Title

Vulvodynia

Journal

Journal of Lower Genital Tract Disease, 21(2)

ISSN

1089-2591

Authors

Havemann, Logan M
Cool, David R
Gagneux, Pascal
et al.

Publication Date

2017-04-01

DOI

10.1097/lgt.0000000000000289

Peer reviewed

Vulvodynia: What We Know and Where We Should Be Going

Logan M. Havemann, BA,[1] David R. Cool, PhD,[1,2] Pascal Gagneux, PhD,[3] Michael P. Markey, PhD,[4] Jerome L. Yaklic, MD, MBA,[1] Rose A. Maxwell, PhD, MBA,[1] Ashvin Iyer, MS,[2] and Steven R. Lindheim, MD, MMM[1]

Objective: The aim of the study was to review the current nomenclature and literature examining microbiome cytokine, genomic, proteomic, and glycomic molecular biomarkers in identifying markers related to the understanding of the pathophysiology and diagnosis of vulvodynia (VVD).

Materials and Methods: Computerized searches of MEDLINE and PubMed were conducted focused on terminology, classification, and "omics" variations of VVD. Specific MESH terms used were VVD, vestibulodynia, metagenomics, vaginal fungi, cytokines, gene, protein, inflammation, glycomic, proteomic, secretomic, and genomic from 2001 to 2016. Using combined VVD and vestibulodynia MESH terms, 7 references were identified related to vaginal fungi, 15 to cytokines, 18 to gene, 43 to protein, 38 to inflammation, and 2 to genomic. References from identified publications were manually searched and cross-referenced to identify additional relevant articles. A narrative synthesis of the articles was conducted; however, meta-analysis was not conducted because of substantial heterogeneity in the studies and limited numbers of control-matched studies.

Results: Varying definitions of VVD complicate a meta-analysis, and standard definitions will better allow for comparisons of studies and enhance the applicability of evidence to patient populations. Although data are still limited, genomic and molecular diagnostic testings continue to be investigated as potential tools for the diagnosis of VVD.

Conclusions: Standardized nomenclature will allow for comparability of studies and progress in research related to the pathophysiology of VVD and to facilitate clinical decision making and treatment choices. Although the current understanding of the pathogenesis of VVD is limited, there are new opportunities to explore potential diagnostic markers differences in women with VVD, which may lead to targeted therapy.

Key Words: vulvodynia, microbiome, genomics, proteomics, glycomics

(*J Low Genit Tract Dis* 2017;21: 00–00)

Vulvodynia (VVD) is a chronic, heterogeneous, and multifactorial disease that has remained an elusive and complex condition despite years of focused research.[1–3] Vulvodynia is highly prevalent with lifetime estimates ranging from 10% to 28% among reproductive-aged women in the general population.[4–7] Diagnosis requires a comprehensive history, physical examination, and specific diagnostic tests.[2,8] However, the diagnosis is one of exclusion, only reached after testing results are negative for bacterial and fungal infections and after ruling out other causes for pain and discomfort.[9] Treatment options are varied and targeted toward managing symptoms rather than toward a specific cause for the condition.[7,8]

Our understanding of VVD has been hindered by the use of varied terminology in the literature complicating research, evolving definitions, and unidentified pathogenesis of disease. The pathogenesis of VVD remains largely unknown and is likely multifactorial. Recent research has focused largely on an inflammatory pathogenesis, and our current understanding suggests an initial vaginal insult with infection[1] followed by an inflammatory response[10] that may result in peripheral and central pain sensitization, mucosal nerve fiber proliferation, hypertrophy, hyperplasia, and enhanced systemic pain perception.[11] With advancements in our ability to measure transcriptomic markers of disease, as well as the progress in mapping the human genome and how variations affect disease states, new avenues of research in the pathogenesis of VVD can now be explored including the potential role for molecular markers in diagnosis, including microbiome cytokine, genomic, proteomic, and glycomic markers of disease.

Thus, we performed computerized searches of MEDLINE and PubMed from 2001 to 2016 focused on nomenclature and potential novel markers of diagnosis in VVD including vaginal microbiome, inflammatory cell composition, cytokines, genomic, proteomic, and glycomic markers.

STANDARDIZED NOMENCLATURE AND ITS RELEVANCE

Research in the area VVD has been hindered by inconsistent terminology use in the literature. The lack of consistent terms and definitions precludes the comparisons of studies and limits the use and applicability of evidence to patient populations whose diagnosis is based on variable criteria. In 1981, the International Society for Study of Vulvovaginal Disease (ISSVD) set up the first task force on vulvar pain. In an attempt to unite researchers, the ISSVD proposed a terminology and classification in 1999 with the universal use of the term "vulvodynia" to define chronic vulvar discomfort, mainly as burning, occurring in the absence of visible relevant findings.[12] The definition has been revised multiple times because research into the causation and treatments modalities of VVD has evolved.[12]

Most recently in 2015, the ISSVD, the International Society for the Study of Women's Sexual Health, and the International Pelvic Pain Society came to a consensus and replaced the original title of the terminology "Terminology and Classification of Vulvodynia" with "Terminology and Classification of Persistent Vulvar Pain" because the new terminology does not pertain to acute vulvar pain or only to VVD.[9] Persistent vulvar pain is now categorized as "vulvar pain caused by a specific disorder" or "vulvodynia."[9] Vulvodynia is now more clearly defined as vulvar pain of at least 3-month duration, without clear identifiable cause and further subcategorized by the following descriptors: (1) localized (e.g., vestibulodynia, clitorodynia) or generalized or mixed (localized and generalized), (2) provoked (e.g., insertional, contact) or spontaneous or mixed (provoked and spontaneous), (3) onset (e.g., primary or secondary), and (4) temporal pattern (e.g., intermittent, persistent, constant, immediate, delayed).[9]

Now with standardized nomenclature, this should allow for progress in the understanding of the pathology of VVD including the comparability of future studies to facilitate clinical decision making and treatment choices. This is based on comprehensive

[1]Department of Obstetrics and Gynecology, Wright State University, Boonshoft School of Medicine, Dayton, OH; [2]Department of Pharmacology and Toxicology, Wright State University, Boonshoft School of Medicine, Dayton, OH; [3]Department of Pathology, University of California San Diego, San Diego, CA; and [4]Department of Biochemistry and Molecular Biology, Wright State University, Boonshoft School of Medicine, Dayton, OH

Reprint requests to: Steven R. Lindheim, MD, MMM, 128 Apple Street, Suite 3800, Weber CHE, Dayton, OH, 45409. E-mail: steven.lindheim@wright.edu

The authors have declared they have no conflicts of interest.

DOI: 10.1097/LGT.0000000000000289

TABLE 1. Differential Diagnosis of VVD

Category	Diagnosis
Infectious	Candidiasis trichomonas, herpes
Inflammatory	Allergic vulvitis, lichen planus, lichen sclerosus
Neoplastic	Paget disease, vulvar intraepithelial neoplasia, squamous cell carcinoma, vulvar melanoma, sarcomas of the vulva
Neurologic	Neuroma, postherpetic neuralgia, pudendal canal syndrome, other nerve compression injury
Trauma	Female genital cutting, forced entry, obstetrical
Iatrogenic	Postoperative, chemotherapy, radiation
Hormonal deficiencies	Menopause, vulvar atrophy, lactational amenorrhea
Psychiatric	Vaginismus

Based on the studies by Bornstein et al.[9] and Reed.[13]

and nuanced parameters that will now be enhanced with the universal classification and diagnosis of patients.

CURRENT STRATEGIES IN THE PATHOGENESIS AND DIAGNOSIS OF VVD

Women with VVD often describe vulvar pain as a burning, stinging, irritation, rawness, and dyspareunia (difficult or painful intercourse).[4,7] The diagnosis of VVD at this time is inherently one of exclusion, because the ISSVD requires that the vulvar pain exists without clear and identifiable cause.[9] Currently, there are no specific tests for markers of disease, and the diagnosis requires a comprehensive history and physical examination[7] and exclusion of other etiologies of vulvar pain (see Table 1).[9,13,14]

A better understanding of the pathogenesis of VVD is an important step in developing better and more specific diagnostic tests for VVD. As medicine moves toward the "-omics" of molecular diagnostic testing (e.g., metagenomic, genomic, proteomic, and glycomic), molecular markers of disease are continuously being investigated as potential tools for more precise and accurate diagnosis (see Figure 1). Given that VVD is a diagnosis of exclusion, elucidation of potential markers of disease moves the field toward discovery of a definitive marker for diagnosis. The following will review current literature on the pathogenesis of VVD, and from this, newer diagnostic strategies will be presented.

Vaginal Microbiome

Recent initiatives including the 2007 Human Microbiome Project have directed efforts toward understanding and better identifying and characterizing microbiomes, deemed to be our "second genome." Many genomes of our symbiotic microbes encode a plethora of genes with important roles for human health.[15–18]

In analysis of the baseline vaginal microbiome in the physiologic healthy women, the Human Microbiome Project using 16S rRNA sequencing found that the *Lactobacillus* genus dominated the vaginal microbiome at the vaginal introitus, midpoint, and posterior fornix.[16] It has additionally been shown that the vaginal microbial environment is usually dominated by 1 or 2 *Lactobacilli* species, most frequently *Lactobacillus iners*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, or *Lactobacillus jensenii*, which can fluctuate in some patients on the basis of the woman's menstrual cycle.[16]

A review of the literature suggests that the vaginal microbiome may be different in women with different geographical ancestry[19] and suggests that these differences may alter predisposition to infection.[19,20] Specifically, a portion of asymptomatic, healthy women, particularly African American and Hispanic women, host a polymicrobial vaginal environment dominated by bacteria other than *Lactobacilli*, including *Prevotella*, *Gardnerella*, *Atopobium*, and *Megasphaera* species.[19] A study from The Vaginal Human Microbiome Project at Virginia Commonwealth University compared the microbiomes of vaginal samples from 1,268 African American women and 416 European American women. The findings revealed that in African American women, the most common vaginal microbiome was *L. iners*, followed by *Gardnerella vaginalis*, BVAB1, "other," and *L. crispatus.*[20] In contrast, the most common vaginal microbiome in women of European ancestry was *L. crispatus*, followed by *L. iners* and *G. vaginalis*, and that the BVAB1 microbial profile was only found in 5 samples.[20]

There are indications of a potential difference in the vaginal microbiome of women with VVD compared with control women.[21] In a double-blind study, vaginal samples for bacterial flora and cytokines of patients with VVD were compared with controls, and cultures from control women showed the presence of *L. crispatus*, which was not present in samples from women with symptomatic VVD or VVD in remission, who alternatively demonstrated the presence of *L. gasseri.*[21] Researchers hypothesized

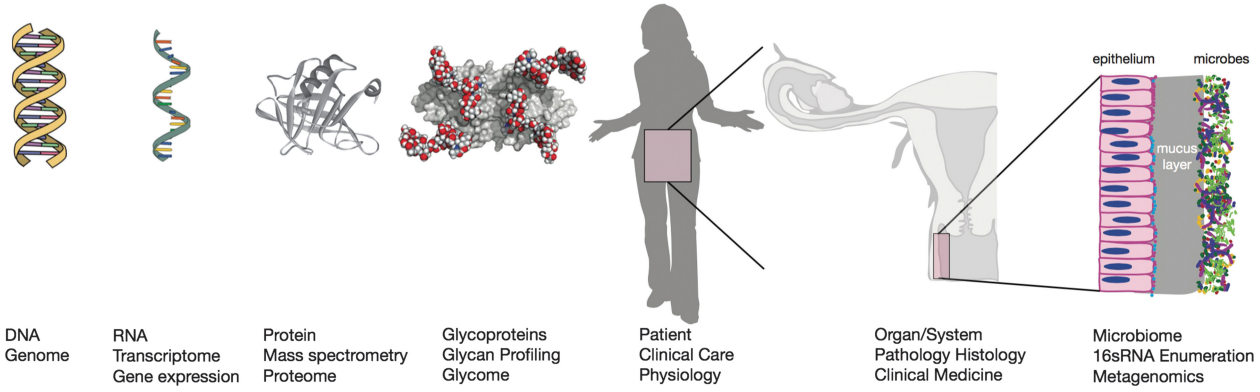

FIGURE 1. Overview of the -omics of diagnostic testing. The -omics of diagnostic testing can be categorized as genomic, transcriptomic, proteomic, glycomic, and metagenomic, and each of these levels can be affected by inflammation and infection. In each of the -omics, specific markers of disease including variations in DNA coding, RNA transcription, protein structure, glycoprotein composition, and composition of the microbiome are sought after to determine definitive markers for diagnosis and to direct treatment strategies.

that this difference in vaginal flora may be the result of an initial vaginal insult causing inflammation that results in abnormal cytokine production.[21] However, metagenomic comparisons are needed to expand these results and identify microbiome differences in an unbiased and comprehensive fashion, expanding on the knowledge of variations in vaginal microbiome composition including ethnic and lifestyle differences.

Inflammatory Pathogenesis

Population-based epidemiologic studies have identified an association between a history of vulvovaginal infections and the subsequent development of VVD.[22] A history of bacterial vaginosis (BV),[23] genital warts, trichomoniasis, urinary tract infections, and yeast infections have all been associated with an increased risk for VVD, with multiple reoccurrences compounding the risk.[24] Mechanisms that link infection to the development of VVD have been widely hypothesized. The current dogma is that during or after an initial vaginal insult with infection, a "susceptible" individual has an inflammatory response that is potentially composed of an abnormally heightened release of proinflammatory cytokines, neurokines, and/or chemokines.[1] The individual is then either unable to successfully clear the inflammation or the infectious process alters and sensitizes the neural tissue in the vulvovaginal area, resulting in localized or generalized allodynia and hyperalgesia.[1]

A significant amount of research has focused on a proposed link between repeat infections with *C. albicans* and VVD. Farmer et al.[25] found that laboratory mice subjected to recurrent infection with *C. albicans* were found to have developed mechanical allodynia localized to the vulva and that upon histological examination, these mice also had hyperinnervation with peptidergic nociceptor and sympathetic fibers compared with controls, with hypersensitivity and hyperinnervation both present for more than 3 weeks after the resolution of infection. Ramirez et al.[25] tested a similar theory through patch testing on humans, and they found that patients reporting VVD were significantly more likely to react to *C. albicans* compared with controls and that lower concentrations of *C. albicans* caused more positive results than higher concentrations.[26] This altered response to lower levels of *C. albicans* has been demonstrated more recently in a matched-control study where vestibular cells may possess an "immunological memory" and produce increased amount of interleukin 6 (IL-6) and Prostaglandin E2 in response to repeated infection with *C. albicans*[27–29] and in response to *C. albicans* in concentrations that are clinically undetectable, compared with external vulvar cell fibroblasts.[29] Although this pattern of response was also present in control samples, the response was greatly accentuated in VVD samples, producing 5 times more IL-6 transcript than vestibular fibroblasts from healthy controls. This suggests that patients with VVD may be responding clinically through production of proinflammatory cytokines to concentrations of *C. albicans* that are normal in the normal vaginal flora.[29]

Additional data demonstrate that the Dectin-1 receptors on fibroblasts, which are innate immune receptors involved in the recognition of fungal β-glucans, similar to those found in the cell walls of *C. albicans* required for maximal proinflammatory mediator response, are comparatively elevated in vestibular compared with external vulvar fibroblasts in women with VVD compared with controls. They seem to work through the *NFκB* pathway, which is associated with the production of IL-6 and PGE-2. Moreover, *C. albicans* activates the *NFκB* pathways more effectively than nonpathogenic *S. cerevisiae*, resulting in more abundant production of IL-6 and PGE-2.[29] This suggests that Dectin-1 receptor and *NFκB* pathway may be implicated in the greater response to *C. albicans* in patients with VVD and might serve as targets for selective treatment options.[29]

Other research on the inflammatory pathogenesis of VVD has focused on histopathologic differences and markers of chronic inflammation. Findings demonstrate an increased density of lymphocytes and lymphocytic infiltration,[30] specifically that CD4-positive T cells have been found more predominantly in vestibular biopsies of women with VVD.[31] In addition, the presence of organized vestibule-associated lymphoid tissue characterized by germinal centers with B lymphocytes and mature mucosal Immunoglobulin A-plasma cells are present in women with VVD compared with controls.[32] This may signify that vestibular tissue is undergoing chronic inflammatory changes in response to antigens that are triggering lymphocyte migration and activation in VVD and perhaps resulting in the chronic pain.

The roles of mast cells and protease inhibitors in nerve proliferation and hyperinnervation have also been investigated. Tender sites, in the absence of clinically visible inflammation, in those with VVD have been found to have greater numbers of mast cells, compared with controls.[30,33] Two proinflammatory mediators released by mast cells, tumor necrosis factor α (TNF-α) and nerve growth factor, have been demonstrated to stimulate nerve proliferation leading to hyperinnervation of nerves. It has been suggested that upon their release, they directly act on nociceptive pain fibers and lower pain thresholds, leading to neurogenic vasodilation and erythema.[33] An increase in nerve thickness and density in painful vestibular regions is also supported by findings from other studies.[30,34] In addition, NGF acts as a chemoattractant for mast cells and binds to NGF receptors on mast cell membranes and nerves, stimulating its own synthesis, perpetuating the response.[33]

Nociceptive pain fibers have also been found to contain a surface protease-activated receptor 2 that upon cleavage induces long-lasting allodynia and hyperalgesia.[35] This coupled with significantly lower concentrations of protease inhibitors in vaginal secretions from women with VVD may be lead to an increase in pain sensitivity as well.[36]

Overall, the literature demonstrates a potential link between infection, inflammation, and VVD through association with repeat vaginal infections, heightened sensitivity and proinflammatory response to *C. albicans*, and histopathologic changes with increased lymphocyte and mast cell density. Some investigators have reported increased levels of mucosal proinflammatory cytokines including IL-6, PGE-2, TNF-α, and NGF. However, the culmination of these findings is not conclusive in elucidating the pathogenesis of VVD because the literature does not identify any consistent inflammatory markers or histologic changes that can be considered pathognomonic for VVD. Furthermore, these findings have yet to lead to adequate treatment modalities. In the absence of pathognomonic findings and targeted treatments, the link between infection, inflammation, and VVD has yet to be definitively elucidated, and further research is needed to elucidate the role of the infection and inflammatory changes in the development of VVD.

Cytokine Markers

Cytokines are detectable and measureable biochemical mediators of inflammation,[37] and an abnormal inflammatory cell response in patients with VVD may subsequently lead to abnormal production and secretion of cytokines in that region. Previous studies discussed previously have only looked at individual cytokines, IL-6, PGE-2, and TNF-α,[27–29] but recent studies have examined a wide array of cytokines in patients with VVD.

In a double-blinded study using the 27-plex cytokine assay, patients with VVD had a 35-fold increase of IL-17, a 7-fold

decrease in macrophage inflammatory protein 1 beta and a 3-fold decrease in IL-12 compared to controls.[21] These results suggest the involvement of an immunological response involving various cytokines; however, there is disagreement in the literature of the role of specific cytokines in VVD. The dramatic increase in IL-17 observed by Ventolini et al.[21] was not seen by Baker et al.,[38] because their recent study found a decrease in IL-17. Further research is needed to extrapolate the importance of these cytokine markers and their potential in the diagnosis of VVD.

Genomic Markers

Separate from repeated infectious assaults predisposing women to VVD, studies have found that the genetic profile of women with VVD includes polymorphisms in genes coding for cytokines, IL-1 receptor antagonist, IL-1β, and mannose-binding lectin (MBL)[39] and proposed that these genetic polymorphisms may lead to an enhanced inflammatory response after an assault with trauma or infection and a reduced capacity to terminate inflammation.[39,40]

Studies suggest that women who were homozygous for allele 2 in the IL-1β receptor antagonist and for allele 2 in the IL-1β gene were more likely to be affected with VVD.[41] These variant alleles result in lower levels of production of the anti-inflammatory mediator, IL-1 receptor antagonist, and higher levels of production of the proinflammatory cytokine, IL-1β, the latter resulting in a heightened inflammatory response and the former in difficulty in terminating it.[41] Homozygosity for these alleles has been associated with an enhanced inflammatory response and is hypothesized to predispose women to VVD.[41]

Buccal swabs from women with and without VVD tested for codons 54 MBL2 gene polymorphisms revealed that the variant MBL2 codon 54, allele B, was more frequent in women with recurrent episodes of vulvovaginal candidiasis and BV than in the women with acute episodes or control women.[42] Additional findings suggest that the MBL2 allele variant is more prevalent in women with primary VVD and that those women with the allele variant also had a reduced ability to produce TNF-α in response to a *Candida* insult.[43] Mannose-binding lectin 2 is another important innate immune receptor that recognizes high-mannose N-glycans characteristic of fungi and other pathogens, and a genetic polymorphism in it may make one more susceptible to various bacterial and *C. albicans* infections.[42–44]

Another area of interest is in the role of single nucleotide polymorphisms in patients with VVD. Ideally, future studies will be able to examine large numbers of patients versus controls by high-throughput NGS to identify single nucleotide polymorphisms and quantitative trait loci that predispose to VVD, giving insight to the etiology of VVD and inform therapeutic targeting.

In addition to genomic DNA markers, we can also define RNA expression differences between VVD and control patients to identify transcriptomic markers of disease. We would expect to see differences in the mRNA expression locally (in biopsies) as well as potential changes in circulating small RNAs. Several studies have identified various circulating small RNAs as noninvasive diagnostic biomarkers of gynecological disease[45–51] that can serve as a "liquid biopsy" (see Figure 2). For example, in the disease state of endometriosis, a genome-wide analysis of lncRNAs in serum identified 5 such lncRNAs that may serve as biomarkers of endometriosis.[45] In addition, mRNA targets of microRNAs may generate new hypotheses about the genes and pathways involved in many disease states. Unfortunately, no such study has yet been undertaken for VVD.

Similar to the principles of the "liquid biopsy" of circulating RNAs, new research has identified DNA-based biomarkers from cell-free circulating DNA (cfcDNA) in the bloodstream, which originate from the normal cells of the body as well as from tumor cells, sparking much research into their use as cancer biomarkers.[52] Work in tumors has demonstrated that cfcDNA from the disease tissue not only carries mutations found in the disease but also carries the methylation pattern (negatively regulate the expression of that gene, even without mutation of the DNA sequence) at the CpG islands within the promoters of disease-related genes.[53] Studies have demonstrated the ability to detect various cancers such as prostate, breast, gastric, testicular, and bladder cancer on the basis of abnormal methylation patterns of the same set of promoters for various diseases in cfcDNA, which are unique to the individual cancer.[52] This suggests that if DNA methylation patterns are unique in individual disease states, we may potentially be able to identify abnormal methylation patterns unique to VVD. To date, no studies have examined correlation between VVD and abnormal methylation patterns, but unique variations in promoter region methylation could potentially serve as a biomarker for VVD.

In addition to DNA methylation, posttranslational modifications of chromatin via chemical modification of histone tails can

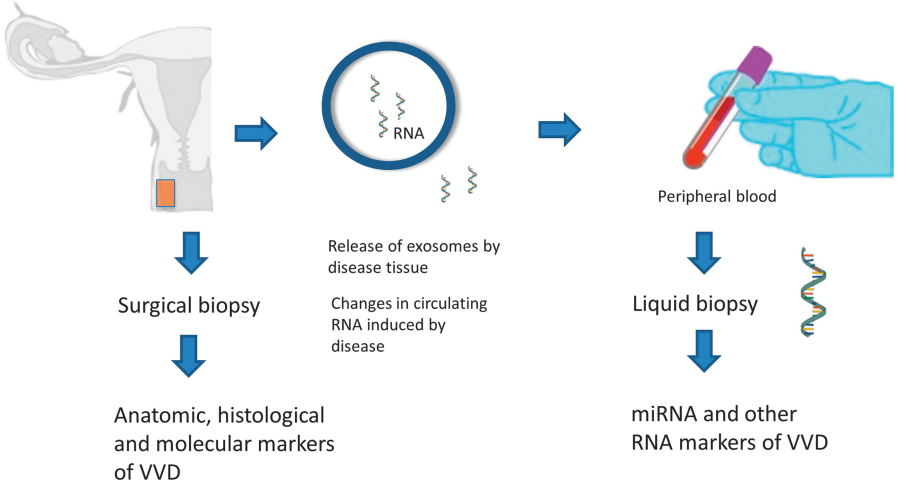

FIGURE 2. Liquid biopsy. Genomic markers of disease include RNA molecules that have entered circulation, both within endosomes and as cell-free RNA. Small RNA molecules in particular have become an area of intense research as biomarkers of disease. Unlike traditional biopsies, these molecules can serve as adjuvant diagnostics without anything more invasive than venipuncture.

have large influence on gene regulation. These include histone methylation, phosphorylation, acetylation, ubiquitination and the addition of a single sugar known as O-GlcNAcylation. Comprehensive studies of such chromatin modification are in their infancy, but it is becoming clear that these are important contributors to regulation of genome function in health and disease.[54]

Proteomic Markers

Alterations in protein expression have been a major target of research since the completion of the Human Genome Project, opening up opportunities to discover novel protein markers for use in diagnosis of human diseases. Although there may be polymorphisms at the gene level, protein expression and their functionality ultimately determine the disease state, and thus, there has been a surge in proteomic studies for the past decade to decipher the proteomic milieu in diseases.[55] This area has identified proteomic markers in obstetrical and gynecologic disease states including pre-eclampsia,[56,57] perinatal infection,[58] preterm birth,[59,60] intrauterine growth restriction,[61] gestational diabetes,[62] ectopic pregnancy,[63] endometriosis,[64] as well as cervical,[65] ovarian,[66] and breast cancers.[67] However, to date, no studies have examined the proteomic differences characterizing patients with VVD and may provide novel biomarkers that are sensitive and specific enough to be used for developing diagnostic tools and treatment strategies in the area of VVD.[68]

Glycomic Markers

One of the most common posttranslational modifications of proteins is glycosylation with various glycans that occurs in more than 70% of all human proteins.[69] The distinct composition and proportion of various glycans located on specific glycoproteins is cell specific, varies depending on the physiologic state, and can be altered by different disease states.[69] Although glycome and glycomic markers have been studied primarily in the field of cancer,[70] newer methods including mass spectrometry, High Performance Liquid Chromatography, as well as glycan arrays and glycan-binding (lectin) arrays have allowed for analysis of novel glycomic markers, particularly because they relate to their role in inflammation.[69,71,72]

The glycans on glycoproteins can be further modified by the addition of sialic acids, which are found abundantly on all human (and other vertebrate) cell surfaces but not on plants or fungi. Sialic acids play a role in the immune system by regulating the alternative pathway of the complement activation, modulating leukocyte trafficking, and controlling immune cell activation, and some organisms, including group B streptococcus, have developed human-like sialytated trisaccharide terminals, mimicking the sialic acids on human cells, that serve to block recognition of underlying glycans by naturally occurring antibodies in humans, thus effectively fooling the immune system.[73]

Given that the glycomic structure of cells can be impacted by disease,[69] it is certainly reasonable that they could also be altered in the vagina by vaginal microbiome.[74] In a study of women with and without BV, samples from women with BV had lower levels of both sialic acid and 2 high-mannose glycans that are known to be targeted by innate (antiviral and antifungal) immune lectins such as DC-SIGN.[74] These alterations associated with the presence of abnormal microbiome may have effects on the innate immune system, making these women more susceptible to infection and subsequent inflammation.

Currently, no research has been published on glycomic markers specific to VVD. However, a strong connection between glycomic markers and inflammation is plausible. In general, it seems that women with VVD seem to have a lower threshold reaction to *C. albicans*. Inflammation in patients with VVD is also linked with previous *Candida* infections.[1] However, *Candida* does not express sialic acids, but like other fungal pathogens, it contains cell wall structures that consist of high-mannose, β-glucans and chitin, and human hosts have a variety of innate receptors for these tell-tale fungal glycans, including Dectin, mannose receptors, TLRs 2, 4, and 6, and DC-SIGN.[75–79] Because *Candida* expresses β-glucans in its cell wall, the link between glycomic markers and inflammation may be a contributing factor in the hypersensitivity of patients with VVD to yeast. Further research is needed to determine whether *Candida* and its effects through alteration of the vaginal microbiome may provide glycomic markers in women that could potentially predispose them to the development of VVD. Additional research is needed specifically to elucidate the glycomic profile in women with VVD compared with healthy control women to determine whether certain glycomic markers are specific to VVD and can be used as markers of disease, similar to investigations in various cancers.

CONCLUSIONS

Progress has been made toward creating standardized nomenclature where the current understanding of VVD has been limited by use of inconsistent terms and definitions, prohibiting comparison of studies and limiting the application of evidence to patient populations whose diagnosis is based on variable criteria. Hopefully, this will allow for progress in facilitating the use of evidence-based medicine in clinical decision making for patients and elucidating the pathogenesis of VVD.

To date, there is limited work in cytokine, genomic, proteomic, and glycomic biomarkers in helping us clarify the pathogenesis of VVD and identifying genetic and molecular diagnostic markers that may ultimately lead to targeted therapeutic interventions. Continued work in the area of secretomics will help clarify this, because the future in research into the pathogenesis and diagnosis of VVD may lie within the cytokine, genomic, proteomic, and glycomic variations in patients.

REFERENCES

1. Akopians AL, Rapkin AJ. Vulvodynia: the role of inflammation in the etiology of localized provoked pain of the vulvar vestibule (vestibulodynia). *Semin Reprod Med* 2015;33:239–45.
2. Ventolini G, Barhan SM. Vulvodynia. *Dermatol Online J* 2008;14:2.
3. Ventolini G. Measuring treatment outcomes in women with vulvodynia. *J Clin Med Res* 2011;3:59–64.
4. Harlow BL, Stewart EG. A population-based assessment of chronic unexplained vulvar pain: have we underestimated the prevalence of vulvodynia? *J Am Med Womens Assoc* 2003;58:82–8.
5. Reed BD, Crawford S, Couper M, et al. Pain at the vulvar vestibule: a web-based survey. *J Low Genit Tract Dis* 2004;8:48–57.
6. Reed BD, Haefner HK, Sen A, et al. Vulvodynia incidence and remission rates among adult women: a 2-year follow-up study. *Obstet Gynecol* 2008;112(2 Pt 1):231–7.
7. Arnold LD, Bachmann GA, Rosen R, et al. Assessment of vulvodynia symptoms in a sample of US women: a prevalence survey with a nested case control study. *Am J Obstet Gynecol* 2007;196:128.e1–6.
8. Sadownik LA. Etiology, diagnosis, and clinical management of vulvodynia. *Int J Womens Health* 2014;6:437–49.
9. 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;13:607–12.
10. Bohm-Starke N. Medical and physical predictors of localized provoked vulvodynia. *Acta Obstet Gynecol Scand* 2010;89:1504–10.

11. Leclair CM, Goetsch MF, Korcheva VB, et al. Differences in primary compared with secondary vestibulodynia by immunohistochemistry. *Obstet Gynecol* 2011;117:1307–13.
12. Moyal-Barracco M, Lynch PJ. 2003 ISSVD terminology and classification of vulvodynia: a historical perspective. *J Reprod Med* 2004; 49:772–7.
13. Reed BD. Vulvodynia: diagnosis and management. *Am Fam Physician* 2006;73:1231–8.
14. Goldstein AT, Pukall CF, Brown C, et al. Vulvodynia: assessment and treatment. *J Sex Med* 2016;13:572–90.
15. Peterson J, Garges S, Giovanni M, et al. NIH HMP Working Group. The NIH Human Microbiome Project. Genome Res 2009;19:2317–23.
16. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207–14.
17. Human Microbiome Project Consortium. A framework for human microbiome research. Nature 2012;486:215–21.
18. Aagaard K, Petrosino J, Keitel W, et al. The Human Microbiome Project strategy for comprehensive sampling of the human microbiome and why it matters. *FASEB J* 2013;27:1012–22.
19. Green KA, Zarek SM, Catherino WH. Gynecologic health and disease in relation to the microbiome of the female reproductive tract. *Fertil Steril* 2015;104:1351–7.
20. Fettweis JM, Brooks JP, Serrano MG, et al. Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology* 2014;160:2272–82.
21. Ventolini G, Gygax SE, Adelson ME, et al. Vulvodynia and fungal association: a preliminary report. *Med Hypotheses* 2013;81:228–30.
22. Pathak D, Agrawal S, Dhali TK. Prevalences of and risk factors for vulvar diseases in Nepal: a hospital-based study. *Int J Dermatol* 2011;50:161–7.
23. Edgardh K, Abdelnor M. Vulvar vestibulitis and risk factors: a population-based case-control study in Oslo. *Acta Derm Venereol* 2007;87:350–4.
24. Nguyen RH, Sanson D, Harlow BL. Urogenital infections in relation to the occurrence of vulvodynia. *J Reprod Med* 2009;54:385–92.
25. Farmer MA, Taylor AM, Bailey AL, et al. Repeated vulvovaginal fungal infections cause persistent pain in a mouse model of vulvodynia. *Sci Transl Med* 2011;3:101ra91.
26. Ramirez De Knott HM, McCormick TS, Do SO, et al. Cutaneous hypersensitivity to Candida albicans in idiopathic vulvodynia. *Contact Dermatitis* 2005;53:214–8.
27. Foster DC, Piekarz KH, Murant TI, et al. Enhanced synthesis of proinflammatory cytokines by vulvar vestibular fibroblasts: implications for vulvar vestibulitis. *Am J Obstet Gynecol* 2007;196:346.e1–8.
28. Foster DC, Falsetta ML, Woeller CF, et al. Site-specific mesenchymal control of inflammatory pain to yeast challenge in vulvodynia-afflicted and pain-free women. *Pain* 2015;156:386–96.
29. Falsetta ML, Foster DC, Woeller CF, et al. Identification of novel mechanisms involved in generating localized vulvodynia pain. *Am J Obstet Gynecol* 2015;213:38.e1–12.
30. Goetsch MF, Morgan TK, Korcheva VB, et al. Histologic and receptor analysis of primary and secondary vestibulodynia and controls: a prospective study. *Am J Obstet Gynecol* 2010;202:614.e1–8.
31. Leclair CM, Leeborg NJ, Jacobson-Dunlop E, et al. CD4-positive T-cell recruitment in primary-provoked localized vulvodynia: potential insights into disease triggers. *J Low Genit Tract Dis* 2014;18:195–201.
32. Tommola P, Bützow R, Unkila-Kallio L, et al. Activation of vestibule-associated lymphoid tissue in localized provoked vulvodynia. *Am J Obstet Gynecol* 2015;212:476.e1–8.
33. Regauer S, Eberz B, Beham-Schmid C. Mast cell infiltrates in vulvodynia represent secondary and idiopathic mast cell hyperplasias. *APMIS* 2015; 123:452–6.
34. Bornstein J, Goldschmid N, Sabo E. Hyperinnervation and mast cell activation may be used as histopathologic diagnostic criteria for vulvar vestibulitis. *Gynecol Obstet Invest* 2004;58:171–8.
35. Vergnolle N, Bunnett NW, Sharkey KA, et al. Proteinase-activated receptor-2 and hyperalgesia: a novel pain pathway. *Nat Med* 2001;7:821–6.
36. Jayaram A, Esbrand F, Dulaveris G, et al. Decreased concentration of protease inhibitors: possible contributors to allodynia and hyperalgesia in women with vestibulodynia. *Am J Obstet Gynecol* 2015;212:184.e1–4.
37. Omoigui S. The biochemical origin of pain: the origin of all pain is inflammation and the inflammatory response. Part 2 of 3 - inflammatory profile of pain syndromes. *Med Hypotheses* 2007;69:1169–78.
38. Baker DA, Peresleni T, Kocis C. Inflammatory markers in vestibulodynia [4]. *Obstet Gynecol* 2016;127(suppl 1):1S–2.
39. Gerber S, Witkin SS, Strucki D. Immunological and genetic characterization of women with vulvodynia. *J Med Life* 2008;1:432–8.
40. Wesselmann U, Bonham A, Foster D. Vulvodynia: current state of the biological science. *Pain* 2014;155:1696–701.
41. Gerber S, Bongiovanni AM, Ledger WJ, et al. Interleukin-1beta gene polymorphism in women with vulvar vestibulitis syndrome. *Eur J Obstet Gynecol Reprod Biol* 2003;107:74–7.
42. Giraldo PC, Babula O, Gonçalves AK, et al. Mannose-binding lectin gene polymorphism, vulvovaginal candidiasis, and bacterial vaginosis. *Obstet Gynecol* 2007;109:1123–8.
43. Babula O, Linhares IM, Bongiovanni AM, et al. Association between primary vulvar vestibulitis syndrome, defective induction of tumor necrosis factor-alpha, and carriage of the mannose-binding lectin codon 54 gene polymorphism. *Am J Obstet Gynecol* 2008;198:101.e1–4.
44. Babula O, Danielsson I, Sjoberg I, et al. Altered distribution of mannose-binding lectin alleles at exon 1 codon 54 in women with vulvar vestibulitis syndrome. *Am J Obstet Gynecol* 2004;191:763–6.
45. Wang WT, Sun YM, Huang W, et al. Genome-wide long non-coding RNA analysis identified circulating LncRNAs as novel non-invasive diagnostic biomarkers for gynecological disease. *Sci Rep* 2016;6:23343.
46. Rekker K, Saare M, Roost AM, et al. Circulating miR-200-family micro-RNAs have altered plasma levels in patients with endometriosis and vary with blood collection time. *Fertil Steril* 2015;104:938–946.e2.
47. Marí-Alexandre J, García-Oms J, Barceló-Molina M, et al. MicroRNAs and angiogenesis in endometriosis. *Thromb Res* 2015;135(suppl 1):S38–40.
48. Cho S, Mutlu L, Grechukhina O, et al. Circulating microRNAs as potential biomarkers for endometriosis. *Fertil Steril* 2015;103:1252–60.e1.
49. Hull ML, Nisenblat V. Tissue and circulating microRNA influence reproductive function in endometrial disease. *Reprod Biomed Online* 2013; 27:515–29.
50. Jia SZ, Yang Y, Lang J, et al. Plasma miR-17-5p, miR-20a and miR-22 are down-regulated in women with endometriosis. *Hum Reprod* 2013;28: 322–30.
51. Wang WT, Zhao YN, Han BW, et al. Circulating microRNAs identified in a genome-wide serum microRNA expression analysis as noninvasive biomarkers for endometriosis. *J Clin Endocrinol Metab* 2013;98:281–9.
52. Levenson VV. DNA methylation as a universal biomarker. *Expert Rev Mol Diagn* 2010;10:481–8.
53. Illingworth RS, Bird AP. CpG islands—'a rough guide'. *FEBS Lett* 2009; 583:1713–20.
54. Lewis BA, Hanover JA. O-GlcNAc and the epigenetic regulation of gene expression. *J Biol Chem* 2014;289:34440–8.
55. Lekhwani S, Shankar V, Vaswani ND. Proteomics in obstetrics and gynecology. *Indian J Hum Genet* 2011;17:3–6.
56. Gharesi-Fard B, Zolghadri J, Kamali-Sarvestani E. Proteome differences of placenta between pre-eclampsia and normal pregnancy. *Placenta* 2010; 31:121–5.
57. Carty DM, Siwy J, Brennand JE, et al. Urinary proteomics for prediction of preeclampsia. *Hypertension* 2011;57:561–9.

58. Buhimschi IA, Christner R, Buhimschi CS. Proteomic biomarker analysis of amniotic fluid for identification of intra-amniotic inflammation. *BJOG* 2005;112:173–81.
59. Liong S, Di Quinzio MK, Fleming G, et al. Prediction of spontaneous preterm labour in at-risk pregnant women. *Reproduction* 2013;146:335–45.
60. Buhimschi CS, Weiner CP, Buhimschi IA. Clinical proteomics: a novel diagnostic tool for the new biology of preterm labor, part I: proteomics tools. *Obstet Gynecol Surv* 2006;61:481–6.
61. Wölter M, Röwer C, Koy C, et al. A proteome signature for intrauterine growth restriction derived from multifactorial analysis of mass spectrometry-based cord blood serum profiling. *Electrophoresis* 2012;33: 1881–93.
62. Page NM, Kemp CF, Butlin DJ, et al. Placental peptides as markers of gestational disease. *Reproduction* 2002;123:487–95.
63. Rausch ME, Beer L, Sammel MD, et al. A disintegrin and metalloprotease protein-12 as a novel marker for the diagnosis of ectopic pregnancy. *Fertil Steril* 2011;95:1373–8.
64. Liu E, Nisenblat V, Farquhar C, et al. Urinary biomarkers for the non-invasive diagnosis of endometriosis. *Cochrane Database Syst Rev* 2015;12:CD012019.
65. Yim EK, Park JS. Role of proteomics in translational research in cervical cancer. *Expert Rev Proteomics* 2006;3:21–36.
66. Tchabo NE, Guancial EA, Czechowicz JA, et al. The role of proteomics in the diagnosis and treatment of ovarian cancer. *Womens Health (Lond)* 2005;1:365–74.
67. Liu XG, Wang XP, Li WF, et al. Ca2+—binding protein S100A11: a novel diagnostic marker for breast carcinoma. *Oncol Rep* 2010;23:1301–8.
68. Hernández-Núñez J, Valdés-Yong M. Utility of proteomics in obstetric disorders: a review. *Int J Womens Health* 2015;7:385–91.
69. Arnold JN, Saldova R, Hamid UM, et al. Evaluation of the serum N-linked glycome for the diagnosis of cancer and chronic inflammation. *Proteomics* 2008;8:3284–93.
70. An HJ, Kronewitter SR, de Leoz MLA, et al. Glycomics and disease markers. *Curr Opin Chem Biol* 2009;13:601–7.
71. Rillahan CD, Paulson JC. Glycan microarrays for decoding the glycome. *Annu Rev Biochem* 2011;80:797–823.
72. Ribeiro JP, Mahal LK. Dot by dot: analyzing the glycome using lectin microarrays. *Curr Opin Chem Biol* 2013;17:827–31.
73. Varki A, Gagneux P. Multifarious roles of sialic acids in immunity. *Ann N Y Acad Sci* 2012;1253:16–36.
74. Wang L, Koppolu S, Chappell C, et al. Studying the effects of reproductive hormones and bacterial vaginosis on the glycome of lavage samples from the cervicovaginal cavity. *PLoS One* 2015;10:e0127021.
75. Pérez-García LA, Csonka K, Flores-Carreón A, et al. Role of protein glycosylation in Candida parapsilosis cell wall integrity and host interaction. *Front Microbiol* 2016;7:306.
76. Usluogullari B, Gumus I, Gunduz E, et al. The role of human Dectin-1 Y238X gene polymorphism in recurrent vulvovaginal candidiasis infections. *Mol Biol Rep* 2014;41:6763–8.
77. Qu X, Che C, Gao A, et al. Association of Dectin-1 and DC-SIGN gene single nucleotide polymorphisms with fungal keratitis in the northern Han Chinese population. *Mol Vis* 2015;21:391–402.
78. Marakalala MJ, Kerrigan AM, Brown GD. Dectin-1: a role in antifungal defense and consequences of genetic polymorphisms in humans. *Mamm Genome* 2011;22:55–65.
79. van der Meer JW, van de Veerdonk FL, Joosten LA, et al. Severe Candida spp. infections: new insights into natural immunity. *Int J Antimicrob Agents* 2010;36(suppl 2):S58–62.