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# A Role for Bradykinin Signaling in Chronic Vulvar Pain

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**Abstract:** **Chronic vulvar pain is alarmingly common in women of reproductive age and is often accompanied by psychological distress, sexual dysfunction, and a significant reduction in quality of life. Localized provoked vulvodynia (LPV) is associated with intense vulvar pain concentrated in the vulvar vestibule (area surrounding vaginal opening). To date, the origins of vulvodynia are poorly understood, and treatment for LPV manages pain symptoms, but does not resolve the root causes of disease. Until recently, no definitive disease mechanisms had been identified; our work indicates LPV has inflammatory origins, although additional studies are needed to understand LPV pain. Bradykinin signaling is one of the most potent inducers of inflammatory pain and is a candidate contributor to LPV. We report that bradykinin receptors are expressed at elevated levels in LPV patient versus healthy control vestibular fibroblasts, and patient vestibular fibroblasts produce elevated levels of proinflammatory mediators with bradykinin stimulation. Inhibiting expression of one or both bradykinin receptors significantly reduces proinflammatory mediator production. Finally, we determined that bradykinin activates nuclear factor (NF)κB signaling (a major inflammatory pathway), whereas inhibition of NFκB successfully ablates this response. These data suggest that therapeutic agents targeting bradykinin sensing and/or NFκB may represent new, more specific options for LPV therapy.**

***Perspective:*** ***There is an unmet need for the development of more effective vulvodynia therapies. As we explore the mechanisms by which human vulvar fibroblasts respond to proinflammatory/propain stimuli, we move closer to understanding the origins of chronic vulvar pain and identifying new therapeutic targets, knowledge that could significantly improve patient care.***



***Key words:*** *Fibroblast, vulvodynia, bradykinin, cytokine, inflammation.*

Pain, although a necessary response to prevent irreparable injury, can be a perplexing condition to resolve when it occurs chronically or without obvious provocation.[71] Pain can affect nearly any body site or it can be generalized; the effects of chronic pain are crippling, even when the pain is not associated with any life-threatening or physically debilitating condition.[71] Although some chronic pain conditions (eg, fibromyalgia) have been the subject of increased public interest, others have received comparatively less attention. Among these, localized provoked vulvodynia (LPV) is a disabling, persistent, and poorly understood pain condition affecting specific regions of the vulvar vestibule (the area immediately surrounding the vaginal opening).[30,32,64]

Like many other chronic pain conditions, the origins of LPV are not clearly delineated.[32,52,64] Consequently, LPV has been relegated to a "diagnosis of exclusion," and no mechanism-based pain treatments have been developed.[31] Women with LPV experience intense pain, yet often show no obvious signs of infection, lesions, or abnormalities.[30-32,64] LPV is common and affects up to 28% of women in the United States at some point during their lifetime,[30] and approximately 8% of the population is currently afflicted.[33,58] Reported prevalence estimates may underestimate true prevalence, based upon the patient's failure to seek evaluation of or the practitioner's failure to diagnose LPV.[33] The diagnostic hallmark of LPV is longstanding exquisite pain to light touching (allodynia), which is highly localized to the vulvar vestibule.[32] As a result, LPV leaves a devastating effect on sexuality, reproduction, self-image, and emotional well-being.[30,64]

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Epidemiologic studies have identified few clinical precursors, with chronic or recurrent infection by the fungus *Candida* being reported as the most common risk factor.[1,59,64] In support of these findings, repeated vulvovaginal infection with *Candida albicans* has been associated with LPV in a mouse model of disease.[21] Our recently published work also supports a *Candida*–LPV association; fibroblasts isolated from sites of LPV allodynia respond strongly to *C. albicans* and other vaginal fungal pathogens through the production of proinflammatory mediators (eg, interleukin (IL)-6 and prostaglandin $E_2$ [$PGE_2$]).[20,23] We also have evidence that other inflammatory triggers (not specific to fungal infection) stimulate the production of proinflammatory mediators.[25] IL-6 and $PGE_2$ have been previously associated with pain in human and animal models.[15-17,19,22,37,45,53,61] Furthermore, we have shown that fibroblast production of proinflammatory mediators can accurately predict the pain threshold measured at the site of fibroblast origin.[23] Therefore, like many other chronic pain conditions, LPV appears to have inflammatory origins.[71]

With the overarching goal of identifying new therapeutic targets, we set out to explore the mechanisms by which vestibular fibroblasts respond to proinflammatory stimuli to generate this maladaptive immune response associated with pain. Presently, therapeutics for vulvodynia manage the symptoms of LPV, but none target the underlying inflammatory origins of disease.[30,32,63] In a previous study, we showed that vestibular fibroblasts derived from LPV patients at sites with low pain thresholds express elevated levels of the Dectin-1 receptor, which is responsible for recognizing fungal cell wall-derived β-glucan.[20] Exposure to fungal cell wall components or fungi resulted in a potent inflammatory response, which was comparatively stronger in patient fibroblasts isolated from pain versus nonpain sites.[20] However, blocking Dectin-1 signaling only partially ameliorated IL-6 and $PGE_2$ release, implying that other receptors/mechanisms contribute to proinflammatory mediator release, and likely pain.[20] Therefore, a broader understanding of the inflammatory pathways that might affect LPV are necessary to design more effective therapeutic agents that will target the underlying biological cause(s) of disease.

Bradykinin, a nonapeptide, is one of the most potent pain-inducing molecules present within the human body.[11,12,48,49,56,70,72] Bradykinin has been linked to inflammatory and neuropathic hyperalgesia,[56] and inhibitors that impair bradykinin recognition have been or are currently being investigated in clinical trials as potential therapies for several painful medical conditions, such as angioedema.[5,6,47,67] Bradykinin also plays important roles in vasodilation and the oxidative stress response.[8,56] Kinins are produced in human plasma and specific body tissues through the cleavage of high molecular weight (HMW) kininogen precursors by plasma and tissue kallikreins, respectively.[56] Kinins are highly inflammatory and can be further degraded to inactive peptides by kininases. Differential cleavage of HMW kininogens generates distinct species of kinins (eg, Des-Arg[9]-bradykinin and bradykinin) that are recognized by 2 bradykinin receptors (BDKRs), BDKRB1 and BDKRB2, whereas the ligand affinity for each receptor is not equal.[56] BDKRB1 is absent in most body tissues, but is readily inducible and can be detected when inflammation is present.[56] BDKRB2, however, is considered to be a constitutive receptor for bradykinin and is highly expressed in all body tissues,[56] although several studies show that it can be further induced under inflammatory conditions.[14,44,60]

We became interested in bradykinin because of its inflammatory and pain-inducing qualities[56] and the observation that *Candida*, which has been implicated in vulvar pain, may play a role in exacerbating the responses to bradykinin by generating locally elevated concentrations of bradykinin.[9,10,35,36,39,57] *Candida* species produce serine aspartyl proteases that cleave human HMW kininogens, which is believed vital to pathogenesis and aids in tissue invasion by *C. albicans.*[9,10,35,39,57] Therefore, we hypothesized that bradykinin may also play important roles in inflammation and pain within the vulvar vestibule. To evaluate our hypothesis, we first examined whether vestibular and external vulvar fibroblasts express the machinery required for bradykinin processing and sensation, then evaluated the influence of bradykinin on proinflammatory mediator release (IL-6). Furthermore, we investigated the mechanism by which bradykinin is sensed in pain-associated vestibular fibroblasts.

## Methods

### *Patient/Sample Selection*

LPV-afflicted cases (fullfilling Friedrich's Criteria[7]) and age- and race-matched pain-free controls were recruited from the Division of General Obstetrics and Gynecology clinical practice at the University of Rochester between December 2012 and February 2014. All subjects provided informed consent, and the research was approved by the University of Rochester Institutional Review Board (RSRB 42136). Expanded details on our selection criteria and sampling procedures have been previously published.[23,25] In brief, cases and controls were age- and race-matched with a mean age of 33.5 years for both groups. All case and control subjects were Caucasian, non-Hispanic. Furthermore, all subjects denied the use of corticosteroids and nonsteroidal anti-inflammatory medications and had no chronic inflammatory illnesses other than LPV. Before biopsy of the vestibular and external vulvar sites, sampling sites underwent mechanical Wagner algometry. The Wagner algomter (Wagner Instruments, Greenwich, CT) method used a method of limits technique initially described by Zolnoun et al[73] and further elaborated on in our earlier publication.[23] Using the Wagner algometer (Wagner Instruments), an increasing .5 N per second force (range 0–5 N) was applied perpendicular to the mucocutaneous surface using a moistened dacron tipped swab affixed to the Wagner algometer (Wagner Instruments). Force was