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# Cutaneous targets for topical pain medications in patients with neuropathic pain: individual differential expression of biomarkers supports the need for personalized medicine

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**Abstract**

**Introduction:** Numerous potential cutaneous targets exist for treating chronic pain with topically applied active pharmaceutical ingredients. This preliminary human skin tissue investigation was undertaken to characterize several key biomarkers in keratinocytes and provide proof-of-principle data to support clinical development of topical compounded formulations for peripheral neuropathic pain syndromes, such as postherpetic neuralgia (PHN).

**Objectives:** The study intended to identify objective biomarkers in PHN skin on a patient-by-patient personalized medicine platform. The totality of biopsy biomarker data can provide a tissue basis for directing individualized compounded topical preparations to optimize treatment efficacy.

**Methods:** Referencing 5 of the most common actives used in topical pain relief formulations (ketamine, gabapentin, clonidine, baclofen, and lidocaine), and 3 well-established cutaneous mediators (ie, neuropeptides, cannabinoids, and vanilloids), comprehensive immunolabeling was used to quantify receptor biomarkers in skin biopsy samples taken from ipsilateral (pain) and contralateral (nonpain) dermatomes of patients with PHN.

**Results:** Epidermal keratinocyte labeling patterns were significantly different among the cohort for each biomarker, consistent with potential mechanisms of action among keratinocytes. Importantly, the total biomarker panel indicates that the enriched PHN cohort contains distinct subgroups.

**Conclusion:** The heterogeneity of the cohort differences may explain studies that have not shown statistical group benefit from topically administered compounded therapies. Rather, the essential need for individual tissue biomarker evaluations is evident, particularly as a means to direct a more accurately targeted topical personalized medicine approach and generate positive clinical results.

**Keywords:** Keratinocyte, Immunochemistry, Skin, Cannabinoid, CGRP, PHN

## 1. Introduction

In numerous chronic pain conditions, morphological and physiological alterations among primary small caliber nociceptor innervation and the peripheral tissues are detected, including potential interactions of intraepidermal nerve fibers (IENF) and epidermal keratinocytes.[2,5,9,22,30,53,66] Small fiber neuropathy (SFN) refers to a *loss* of small caliber IENF detected within the epidermis and has been demonstrated in several diverse conditions with pain as a chief complaint, including, for example, the neuropathic pain (NP) condition postherpetic neuralgia (PHN) and the largely described

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as nociplastic pain condition fibromyalgia.[2,3,24] The epidermis is the richly innervated outermost layer of skin that functions as a sensory transduction organ and includes a nociceptor population, and a β-endorphin–mediated cannabinoid receptor 2 (CB2)–activated inhibitory mechanism among the keratinocytes.[2,37,41,54,62] Although somewhat counterintuitive that a loss of nociceptive fibers would lead to enhanced chronic pain, accumulated evidence demonstrates additional peripheral mechanisms involved in the generation of nociceptor hyperexcitability, particularly signaling from cutaneous terminal structures and cells, including epidermal keratinocytes, dermal vasculature, adnexal organs, and/or immune (mast or Langerhans) cells, as means that damaged or remaining innervation becomes hyperactive and/or ectopically active.[2,13,15,37,48,66] Pathologies related to either the primary sensory endings and/or cutaneous terminal structures, such as epidermal keratinocytes, represent potential targets for topically applied compounded pharmaceuticals.[2,6,17,40,54,55,61]

Peripheral inflammation is often a main culprit of pain and many efficacious analgesics target peripheral inflammatory mechanisms (eg, cyclooxygenase), while the most potent analgesics act via the central nervous system, through well-described mechanisms, particularly the opiate system.[8,64,67,77] Much evidence also implicates the endocannabinoid and endovanilloid lipid signaling systems as essential in pain modulation.[44,63,69,73] Direct neural injury or dysfunction along the nociceptive pathways of the pain matrix underlies the pathophysiology of chronic NP conditions, including the degeneration of nerve fibers, both centrally and/or peripherally, as a result of natural age, trauma, metabolic disease, bacterial/viral infections, toxins, and/or drug-/vaccine-related side effects.[2,23,28,30,47,64,67,77] Central mechanisms of chronic pain, including neural plasticity induced central sensitization, which have more recently been termed nociplastic pain,[27,50] and also microglial/immune activation, have been described at every level of nociceptive transmission (ie, spinal cord dorsal horn, brainstem, thalamus, cortex), and are used to define the etiology and pathophysiology of several chronic pain conditions, including those with medically unexplained symptoms (MUS).[3,7,8] Analgesics derive efficacy via direct/indirect interference of neural transmission along the peripheral nociceptive pathways (nociceptors) and pain matrix of brainstem and cortical structures.[23,26,28,71] However, in most chronic pain conditions, blocking peripheral nociceptor activity/hyperactivity can alleviate painful symptoms,[7,30,31,47,58] indicative of an ongoing peripheral tissue driver. Currently, the majority of drugs used to treat chronic NP conditions are believed to generate efficacy via central mechanisms, which also drives efficacy-limiting side effects, including addiction potential (ie, opiates).[25,28]

Neuropathic pain conditions (ie, PHN) involve hypersensitive peripheral signaling, which is demonstrated as altered ion channels, increased N-methyl-D-aspartate (NMDA) receptor function, and loss of inhibitory neural tone, among other mechanisms.[2,71] Anti-inflammatory analgesics commonly used for acute pain are often ineffective in these chronic NP conditions.[2,68,74] Compounded topical pain medication has been prescribed by practitioners in managing various NP conditions in patients who do not respond to standard treatments.[17,60,80] Therapeutic classes of actives often seen in topical compounds for NP include NMDA receptor antagonists, alpha-2 adrenergic receptor agonists, anticonvulsants, ion channel modulators, vasodilators, NSAIDs, muscle relaxants, opioids, cannabinoids, and others that can be concentrated locally in the periphery with potential reduction of systemic exposure.[6,40,55]

Since our initial discovery of the opiate peptide beta-endorphin in epidermal keratinocytes in 2003, the role of keratinocytes in communicating between the environment and the IENF, particularly via the release of neural signaling mediators, has become widely recognized.[2,34,37,41,65,66] The epidermis is a vital sensory organ that functions as a polymodal nociceptive structure.[11,21,35] In particular, epidermal keratinocytes have been shown to express numerous mediators of nociceptor hyperexcitability in vitro and/or in vivo, including NMDA and AMPA glutamate receptors,[13,49] voltage-gated calcium channels and sodium channels,[20,78] alpha-adrenergic receptors,[52] GABA receptors,[21] the neuropeptide calcitonin gene-related peptide (CGRP),[34] endothelin-1 (ET1) and the cognate receptors type A (endothelin receptor type A [ETA]) and type B (ETB),[41] the transient receptor potential vanilloid 1 (TrpV1), and, as noted, the cannabinoid receptor 2 (CB2),[19,33,76] among several others. Many of these various signaling family members have been used as drug targets for topical agents, and clear advantages exist when using compounded pharmacotherapy, including the personalized medicine principle and flexibility to be able to make adjustments based on patients' identified biomarkers and response. Presumptive druggable targets associated with nociceptor hyperactivity identified among cutaneous structures, such as epidermal keratinocytes, could provide novel avenues of treatment for PHN and other NP conditions, using currently available active pharmaceutical agents via targeted topical delivery. Therefore, based on the pathogenesis of PHN, hypothesized peripheral mechanisms of NP, and current topical treatment strategies, we selected the following 9 biomarkers to investigate in a cohort of well-characterized PHN patient skin biopsies: N-methyl-D-aspartate receptor 2b (NMDAR2b), calcium voltage-gated channel subunit alpha1 S (CACNA1S), alpha-2A adrenergic receptor (Alpha2AR), gamma-aminobutyric acid type B receptor 2 ($GABA_BR2$), CGRP, CB2, TrpV1, ETA, and sodium voltage-gated channel subunit 1.6 (NaV1.6). The objective of the study was to examine the presence of these nociceptive signal mediators in keratinocytes from PHN (pain) skin and contralateral (nonpain) skin to determine whether potential keratinocyte mechanisms of action are involved.

## 2. Methods

### 2.1. Tissue specimen

Deidentified PHN human research tissue for this study was collected before any drug treatments from an unrelated clinical study (ClinTrials.gov NCT02365636/TEVA Pharmaceuticals) where each subject provided a contralateral and ipsilateral biopsy from the same thoracic dermatome.[24] All patients fully consented to the deidentified use of biopsy material in additional basic research projects under the direction of Integrated Tissue Dynamics, LLC (INTiDYN). Previous research has demonstrated ipsilateral SFN among cohorts of patients with PHN, as well as other innervation alterations; however, not all patients with PHN show ipsilateral fiber loss.[24,56,59] Therefore, for this study, we used an enriched cohort of PHN patient biopsies that were known to fit the prevailing notion that a loss of IENF occurs on the ipsilateral (pain) side compared with contralateral (nonpain). The following criteria were used for the selection of the innervation-standardized PHN patient cohort biopsies used for this research study from review of the 271 total PHN patient tissue database:

(1) An initial study VAS score ≥5.5 (average VAS score for the 271 patient study was 5.5).
(2) A contralateral IENF density that was within one standard deviation of the 271 patient study average (10.3 fibers/mm length of epidermis).
(3) A 30% to 50% reduction of the ipsilateral IENF density compared with contralateral IENF density.

### 2.2. Assessments

The ChemoMorphometric Analysis (CMA) methodology platform was designed by INTiDYN to provide comprehensive immunolabeling analysis in complex tissues. As per CMA protocol, 20x montage images were collected using computer-assisted digital microscopy. The images were then assessed by each biomarker label for measures of epidermal keratinocyte average pixel intensity (API), taken at 5 evenly spaced locations on each of 3 different sections for each biopsy analyzed (n = 15 measures/biopsy). Details of the immunolabeling methodology, analysis, and antibodies used for this study are provided in the supplemental material, http://links.lww.com/PR9/A216.

### 2.3. Statistical analysis

Ipsilateral and contralateral biopsy API averages were calculated from each slide, analyzed for differences by using the paired Student *t* test, and within-patient API ratios [Ipsilateral/Contralateral] were created for cohort analysis. A ratio value of 1 indicated no differences between sides, whereas ratio values less than 1 indicated an increased contralateral expression, and ratio values greater 1 indicated increased ipsilateral expression. The API % difference of ipsilateral to contralateral biopsy expressions were also calculated as [(ipsilateral − contralateral)/contralateral × 100]. A paired Student *t* test statistic was used to determine significant differences ($P \leq 0.05$) between contralateral and ipsilateral biopsies from each slide (within patient), and to determine ratio differences among the cohort (between patient).

## 3. Results

Demographic characteristics and neural innervation status of the enriched PHN patient cohort tissue (n = 20) are presented in **Table 1**. Representative images of the biomarker immunolabeling demonstrate that individualized variable results were observed across the enriched cohort, including significantly greater contralateral labeling and significantly greater ipsilateral labeling for each biomarker evaluated (**Fig. 1**).

### 3.1. Variable expression of biomarkers in contralateral and ipsilateral biopsies

#### 3.1.1. N-methyl-D-aspartate receptor 2b

Significant ipsilateral to contralateral differences of NMDAR2b keratinocyte API were observed in 11/20 PHN cohort biopsy pairs, with lower ipsilateral labeling in 5/20 patients (**Table 2**, green) and higher ipsilateral labeling in 6/20 patients (**Table 2**, red). The group average API ratio (1.09) and the % difference of ipsilateral from contralateral (8.8) indicate a slight trend toward higher ipsilateral biopsy labeling but did not test significant across the cohort (**Table 2**; **Fig. 2**).

#### 3.1.2. Calcium voltage-gated channel subunit alpha1 S

Significant ipsilateral to contralateral differences of CACNA1S keratinocyte API were observed in 10/20 PHN cohort biopsy pairs, with lower ipsilateral labeling in 7/20 patients (**Table 3**, green) and higher ipsilateral labeling in 3/20 patients (**Table 3**, red). The group average API ratio (0.97) and the % difference of

**Table 1**

**Patient and tissue innervation characteristics.**

| Cohort ID | Sex | Age, years | Baseline VAS score | Contralateral IENF | Ipsilateral IENF |
|---|---|---|---|---|---|
| 1 | F | 69 | 5 | 15.9 | 9.6 |
| 2 | F | 52 | 6 | 14.3 | 8.6 |
| 3 | M | 83 | 7 | 8.2 | 4.3 |
| 4 | F | 24 | 5 | 12.3 | 5.3 |
| 5 | F | 78 | 7 | 7.1 | 2.9 |
| 6 | M | 65 | 7 | 7.0 | 2.4 |
| 7 | F | 89 | 5 | 7.5 | 2.3 |
| 8 | F | 70 | 6 | 8.6 | 2.2 |
| 9 | F | 74 | 6 | 14.2 | 9.7 |
| 10 | M | 46 | 6 | 9.0 | 4.8 |
| 11 | M | 37 | 6 | 11.0 | 5.3 |
| 12 | M | 31 | 5 | 14.6 | 6.9 |
| 13 | F | 44 | 6 | 10.6 | 4.8 |
| 14 | F | 39 | 7 | 7.1 | 2.8 |
| 15 | F | 55 | 6 | 13.4 | 4.8 |
| 16 | F | 70 | 5 | 10.9 | 3.9 |
| 17 | M | 74 | 6.5 | 7.4 | 2.1 |
| 18 | F | 71 | 6 | 11.0 | 2.9 |
| 19 | M | 57 | 5 | 14.3 | 3.5 |
| 20 | F | 60 | 5 | 10.7 | 2.2 |
| Group ave (SEM) | 13 F 7 M | 59 (4.0) | 5.8 (0.2) | 10.8 (0.7) | 4.6 (0.5) |

PHN cohort demographic information, pain rating (VAS), and cutaneous intraepidermal nerve fiber (IENF) innervation from the contralateral (nonpain) and ipsilateral (PHN, painful) biopsy sites. The IENF densities are expressed as the number of fibers/mm length of epidermis.

PHN, postherpetic neuralgia; VAS, visual analogue scale.

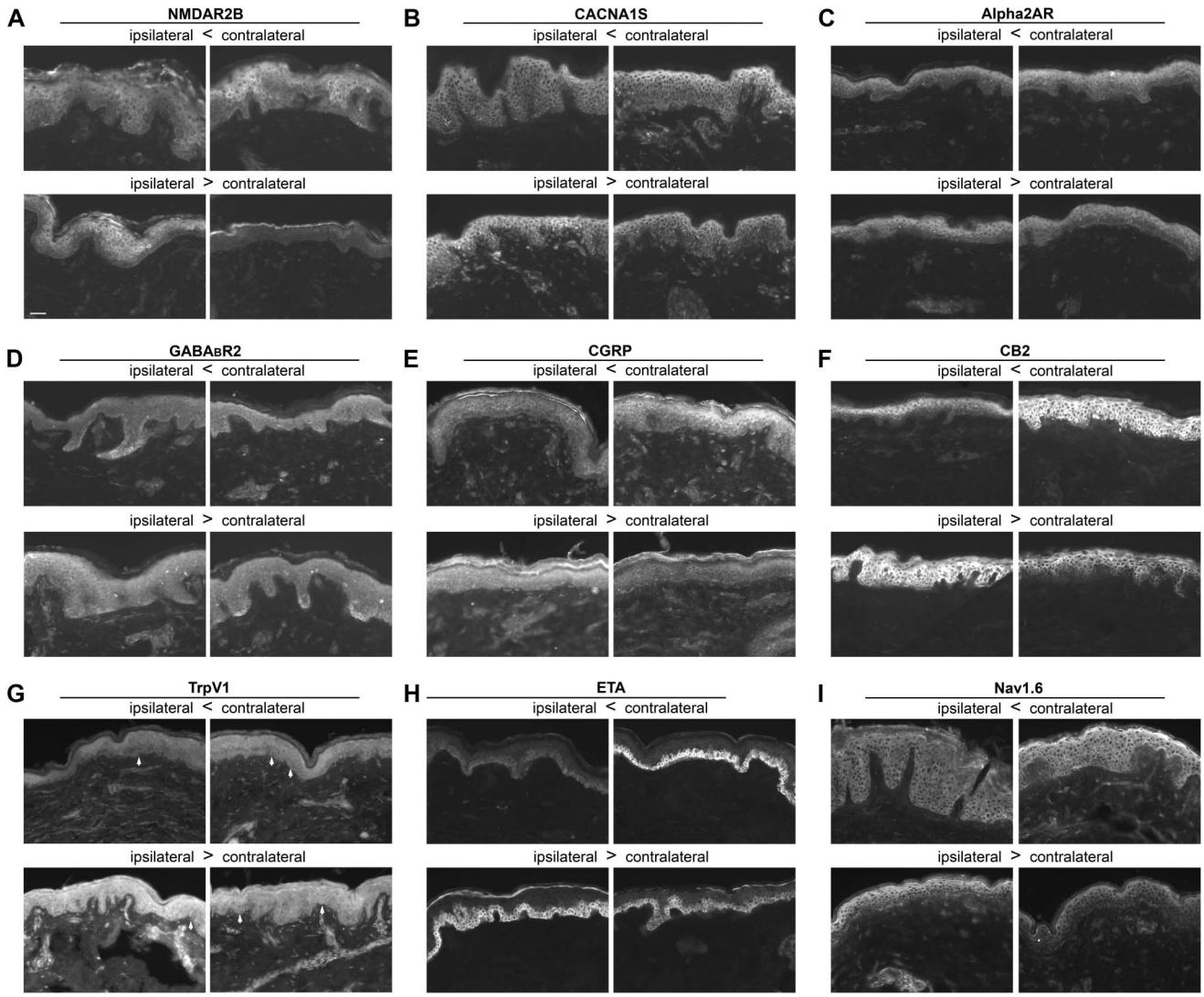


Figure 1. Keratinocyte biomarker immunolabeling examples demonstrate the discreet variability among individual patients of the innervation pathology-matched PHN cohort. For every biomarker (A–I), at least one individual patient within the total cohort had significantly less ipsilateral labeling than contralateral (top plates), and at least one individual patient within the total cohort also had significantly more ipsilateral labeling than contralateral (bottom plates). A few TrpV1-positive small fiber IENF are also seen (G, white arrows). Mag bar = 50 μm for all images. IENF, intraepidermal nerve fibers; PHN, postherpetic neuralgia; TrpV1, transient receptor potential vanilloid 1.

ipsilateral from contralateral (−3.2) indicate a slight trend toward lower ipsilateral biopsy labeling but did not test significant across the cohort (**Table 3**; **Fig. 2**).

### 3.1.3. Alpha-2A adrenergic receptor

Significant ipsilateral to contralateral differences of Alpha2AR keratinocyte API were observed in 13/20 PHN cohort biopsy pairs, with lower ipsilateral labeling in 9/20 patients (**Table 4**, green) and higher ipsilateral labeling in 4/20 patients (**Table 4**, red). The group average API ratio (0.91) and the % difference of ipsilateral from contralateral (−9.1) demonstrate significantly lower ipsilateral biopsy labeling (**Table 4**, green; **Fig. 2**).

### 3.1.4. Gamma-aminobutyric acid type B receptor 2

Significant ipsilateral to contralateral differences of $GABA_BR2$ keratinocyte API were observed in 10/20 PHN cohort biopsy pairs, with lower ipsilateral labeling in 5/20 patients (**Table 5**, green) and higher ipsilateral labeling in 5/20 patients (**Table 5**, red). The group average API ratio (0.99) and the % difference of ipsilateral from contralateral (−0.5) indicate equally split differences among the biopsy pairs (**Table 5**; **Fig. 2**).

### 3.1.5. Calcitonin gene-related peptide

Significant ipsilateral to contralateral differences of CGRP keratinocyte API were observed in 10/20 PHN cohort biopsy pairs, with lower ipsilateral labeling in 7/20 patients (**Table 6**, green) and higher ipsilateral labeling in 3/20 patients (**Table 6**, red). The group average API ratio (0.96) and the % difference of ipsilateral from contralateral (−3.7) indicate a slight trend toward lower ipsilateral biopsy labeling but did not test significant across the cohort (**Table 6**; **Fig. 2**).

### 3.1.6. Cannabinoid receptor 2

Significant ipsilateral to contralateral differences of CB2 keratinocyte API were observed in 13/20 PHN cohort biopsy pairs, with lower ipsilateral labeling in 10/20 patients (**Table 7**, green) and

**Table 2**

**N-methyl-D-aspartate R2b immunolabeling average pixel intensity.**

| NMDA R2b | Individual API Ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs Contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 0.89 | −10.8 | 0.05 |
| 2 | 0.74 | −26.4 | 0.00 |
| 3 | 1.06 | 6.3 | 0.47 |
| 4 | 0.92 | −7.9 | 0.10 |
| 5 | 0.78 | −22.2 | 0.00 |
| 6 | 1.31 | 31.1 | 0.00 |
| 7 | 0.86 | −14.3 | 0.00 |
| 8 | 1.57 | 57.1 | 0.00 |
| 9 | 1.29 | 29.3 | 0.03 |
| 10 | 0.98 | −2.1 | 0.71 |
| 11 | 1.06 | 5.7 | 0.43 |
| 12 | 1.11 | 10.8 | 0.17 |
| 13 | 1.00 | 0.0 | 0.97 |
| 14 | 1.14 | 14.5 | 0.10 |
| 15 | 1.45 | 45.2 | 0.00 |
| 16 | 1.01 | 0.8 | 0.84 |
| 17 | 1.26 | 25.7 | 0.00 |
| 18 | 1.54 | 54.1 | 0.00 |
| 19 | 0.90 | −10.1 | 0.03 |
| 20 | 0.88 | −11.6 | 0.26 |
| *Group Ave.* | 1.09 | 8.8 | 0.21 |

The NMDA R2b average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values $< 1$ indicate higher contralateral (nonpain) expression, and values $> 1$ indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

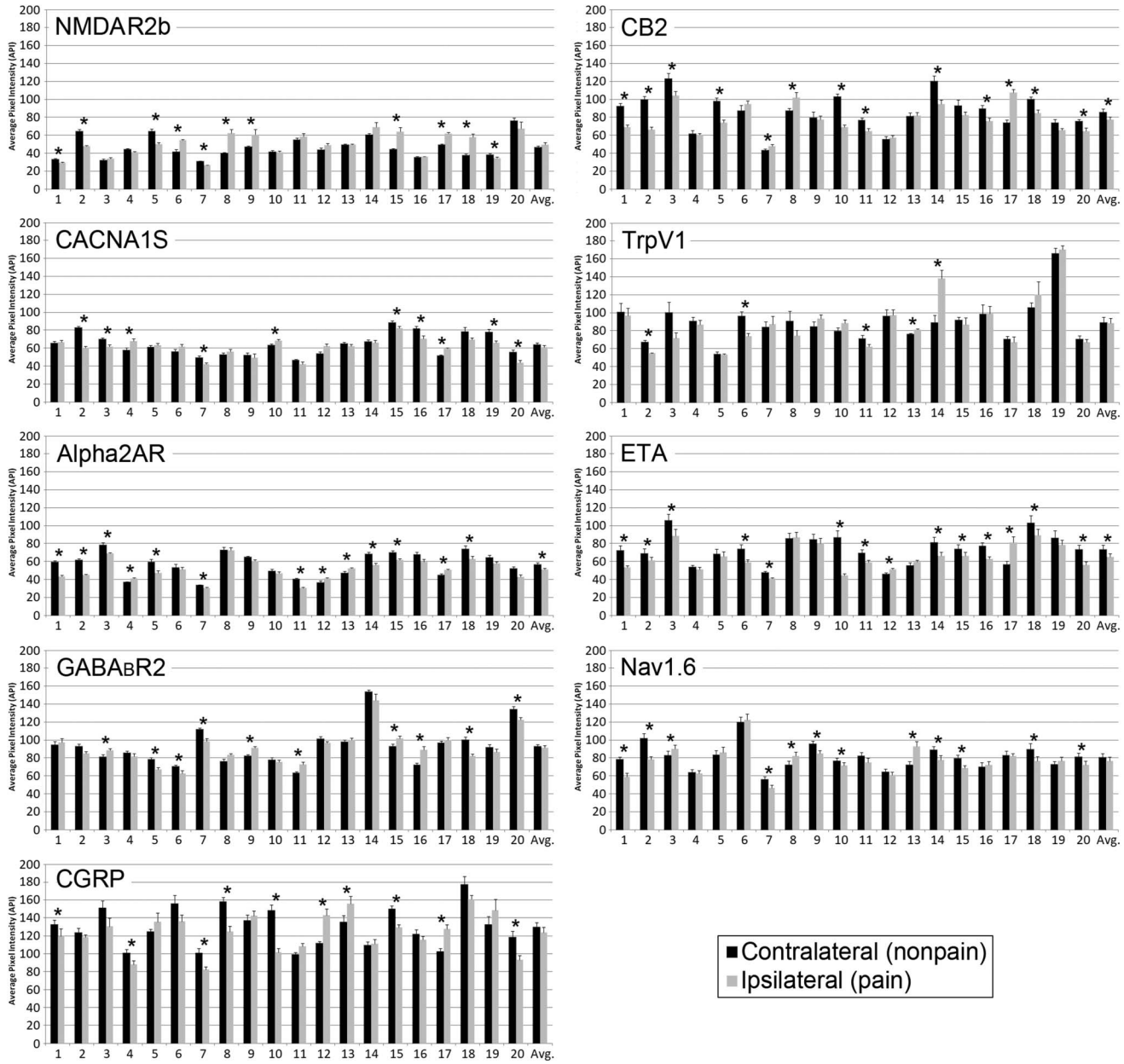


Figure 2. Keratinocyte biomarker immunolabeling average pixel intensity (API) in contralateral (black) and ipsilateral (grey) biopsies from the total PHN patient cohort. Asterisks indicate a significant ($P \leq 0.05$) within-patient difference between the contralateral (nonpain) and ipsilateral (pain) biopsy labeling. PHN, postherpetic neuralgia.

higher ipsilateral labeling in 3/20 patients (**Table 7**, red). The group average API ratio (0.92) and the % difference of ipsilateral from contralateral (−7.8) demonstrate significantly lower ipsilateral biopsy labeling (**Table 7**, green; **Fig. 2**).

### 3.1.7. Transient receptor potential vanilloid 1

Significant ipsilateral to contralateral differences of TrpV1 keratinocyte API were observed in 5/20 PHN cohort biopsy pairs, with lower ipsilateral labeling in 3/20 patients (**Table 8**, green) and higher ipsilateral labeling in 2/20 patients (**Table 8**, red). The group average API ratio (0.99) and the % difference of ipsilateral from contralateral (−1.0) indicate equally split differences among the biopsy pairs (**Table 8**; **Fig. 2**).

### 3.1.8. Endothelin receptor type A

Significant ipsilateral to contralateral differences of ETA keratinocyte API were observed in 14/20 PHN cohort ipsilateral and contralateral biopsy labeling pairs, with lower ipsilateral labeling in 12/20 patients (**Table 9**, green) and higher ipsilateral labeling in 2/20 patients (**Table 9**, red). The group average API ratio (0.90) and the % difference of ipsilateral from contralateral (−10.0) demonstrate significantly lower ipsilateral biopsy labeling (**Table 9**, green; **Fig. 2**).

### 3.1.9. Sodium voltage-gated channel subunit 1.6

Significant ipsilateral to contralateral differences of Nav1.6 keratinocyte API were observed in 12/20 PHN cohort biopsy pairs, with lower ipsilateral labeling in 9/20 patients (**Table 10**, green) and

**Table 3**

**CACN A1S immunolabeling.**

| CACN A1S | Individual API ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 1.02 | 1.6 | 0.73 |
| 2 | 0.73 | −26.9 | 0.00 |
| 3 | 0.88 | −12.1 | 0.00 |
| 4 | 1.17 | 17.4 | 0.04 |
| 5 | 1.04 | 3.6 | 0.52 |
| 6 | 1.10 | 9.9 | 0.20 |
| 7 | 0.85 | −14.7 | 0.02 |
| 8 | 1.06 | 6.1 | 0.36 |
| 9 | 0.95 | −5.0 | 0.53 |
| 10 | 1.08 | 8.1 | 0.02 |
| 11 | 0.91 | −8.9 | 0.24 |
| 12 | 1.16 | 16.0 | 0.08 |
| 13 | 0.96 | −4.0 | 0.37 |
| 14 | 0.98 | −2.2 | 0.65 |
| 15 | 0.93 | −7.0 | 0.03 |
| 16 | 0.87 | −13.5 | 0.05 |
| 17 | 1.15 | 15.2 | 0.01 |
| 18 | 0.88 | −11.7 | 0.08 |
| 19 | 0.85 | −15.2 | 0.00 |
| 20 | 0.79 | −21.4 | 0.01 |
| *Group Ave.* | 0.97 | −3.2 | 0.16 |

The CACN A1S average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values <1 indicate higher contralateral (nonpain) expression, and values >1 indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

**Table 4**

**Alpha2AR immunolabeling.**

| Alpha2AR | Individual API ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 0.73 | −27.3 | 0.00 |
| 2 | 0.72 | −27.7 | 0.00 |
| 3 | 0.88 | −12.1 | 0.02 |
| 4 | 1.10 | 9.7 | 0.05 |
| 5 | 0.79 | −20.5 | 0.01 |
| 6 | 0.96 | −4.1 | 0.65 |
| 7 | 0.91 | −9.3 | 0.01 |
| 8 | 0.99 | −1.0 | 0.84 |
| 9 | 0.93 | −6.7 | 0.07 |
| 10 | 0.94 | −5.8 | 0.19 |
| 11 | 0.76 | −24.5 | 0.00 |
| 12 | 1.11 | 10.9 | 0.05 |
| 13 | 1.10 | 10.2 | 0.01 |
| 14 | 0.82 | −18.2 | 0.00 |
| 15 | 0.88 | −12.4 | 0.00 |
| 16 | 0.88 | −12.4 | 0.06 |
| 17 | 1.13 | 13.2 | 0.01 |
| 18 | 0.85 | −14.9 | 0.01 |
| 19 | 0.90 | −10.3 | 0.09 |
| 20 | 0.82 | −18.0 | 0.07 |
| *Group Ave.* | 0.91 | −9.1 | 0.00 |

The Alpha2AR average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values <1 indicate higher contralateral (nonpain) expression, and values >1 indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

**Table 5**

**$GABA_B$ R2 immunolabeling.**

| $GABA_B$ R2 | Individual API Ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 1.02 | 2.4 | 0.62 |
| 2 | 0.91 | −8.7 | 0.06 |
| 3 | 1.09 | 8.7 | 0.01 |
| 4 | 0.96 | −4.2 | 0.25 |
| 5 | 0.86 | −14.0 | 0.00 |
| 6 | 0.89 | −10.6 | 0.04 |
| 7 | 0.88 | −11.6 | 0.01 |
| 8 | 1.09 | 9.0 | 0.06 |
| 9 | 1.11 | 11.0 | 0.00 |
| 10 | 0.97 | −2.6 | 0.46 |
| 11 | 1.15 | 15.0 | 0.02 |
| 12 | 0.95 | −4.6 | 0.11 |
| 13 | 1.02 | 2.5 | 0.39 |
| 14 | 0.94 | −6.1 | 0.22 |
| 15 | 1.09 | 9.1 | 0.00 |
| 16 | 1.23 | 23.2 | 0.00 |
| 17 | 1.04 | 3.8 | 0.23 |
| 18 | 0.82 | −18.3 | 0.00 |
| 19 | 0.94 | −5.6 | 0.29 |
| 20 | 0.91 | −8.9 | 0.00 |
| Group Ave. | 0.99 | −0.5 | 0.50 |

The $GABA_B$ R2 average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values <1 indicate higher contralateral (nonpain) expression, and values >1 indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

**Table 6**

**Calcitonin gene-related peptide immunolabeling.**

| CGRP | Individual API ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 0.90 | −9.9 | 0.05 |
| 2 | 0.96 | −4.3 | 0.43 |
| 3 | 0.86 | −13.9 | 0.14 |
| 4 | 0.87 | −13.3 | 0.02 |
| 5 | 1.08 | 8.4 | 0.25 |
| 6 | 0.87 | −12.7 | 0.16 |
| 7 | 0.81 | −18.9 | 0.01 |
| 8 | 0.79 | −21.0 | 0.01 |
| 9 | 1.04 | 3.7 | 0.63 |
| 10 | 0.68 | −31.6 | 0.00 |
| 11 | 1.09 | 8.9 | 0.08 |
| 12 | 1.28 | 27.7 | 0.00 |
| 13 | 1.15 | 14.7 | 0.03 |
| 14 | 1.02 | 1.6 | 0.74 |
| 15 | 0.86 | −13.9 | 0.00 |
| 16 | 0.95 | −4.8 | 0.28 |
| 17 | 1.24 | 24.2 | 0.00 |
| 18 | 0.91 | −9.1 | 0.13 |
| 19 | 1.12 | 12.1 | 0.44 |
| 20 | 0.78 | −21.8 | 0.02 |
| *Group Ave.* | 0.96 | −3.7 | 0.20 |

The CGRP average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values <1 indicate higher contralateral (nonpain) expression, and values >1 indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

CGRP, calcitonin gene-related peptide.

**Table 7**

**CB2 immunolabeling.**

| CB2 | Individual API ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 0.75 | −25.5 | 0.00 |
| 2 | 0.66 | −33.6 | 0.00 |
| 3 | 0.85 | −15.1 | 0.01 |
| 4 | 0.98 | −1.8 | 0.80 |
| 5 | 0.75 | −24.7 | 0.00 |
| 6 | 1.09 | 8.8 | 0.29 |
| 7 | 1.12 | 11.7 | 0.04 |
| 8 | 1.17 | 16.7 | 0.03 |
| 9 | 0.98 | −2.4 | 0.75 |
| 10 | 0.67 | −32.8 | 0.000 |
| 11 | 0.84 | −15.5 | 0.02 |
| 12 | 1.02 | 2.4 | 0.74 |
| 13 | 1.01 | 0.8 | 0.84 |
| 14 | 0.79 | −21.4 | 0.00 |
| 15 | 0.88 | −11.9 | 0.11 |
| 16 | 0.85 | −15.4 | 0.03 |
| 17 | 1.45 | 45.0 | 0.00 |
| 18 | 0.85 | −15.5 | 0.00 |
| 19 | 0.88 | −11.6 | 0.07 |
| 20 | 0.85 | −14.8 | 0.04 |
| *Group Ave.* | 0.92 | −7.8 | 0.03 |

The CB2 average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values <1 indicate higher contralateral (nonpain) expression, and values >1 indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

**Table 8**

**Transient receptor potential vanilloid 1 immunolabeling.**

| TrpV1 | Individual API ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 0.96 | −4.2 | 0.73 |
| 2 | 0.81 | −18.6 | 0.00 |
| 3 | 0.71 | −28.7 | 0.08 |
| 4 | 0.96 | −4.4 | 0.63 |
| 5 | 0.98 | −1.6 | 0.76 |
| 6 | 0.77 | −23.3 | 0.01 |
| 7 | 1.04 | 4.3 | 0.50 |
| 8 | 0.82 | −17.8 | 0.06 |
| 9 | 1.10 | 10.3 | 0.33 |
| 10 | 1.12 | 11.7 | 0.08 |
| 11 | 0.88 | −12.0 | 0.02 |
| 12 | 1.01 | 1.3 | 0.64 |
| 13 | 1.06 | 6.4 | 0.00 |
| 14 | 1.55 | 54.9 | 0.00 |
| 15 | 0.94 | −5.5 | 0.45 |
| 16 | 1.01 | 0.5 | 0.97 |
| 17 | 0.95 | −4.9 | 0.67 |
| 18 | 1.14 | 13.8 | 0.41 |
| 19 | 1.03 | 2.6 | 0.55 |
| 20 | 0.95 | −4.6 | 0.62 |
| *Group Ave.* | 0.99 | −1.0 | 0.86 |

The TrpV1 average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values <1 indicate higher contralateral (nonpain) expression, and values >1 indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

TrpV1, transient receptor potential vanilloid 1.

**Table 9**

**Endothelin receptor type A immunolabeling.**

| ETA | Individual API Ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 0.74 | −26.5 | 0.00 |
| 2 | 0.88 | −11.8 | 0.04 |
| 3 | 0.84 | −16.4 | 0.01 |
| 4 | 0.95 | −4.8 | 0.22 |
| 5 | 0.96 | −4.5 | 0.51 |
| 6 | 0.80 | −20.2 | 0.05 |
| 7 | 0.86 | −14.1 | 0.00 |
| 8 | 1.01 | 1.4 | 0.81 |
| 9 | 0.95 | −4.9 | 0.46 |
| 10 | 0.51 | −49.0 | 0.00 |
| 11 | 0.85 | −15.4 | 0.00 |
| 12 | 1.11 | 11.4 | 0.00 |
| 13 | 1.06 | 5.5 | 0.17 |
| 14 | 0.81 | −18.5 | 0.00 |
| 15 | 0.90 | −10.3 | 0.00 |
| 16 | 0.81 | −18.7 | 0.00 |
| 17 | 1.43 | 42.9 | 0.00 |
| 18 | 0.87 | −13.3 | 0.00 |
| 19 | 0.91 | −8.7 | 0.18 |
| 20 | 0.76 | −23.5 | 0.00 |
| *Group Ave.* | 0.90 | −10.0 | 0.01 |

The endothelin receptor type A (ETA) average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values <1 indicate higher contralateral (nonpain) expression, and values >1 indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

**Table 10**

**Nav1.6 immunolabeling.**

| Nav1.6 | Individual API Ratio | API % difference: pain from nonpain | Within Pt. API Ipsi (n = 15) vs contra (n = 15) |
|---|---|---|---|
| Patient ID | (Ipsi/Contra) | (I-C)/C × 100 | Paired *t*-test |
| 1 | 0.75 | −24.6 | 0.00 |
| 2 | 0.77 | −23.2 | 0.00 |
| 3 | 1.09 | 8.8 | 0.04 |
| 4 | 0.98 | −1.9 | 0.71 |
| 5 | 1.03 | 3.3 | 0.72 |
| 6 | 1.03 | 2.5 | 0.60 |
| 7 | 0.83 | −17.4 | 0.00 |
| 8 | 1.14 | 13.7 | 0.02 |
| 9 | 0.89 | −11.1 | 0.01 |
| 10 | 0.94 | −6.4 | 0.05 |
| 11 | 0.91 | −8.7 | 0.09 |
| 12 | 0.95 | −5.4 | 0.32 |
| 13 | 1.29 | 28.9 | 0.00 |
| 14 | 0.87 | −12.5 | 0.01 |
| 15 | 0.87 | −13.0 | 0.00 |
| 16 | 1.03 | 3.1 | 0.58 |
| 17 | 0.99 | −1.0 | 0.85 |
| 18 | 0.85 | −14.5 | 0.00 |
| 19 | 1.05 | 4.9 | 0.31 |
| 20 | 0.88 | −11.6 | 0.05 |
| *Group Ave.* | 0.96 | −4.3 | 0.12 |

The Nav1.6 average pixel intensity (API) individual patient ratios [ipsilateral (pain)/contralateral (nonpain)] and the within-patient API percent difference of ipsilateral (pain) from contralateral (nonpain) biopsy biomarker immunolabeling are shown with paired Student *t*test results. Ratio values of 1 indicate no difference, values <1 indicate higher contralateral (nonpain) expression, and values >1 indicate higher ipsilateral (pain) expression. Positive % difference values correspond to higher ipsilateral expression. Patients with significantly ($P \leq 0.05$) different expression between ipsilateral (pain) and contralateral (nonpain) biopsies are color-coded: higher contralateral (nonpain; green); higher ipsilateral (pain; red).

higher ipsilateral labeling in 3/20 patients (**Table 10**, red). The group average API ratio (0.96) and the % difference of ipsilateral from contralateral (−4.3) indicate a slight trend toward lower ipsilateral biopsy labeling but did not test significant across the cohort (**Table 10**; **Fig. 2**).

Altogether, these data demonstrate that each of the 9 evaluated biomarkers is differentially expressed in keratinocytes across the PHN cohort skin biopsies. Of the 9 biomarkers evaluated, 3 (Alpha2AR, CB2, and ETA) tested as significantly lower on the ipsilateral biopsy compared with the contralateral biopsy across the cohort, 3 others (CACNA1S, CGRP, and Nav1.6) showed insignificant trends toward lower ipsilateral labeling, 2 others ($GABA_BR2$ and TrpV1) showed an equal degree of sided-differences, and 1 biomarker (NMDAR2b) showed an insignificant trend toward higher ipsilateral labeling. Importantly, across this innervation morphology enriched cohort of patients with PHN, significant differences were found in both directions for each biomarker. The API % difference of ipsilateral from contralateral biopsies demonstrates the extent of these differences (**Fig. 3**, significant differences shown with filled circles).

### *3.2. Differential biomarker expression across cohort individuals*

For each individual patient, a single result code was calculated based upon the individual API ratio of labeling for each of the 9 biomarkers. Ipsilateral to contralateral API labeling ratios were calculated; ratio values close to 1 (0.99–1.01) indicated no difference, whereas ratio values $< 1.01$ indicated higher contralateral labeling and ratio values $> 0.99$ indicated higher ipsilateral labeling. To create a single result code encompassing the degree of difference for each biomarker, the API labeling ratios for all 9 biomarkers were averaged for each patient. Each individual ratio result was then color-coded as higher contralateral biomarker labeling (**Fig. 4**, green), higher ipsilateral biomarker labeling (**Fig. 4**, red), or equal labeling differences (**Fig. 4**, blue). The individual results ratio codes were then used to sort the enriched PHN cohort and demonstrated 3 distinct subsets of patients. Among the enriched PHN patient cohort (n = 20), there were 14 patients with biomarker labeling always or more often significantly lower in ipsilateral biopsies (**Fig. 4**, green code), 4 patients with biomarker labeling always or most often significantly

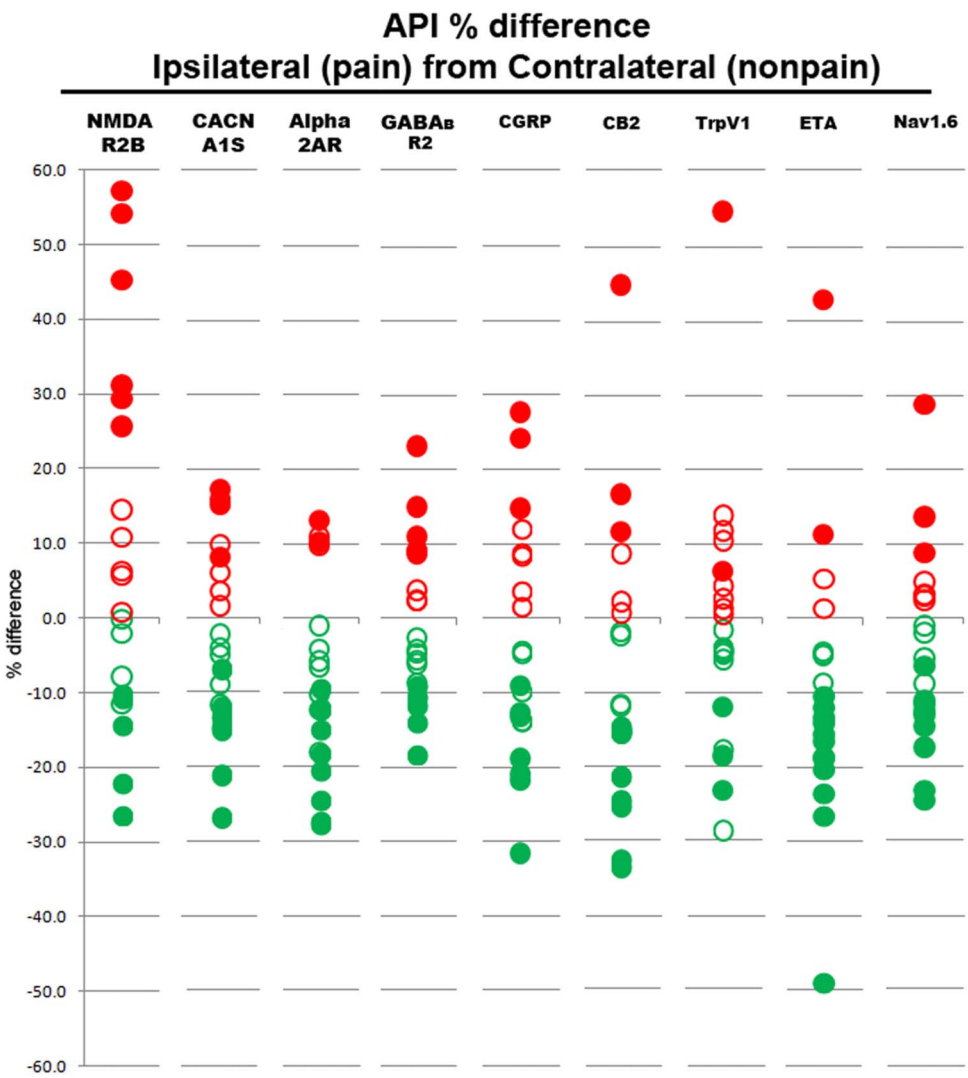


Figure 3. Keratinocyte biomarker API % difference of ipsilateral (pain) from contralateral (nonpain). Filled circles represent significant difference. The data show that for each of the biomarkers evaluated, a significant difference was seen in both directions among the total PHN cohort (n = 20). These data demonstrate the differential keratinocyte labeling for each biomarker, emphasizes the existence of patient subgroups within the enriched cohort, and highlights the potential for individualized topical treatment strategies based from skin biopsy analysis. API, average pixel intensity; PHN, postherpetic neuralgia.

| RESULT CODE SORTED | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cohort ID/MARKER: | NMDA R2B | CACN A1S | Alpha 2AR | GABAb R2 | CGRP | CB2 | TrpV1 | ETA | Nav1.6 | INDIVIDUAL SIGNIFICANCE SUMMARY | API ratios Ave. / RESULT CODE | POTENTIAL EFFECTIVE TOPICAL REGIMEN |
| 2 | | | | | | | | | | 7/9 different; 7 decreased Ipsi | 0.81 | Clonidine; THC or CBD; Capsaicin |
| 1 | | | | | | | | | | 6/9 different; 6 decreased Ipsi | 0.83 | Clonidine; THC or CBD |
| 20 | | | | | | | | | | 6/9 different; 6 decreased Ipsi | 0.86 | Baclofen; THC or CBD |
| 10 | | | | | | | | | | 5/9 different; 4 decreased Ipsi | 0.87 | Gabapentin; THC or CBD |
| 11 | | | | | | | | | | 5/9 different; 4 decreased Ipsi | 0.90 | Clonidine; THC or CBD; Capsaicin |
| 3 | | | | | | | | | | 6/9 different; 4 decreased Ipsi | 0.91 | Clonidine; THC or CBD; Lidocaine |
| 5 | | | | | | | | | | 4/9 different; 4 decreased Ipsi | 0.91 | Clonidine; Baclofen; THC or CBD |
| 7 | | | | | | | | | | 8/9 different; 7 decreased Ipsi | 0.93 | Clonidine; Baclofen; CBD |
| 16 | | | | | | | | | | 4/9 different; 3 decreased Ipsi | 0.93 | THC or CBD |
| 15 | | | | | | | | | | 7/9 different; 5 decreased Ipsi | 0.96 | Ketamine; Clondine |
| 19 | | | | | | | | | | 2/9 different; 2 decreased Ipsi | 0.97 | Not Available |
| 6 | | | | | | | | | | 4/9 different; 3 decreased Ipsi | 0.98 | Ketamine; Baclofen; Capsaicin or CBD |
| 14 | | | | | | | | | | 5/9 different; 4 decreased Ipsi | 0.98 | Clonidine; THC |
| 18 | | | | | | | | | | 6/9 different; 5 decreased Ipsi | 0.98 | Ketamine; Clondine; Baclofen; THC or CBD |
| 4 | | | | | | | | | | 3/9 different; 1 decreased Ipsi | 0.99 | Gabapentin |
| 9 | | | | | | | | | | 3/9 different; 1 decreased Ipsi | 1.01 | Ketamine |
| 8 | | | | | | | | | | 4/9 different; 1 decreased Ipsi | 1.05 | Ketamine; CBD; Lidocaine |
| 12 | | | | | | | | | | 3/9 different; 0 decreased Ipsi | 1.08 | Not Available |
| 13 | | | | | | | | | | 4/9 different; 0 decreased Ipsi | 1.09 | Lidocaine |
| 17 | | | | | | | | | | 6/9 different; 0 decreased Ipsi | 1.18 | Ketamine; Gabapentin; CBD |
| *API Group Ave.* | | | | | | | | | | 3/9 different; 3 decreased Ipsi | | |
| color codes indicate significant ($p \leq 0.05$) difference by T-test found between ipsilateral (pain) and contralateral (nonpain) biopsies (see Tables 2-10) | | | | | | | | | | | *Ratio values below 0.99 indicate higher contralateral labeling (green)* | |
| Ipsi decrease = Green | | | | | | | | | | | *Ratio values equal to 0.99 - 1.01 indicate no difference in labeling (blue)* | |
| Ipsi increase = Red | | | | | | | | | | | *Ratio values above 1.01 indicate higher ipsilateral labeling (red)* | |

Figure 4. Individual patient biomarker response codes were ordered to demonstrate the differential labeling among individual patients of the enriched PHN cohort. Based from the observed tissue biomarker expression patterns, potential individualized topical treatment regimen with appropriate effective compounds can be rationally advised. PHN, postherpetic neuralgia.

higher in ipsilateral biopsies (**Fig. 4**, red code), and 2 patients with biomarker labeling differences that were equally mixed (**Fig. 4**, blue code). Based from the biomarker labeling profiles, individual topical regimen can be rationally suggested to increase treatment efficacy.

## 4. Discussion

This human tissue research study demonstrates keratinocyte protein immunolabeling for several potential mediators of neuropathic (or potentially other) pain. The cutaneous neurosignaling biomarkers were found to be different in the painful ipsilateral vs the nonpainful contralateral biopsies from several patients, demonstrating the biomarkers as clinically useful for subcategorizing patients with PHN enriched for innervation similarities and validating them as potential drug targets for topical therapy. Importantly, the degree of differential labeling of keratinocyte biomarkers across the small fiber innervation-similar enriched cohort identifies discreet subgroups of patients with PHN. This suggests that each of these 9 distinct molecular signaling systems may contribute to PHN pathology on an individual basis. Furthermore, these data highlight the essential need for assessing individual patient biomarkers to direct personalized and targeted efficacious therapy.

The neurosignaling systems associated with each of the keratinocyte biomarkers have been shown to be involved in functional pain processing and also have specific compounds available for targeted pharmacologic manipulation. Specifically, the NMDA receptor can be inhibited by ketamine, the voltage-gated calcium ion channel CACNA1S can be inhibited by gabapentin, and the voltage-gated sodium channel Nav1.6 can be inhibited by lidocaine. The alpha2A receptor can be activated by clonidine, and $GABA_B$ receptors can be activated by baclofen. Calcitonin gene-related peptide inhibitors have been developed and recently approved for treating migraine, although a topical CGRP antagonist is not yet available. The CB2 receptor can be activated by endocannabinoids and by exogenous cannabinoids (THC and CBD), and have recently been shown to be effective in animal models of chemotherapeutic-induced peripheral neuropathy (CIPN).[10,18,32,43,45] ETA can be activated by the endogenous molecule endothelin-1 and also by specific pharmacologic compounds.[41] As well, the TrpV1 receptor can be activated by capsaicin and modulated by CBD.[36,38,72] The use of any of these compounds alone or in combination may provide relief for individual patients; however, choosing the correct active ingredients to achieve that benefit remains based mostly upon the diagnosed conditions with limited other considerations.

The current data demonstrate that not all similarly diagnosed patients will respond to the same mix of active ingredients and individual biomarker evaluations will greatly hasten the process of finding the correct combinations for each patient. As well, the effects of these compounds on keratinocyte sensory signaling mechanisms remain unclear. Keratinocyte CB2 receptor function may be indirectly regulated by exogenous cannabinoids, such as CBD and/or THC. Particularly, CBD, the major nonpsychoactive constituent of the plant *C. sativa*, has recently been incorporated in topical preparations. CBD can inhibit the fatty acid amide hydrolase (FAAH) enzyme that degrades the endocannabinoids *N*-arachidonoylethanolamine (AEA, anandamide) and 2-arachidonoylglycerol (2-AG), thereby leading to increased availability and potential activity on CB2 and also Trpv1 receptors.[16,18,32] CBD may function peripherally to normalize aberrant CB2 and/or TrpV1 expression in keratinocytes and may have positive or potentially negative effects, depending upon individual keratinocyte biomarker expressions.

Interestingly, ETA labeling was consistently lower in keratinocytes from ipsilateral biopsies among the cohort, although ETA has been shown to be a mediator of nocifensive behaviors to ET-1.[29,42,79] Keratinocytes can produce ET-1 and express ETA, and paracrine activation of ETA receptors induces keratinocyte proliferation[70]; however, the function of ETA in keratinocytes as it relates to pain mechanisms remains obscure. The decreased ETA labeling in ipsilateral biopsies may be indicative of delayed keratinocyte turnover, which potentially also contributes to chronic pain conditions.[34,57] Surprisingly, keratinocyte labeling for CACNA1S, Nav1.6, and CGRP demonstrated a trend toward lower labeling in ipsilateral biopsies, although not significant. All 3 of these biomarkers have been previously shown to be increased among keratinocytes in other chronic painful conditions and after nerve injury. Although gabapentin and lidocaine block the ion channels on nerve fibers and inhibit conduction, the potential of inhibiting these channels in keratinocyte function(s) remains

undefined. As well, CGRP has been shown to play a prominent role in neurogenic inflammation and peripheral pain mechanisms, and anti-CGRP medications have now been approved for migraine treatment. Currently, the full range of functions for CGRP receptor activation in keratinocytes remains largely unexplored; however, recent evidence suggests a role for keratinocyte CGRP in the pain associated with diabetic peripheral neuropathy (PDPN).[4,34] Mixed results were found for both $GABA_B$ and TrpV1 keratinocyte labeling, demonstrating the equally differential individual labeling patterns. Baclofen has been used topically with variable benefit,[1,39] and topical capsaicin has been used for some chronic pain conditions, particularly PHN,[14,46,51,75] and both are associated with activity directly on nerve fibers. However, differential $GABA_B$ and/or TrpV1 keratinocyte biomarker function may be associated with the variable outcomes of topical use. Yet, the actions of these active ingredients on keratinocyte function(s) remain unknown.

An examination of individual patient keratinocyte labeling reveals a differential profile of the 9 biomarkers among the enriched PHN cohort. This type of heterogeneous functional biomarker expression among the cohort likely underlies why PHN is a difficult-to-treat disease and why so many patients fail to achieve benefit from standard therapies. Compounding a personalized and targeted topical preparation would potentially be a more viable therapeutic approach. For example, one patient profile revealed significantly decreased Alpha2AR, CB2, and NMDAR2b keratinocyte labeling in the ipsilateral painful skin, suggesting that topical clonidine and THC might act to lower the algesic signals, whereas ketamine would likely not be a beneficial addition. By contrast, a different patient had significantly increased labeling of 7 of the 9 biomarkers. For that patient, targeting NMDAR with ketamine, Nav1.6 with lidocaine, GABA with baclofen, and maybe the CGRP would likely be effective; however, the use of clonidine, THC, or gabapentin might exacerbate painful symptoms. A list of potentially effective topical regimen based from the keratinocyte biomarker profiles of each patient is presented (**Fig. 4**). Additionally, the cohort contained patients who might be representative of the treatment-resistant population, demonstrated with lowered CACNA1S and NMDAR labeling, and only small changes of the other biomarkers. This type of biomarker profile would likely limit the success of many common therapies. For this 20-patient PHN cohort with very similar clinical presentations, including pain VAS scores and ipsilateral IENF loss, using the same topical regimen for each patient would most likely be ineffective in many of them and would therefore not reach a statistically significant outcome for treatment efficacy. Perhaps, this is a fundamental issue with the data from a recent study demonstrating a limited effect of topical therapies, in so much as that study included patients with pain across a very wide and diverse population.[12] Despite the failure of compounded topical pain creams in that clinical trial, the authors agreed with the possibility that only specific pain conditions responded to the compounded cream that was administered to all patients.[12] Overall, there have not been consistent results from multiple independent clinical studies regarding the efficacy of compounded topical pain medications.[6,12,80]

The heterogeneity of pain mechanisms have also been acknowledged by other researchers. The heterogeneity may underlie the difficulties in treating neuropathic pain using standard approaches; however, compounding using a personalized approach and flexibility should be able to overcome this barrier. The value of an extemporaneous compounding approach is in its capability to deliver potentially effective active ingredients that are specific to the individual patient-verified biomarkers by a topical route that mitigates the risk of systemic adverse reactions and allows for relatively simple therapeutic adjustment based on the patient's response.

Another clinical implication brought about by this study is that biopsy profiling may provide a means to stratify patients and enhance clinical study design. Whether for clinical practice or for designing a clinical trial to evaluate a topical pain therapy, performing a skin punch biopsy and creating keratinocyte biomarker profiles before starting treatment will provide essential data and valuable guidance on regimen selection and patient stratification. However, we do not yet know how to correlate the levels of increased or decreased keratinocyte biomarker expression to the level of pain perception and a prediction of therapeutic outcomes. We also do not know whether targeting one pathway would affect the expression of other pathways, eventually affecting the choice of concurrent targeted drugs. Further studies are desired to answer these questions.

Several limitations exist with the current study, including that only two 3-mm punch biopsies are being compared in each patient with PHN, each representing a very small fractional area of the overall ipsilateral and contralateral dermatome. Previous work on PHN biopsies has demonstrated a wide variability of innervation among the affected dermatome site compared with healthy control sites. It remains unknown how this rash-site innervation variability effects keratinocyte expression of various signaling mediators. Although some differences may be due to random variations, the complete data set demonstrated a strong consistency among all the biomarkers in most of the patients (18 of 20 had result codes for ipsilateral decreases or ipsilateral increases), which indicates that the biomarker profile should provide a rationale for designing an efficacious topical treatment regimen. Another important potential limitation is that the precise time from PHN onset to skin biopsy may be variable among this cohort, and no current data exist that examine changes of keratinocyte biomarkers over the duration of PHN. These keratinocyte biomarkers may change with PHN duration. Furthermore, potential targets investigated in this study were determined based on druggability and existing knowledge of neuropathic pain pathogenesis. Without a comprehensive signaling pathway screening to compare between the contralateral side and the ipsilateral side in a larger-scale patient population, potential targets will most likely be missed.

In conclusion, this is a first-of-its-kind study to show that there are differentially altered biomarker levels among epidermal keratinocytes in the skin of patients with neuropathic pain (ie, PHN). These signaling mediators are also viable targets of common active pharmacologic ingredients used in compounded topical pain creams. This important study points to the potential to use a skin biopsy profiling method in determining a therapeutic regimen for treatment-naïve and -resistant patients. Under the guidance of biopsy profiling, a potentially effective and safe therapy may be achieved through topical delivery of compounded targeted drugs.

## Disclosures

The authors have no conflict of interest to declare.

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