthesis in melanocytes). Epigenetic regulation modulates how polymorphisms are expressed in a particular anatomical context, influencing transcription in response to environmental cues.[45]

This may express a peculiar vestibular phenotype that is crucial to the development of the disease, achieved through two mechanisms: (1) an altered trophism leading to vestibular thinning and increased sensitivity of nerve endings, likely resulting in higher vulnerability to irritative and infectious stimuli that may cause "neuro-proliferation"; (2) direct action of hormonal changes on nociception (genetic predisposition to high levels of progesterone receptor, as well as high PRL and MR levels).

The results of present study suggest that is pivotal to highlight the genetic and microscopic patterns of the vulvar vestibule to build the tailored treatment of pain conditions affecting this region.

To prevent future pain problems in these patients, it is necessary to conduct prospective studies that include subgroups with different risk profiles, as well as improve methods to accelerate VBD diagnosis. Finally, we believe that our approach may offer potential diagnostic markers and lead to novel targets for therapeutic intervention.

Acknowledgments

The study was supported by Polo d'Innovazione di Genomica, Genetica e Biologia (Polo GGB), an Italian health tech company, dedicated to providing innovation in human health.

Author contributions

F.M.: Conceptualization-Lead, Data curation-Equal, Formal analysis-Equal, Validation-Equal, Writing—original draft-Equal, Writing—review & editing-Equal. C.F.: Data curation-Equal, Formal analysis-Equal, Writing—original draft-Equal. C.L.: Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Writing—original draft-Equal. G.C.: Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Writing—original draft-Equal. A.R.: Data curation-Equal, Formal analysis-Equal, Writing—original draft-Equal. S.A.:

Downloaded from https://academic.oup.com/jsm/advance-article/doi/10.1093/jsxmed/qdae201/7951995 by ISSM Member Access user on 16 January 2025

Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Writing—original draft-Equal. V.N.: Data curation-Equal, Writing—original draft-Equal. V.S.: Supervision-Equal, Writing—review & editing-Equal).

Supplementary material

Supplementary material is available at *The Journal of Sexual Medicine* online.

Funding

None declared.

Conflicts of interest

None declared.

Data availability

Data submitted to the European Nucleotide Archive (EMBL) under the accession number PRJEB75751.

References

1. Bornstein J, Goldstein AT, Stockdale CK, *et al.* 2015 ISSVD, ISSWSH, and IPPS consensus terminology and classification of persistent vulvar pain and vulvodynia. *J Sex Med*. 2016;**13**(4):607–612. https://doi.org/10.1016/j.jsxm.2016.02.167.
2. Harlow BL, Kunitz CG, Nguyen RH, Rydell SA, Turner RM, MacLehose RF. Prevalence of symptoms consistent with a diagnosis of vulvodynia: population-based estimates from 2 geographic regions. *Am J Obstet Gynecol*. 2014;**210**(1):40.e1–40.e8. https://doi.org/10.1016/j.ajog.2013.09.033.
3. Graziottin A, Murina F, Gambini D, *et al.* Vulvar pain: the revealing scenario of leading comorbidities in 1183 cases. *Eur J Obstet Gynecol Reprod Biol*. 2020 Sep;**252**:50–55. https://doi.org/10.1016/j.ejogrb.2020.05.052.
4. Pukall CF, Goldstein AT, Bergeron S, *et al.* Vulvodynia: definition, prevalence, impact, and pathophysiological factors. *J Sex Med*. 2016;**13**(3):291–304. https://doi.org/10.1016/j.jsxm.2015.12.021.
5. Tomalty D, Giovannetti O, Magliocchetti S, *et al.* Characterizing the innervation of the vulvar vestibule and the immunohistochemical features of neuroproliferative vestibulodynia. *J Sex Med*. 2023;**20**(6):716–731. https://doi.org/10.1093/jsxmed/qdad040.
6. Meister MR, Sutcliffe S, Ghetti C, *et al.* Development of a standardized, reproducible screening examination for assessment of pelvic floor myofascial pain. *Am J Obstet Gynecol*. 2019;**220**(255):e1–255.e9.
7. Morgan TK, Allen-Brady KL, Monson MA, Leclair CM, Sharp HT, Cannon-Albright LA. Familiarity analysis of provoked vestibulodynia treated by vestibulectomy supports genetic predisposition. *Am J Obstet Gynecol*. 2016;**214**(e609):e1–e7.
8. Gerber S, Bongiovanni AM, Ledger WJ, Witkin SS. Interleukin-1beta gene with vulvar vestibulitis syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2003;**107**(1):74–77. https://doi.org/10.1016/S0301-2115(02)00276-2.
9. Babula O, Danielsson I, Sjoberg I, Ledger WJ, Witkin SS. Altered distribution of mannose-binding lectin alleles at exon I codon 54 in women with vulvar vestibulitis syndrome. *Am J Obstet Gynecol*. 2004;**191**(3):762–766. https://doi.org/10.1016/j.ajog.2004.03.073.
10. Portman DJ, MLS G, Vulvovaginal Atrophy Terminology Consensus Conference Panel. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the International Society for the Study of Women's Sexual Health and the North American Menopause Society. *Menopause*. 2014;**21**(10):1063–1068. https://doi.org/10.1097/GME.0000000000000329.
11. Murina F, Graziottin A, Felice R, Di Francesco S. Coital pain in the elderly: could a low dose estriol gel thrill the vulvar vestibule? *Eur J Obstet Gynecol Reprod Biol*. 2016 Dec ;**207**:121–124. https://doi.org/10.1016/j.ejogrb.2016.10.016.
12. Murina F, Torraca M, Graziottin A, Nappi RE, Villa P, Cetin I. Validation of a clinical tool for vestibular trophism in postmenopausal women. *Climacteric*. 2023;**26**(2):149–153. https://doi.org/10.1080/13697137.2023.2171287.
13. Murina F, Barbieri S, Lubrano C, Cetin I. Vestibular mucosa thickness measured by ultrasound in patients affected by vestibulodynia: a case-control study. *Sex Med*. 2021;**9**(2):page 100320. https://doi.org/10.1016/j.esxm.2020.100320.
14. Lowenstein L, Davis C, Jesse K, Durazo-Arvizu R, Kenton K. Comparison between sensory testing modalities for the evaluation of afferent nerve functioning in the genital area. *Int Urogynecol J Pelvic Floor Dysfunct*. 2009;**20**(1):83–87. https://doi.org/10.1007/s00192-008-0733-5.
15. Andrews, S. *FastQC: A Quality Control Tool for High Throughput Sequence F1000 Res*. 2018;**7**:1338. https://doi.org/10.12688/f1000research.15931.2.
16. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;**30**(15):2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
17. Li H, Durbin R. Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*. 2009;**25**(14):1754–1760. https://doi.org/10.1093/bioinformatics/btp324.
18. Rubinacci S, Ribeiro DM, Hofmeister RJ, Delaneau O. Efficient phasing and imputation of low-coverage sequencing data using large reference panels. *Nat Genet*. 2021;**53**(1):120–126. https://doi.org/10.1038/s41588-020-00756-0.
19. Rubinacci S, Hofmeister RJ, Sousa da Mota B, Delaneau O. Imputation of low-coverage sequencing data from 150,119 UK biobank genomes. *Nat Genet*. 2023;**55**(7):1088–1090. https://doi.org/10.1038/s41588-023-01438-3.
20. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 2015;**4**(1):7. https://doi.org/10.1186/s13742-015-0047-8.
21. 1000 Genomes Project Consortium, Auton A, Brooks LD, *et al.* A global reference for human genetic variation. *Nature*. 2015;**526**(7571):68–74.
22. R Core Team. *R: A Language and Environment for Statistical Computing*. <https://www.R-project.org/>. 2023. R Foundation for Statistical Computing, Vienna, Austria.
23. Hemani G, Zheng J, Elsworth B, *et al.* The MR-base platform supports systematic causal inference across the human phenome. *elife*. 2018;**7**(7):e34408. https://doi.org/10.7554/eLife.34408.
24. Elsworth B, Lyon M, Alexander T, *et al.* The MRC IEU OpenGWAS data infrastructure. *bioRxiv* 2020.08.10.244293. https://doi.org/10.1101/2020.08.10.244293.
25. Lyon MS, Andrews SJ, Elsworth B, Gaunt TR, Hemani G, Marcora E. The variant call format provides efficient and robust storage of GWAS summary statistics. *Genome Biol*. 2021;**22**(1):32. https://doi.org/10.1186/s13059-020-02248-0.
26. Krapf JM, Goldstein AT. Combined estrogen-progestin oral contraceptives and female sexuality: an updated review. *Sex Med Rev*. 2024;**12**(3):307–320. https://doi.org/10.1093/sxmrev/qeae011.
27. Labus JS, Mayer EA, Aagaard K, Stains J, Broniowska K, Rapkin A. Reduced concentrations of vaginal metabolites involved in steroid hormone biosynthesis are associated with increased vulvar vestibular pain and vaginal muscle tenderness in provoked vestibulodynia: an exploratory metabolomics study. *Mol Pain*. 2021 Jan-Dec;**17**:17448069211041853. https://doi.org/10.1177/17448069211041853.
28. Champaneria R, D'Andrea RM, Latthe PM. Hormonal contraception and pelvic floor function: a systematic review. *Int Urogynecol J*. 2016;**27**(5):709–722. https://doi.org/10.1007/s00192-015-2833-3.

Downloaded from https://academic.oup.com/jsm/advance-article/doi/10.1093/jsxmed/qdae201/7951995 by ISSM Member Access user on 16 January 2025

29. Arnold LD, Bachmann GA, Rosen R, Rhoads GG. Assessment of vulvodynia symptoms in a sample of US women: a prevalence survey with a nested case control study. *Am J Obstet Gynecol*. 2007;**196**(2):128.e1–e6. https://doi.org/10.1016/j.ajog.2006.07.047.
30. Reed BD, Harlow SD, Legocki LJ, *et al*. Oral contraceptive use and risk of vulvodynia: a population-based longitudinal study. *BJOG*. 2013;**120**(13):1678–1684. https://doi.org/10.1111/1471-0528.12407.
31. Goldtein AT, Belkin ZR, Krapf JM, *et al*. Polymorphisms of the androgen receptor gene and hormonal contraceptive induced provoked vestibulodynia. *J Sex Med*. 2014;**11**(11):2764–2771. https://doi.org/10.1111/jsm.12668.
32. Patil MJ, Ruparel SB, Henry MA, Akopian AN. Prolactin regulates TRPV1, TRPA1, and TRPM8 in sensory neurons in a sex-dependent manner: contribution of prolactin receptor to inflammatory pain. *Am J Physiol Endocrinol Metab*. 2013;**305**(9):E1154–E1164. https://doi.org/10.1152/ajpendo.00187.2013.
33. Shaqura M, Li L, Mohamed DM, *et al*. Neuronal aldosterone elicits a distinct genomic response in pain signaling molecules contributing to inflammatory pain. *J Neuroinflammation*. 2020;**17**(1):183. https://doi.org/10.1186/s12974-020-01864-8.
34. Chen Y, Navratilova E, Dodick DW, Porreca F. An emerging role for prolactin in female-selective pain. *Trends Neurosci*. 2020;**43**(8):635–648. https://doi.org/10.1016/j.tins.2020.06.003.
35. Patil MJ, Henry MA, Akopian AN. Prolactin receptor in regulation of neuronal excitability and channels. *Channels (Austin)*. 2014;**8**(3):193–202. https://doi.org/10.4161/chan.28946.
36. Zhang JM, An J. Cytokines, inflammation, and pain. *Int Anesthesiol Clin*. 2007;**45**(2):27–37. https://doi.org/10.1097/AIA.0b013e318034194e.
37. Goffin V, Bernichtein S, Touraine P, Kelly PA. Development and potential clinical uses of human prolactin receptor antagonists. *Endocr Rev*. 2005;**26**(3):400–422. https://doi.org/10.1210/er.2004-0016.
38. À-González SL, Meyer L, Raggio MC, *et al*. Allopregnanolone and progesterone in experimental neuropathic pain: former and new insights with a translational perspective. *Cell Mol Neurobiol*. 2019;**39**(4):523–537. https://doi.org/10.1007/s10571-018-0618-1.
39. Kim MJ, Shin HJ, Won KA, *et al*. Progesterone produces antinociceptive and neuroprotective effects in rats with microinjected lysophosphatidic acid in the trigeminal nerve root. *Mol Pain*. 2012;**8**(8):16. https://doi.org/10.1186/1744-8069-8-16.
40. Coronel MF, Labombarda F, Villar MJ, De Nicola AF, González SL. Progesterone prevents allodynia after experimental spinal cord injury. *J Pain*. 2011;**12**(1):71–83. https://doi.org/10.1016/j.jpain.2010.04.013.
41. Goldstein SW, Goldstein I, Kim NN, Kellogg-Spadt S, Murina F. Safety and efficacy of fractional CO2 laser treatment to the vestibule: a randomized, double-blind, sham-controlled, prospective 3-site clinical study in women with vestibular pain. *J Sex Med*. 2023;**20**(6):800–812. https://doi.org/10.1093/jsxmed/qdac053.
42. Murina F, Karram M, Salvatore S, Felice R. Fractional CO2 laser treatment of the vestibule for patients with vestibulodynia and genitourinary syndrome of menopause: a pilot study. *J Sex Med*. 2016;**13**(12):1915–1917. https://doi.org/10.1016/j.jsxm.2016.10.006.
43. Velikonja L, Giovannetti O, Adams MA, Tomalty D. Innervation of the human vulvar vestibule: a comprehensive review. *Clin Anat*. 2023;**36**(1):18–27. https://doi.org/10.1002/ca.23966.
44. Mwaura AN, Marshall N, Anglesio MS, Yong PJ. Neuroproliferative dyspareunia in endometriosis and vestibulodynia. *Sex Med Rev*. 2023;**11**(4):323–332. https://doi.org/10.1093/sxmrev/qead033.
45. Iannaccone PM, Ryznar RJ, Van Winkle LJ. Genetics, epigenetics, and the environment: are precision medicine, provider compassion, and social justice effective public health measures to mitigate disease risk and severity? *Int J Environ Res Public Health*. 2024;**21**(11):1522. https://doi.org/10.3390/ijerph21111522.

Downloaded from https://academic.oup.com/jsm/advance-article/doi/10.1093/jsxmed/qdae201/7951995 by ISSM Member Access user on 16 January 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of The International Society for Sexual Medicine. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com
The Journal of Sexual Medicine, 2025, 1–8
https://doi.org/10.1093/jsxmed/qdae201
Original Research