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*Published in:*
British Journal of Dermatology

*DOI:*
10.1111/bjd.16217

*Publication date:*
2017

*Document Version*
Peer reviewed version

(4007-2719-0002-0000 :Orcid ID )PROFESSOR RUTH DEFRIN

Article type : Original Article



**Chronic pain in Pachyonychia Congenita: evidence for neuropathic origin**

S. Brill[1], E. Sprecher[2], F.J.D. Smith[3], N. Geva[4], H. Gruener[4], H. Nahman-Averbuch[5], R. Defrin[4]

[1]Center for Pain Medicine, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel.

[2]Department of Dermatology, Tel-Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv Israel.

[3]Pachyonychia Congenita Project, School of Life Sciences, University of Dundee, Dundee, UK.

[4]Department of Physical Therapy, Sackler Faculty of Medicine & Sagol School of Neuroscience, Tel-Aviv University, Tel-Aviv, 69978, Israel.

[5]Department of Anesthesia, Cincinnati Children's Hospital Medical Center, Ohio, USA

**Corresponding author:**

Ruth Defrin

Department of Physical Therapy

Sackler Faculty of Medicine & Sagol School of Neuroscience

Tel-Aviv University, Tel-Aviv, 69978, Israel

Email: rutidef@post.tau.ac.il

Tel: +972-3-6405431

Fax: +972-3-6409223

The authors have no conflicts of interests.

This work was supported by the Pachyonychia Congenita Project

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bjd.16217

**What's already known about this topic?**

- Chronic pain is the most prominent and debilitating complaint in Pachyonychia congenita (PC)
- The origin of the pain is not established hence also an appropriate intervention
- Histological studies found alterations in sensory innervation

**What does this study add?**

- Systemic sensory testing revealed hypoesthesia along with mechanical hyperalgesia and allodynia (pain due to a non-painful stimulus) in the painful body regions
- Causative mutation and gene harboring did not affect any of these features
- The clinical and physiological characteristics of the pain suggest a possible neuropathic origin
- Neuropathic pain medications may be beneficial for PC patients

**Abstract**

**Background:** Pachyonychia congenita (PC) is a rare autosomal dominant skin disease with chronic pain being the most prominent complaint. Histological studies showing alterations in sensory innervation along with few reports on alterations in mechanical sensitivity suggest that PC may be a form of neuropathy.

**Objective:** To systematically evaluate sensory function of PC patients vs. controls, here for the first time, in order to investigate PC pathophysiology.

**Methods:** Patients (n=62) and controls (n=45) completed the McGill and Douleur Neuropathique-4 (DN4) questionnaires. Sensory testing included: detection and pain thresholds, pathological sensations, conditioned pain modulation (CPM) and temporal summation of pain (TSP).

**Results:** A moderate-severe chronic pain in the feet, throbbing and stabbing in quality, was highly prevalent among PC patients (86%) and especially debilitating during weight bearing. In addition, the majority of patients had DN4 score $\geq$4 (62%), static allodynia (55%) and tingling (53%) in the feet. Compared to controls, PC patients exhibited thermal and mechanical hypoesthesia and

mechanical hyperalgesia in the feet. CPM was reduced among the patients, and associated with more enhanced feet mechanical hyperalgesia. The specific gene and nature of the causative mutation did not affect any of these features.

**Conclusion:** Although thermal and mechanical hypoesthesia may result from thicker skin, its presentation in painful regions along with mechanical hyperalgesia and allodynia point towards the possibility of neuropathic changes in PC. The clinical features and DN4 scores support this possibility and therefore neuropathic pain medications may be beneficial for PC patients.

**Keywords:** Pachyonychia congenita, chronic pain, diagnosis, neuropathic pain

## Introduction

Pachyonychia congenita (PC) is an autosomal dominant skin disease caused by a mutation in one of 5 genes encoding different keratins: *KRT6A* (K6a), *KRT6B* (K6b), *KRT6C* (K6c), *KRT16* (K16) or *KRT17* (K17). These keratins are expressed in keratinocytes of the nail, palmoplantar skin, mucosa, and hair, leading to clinical manifestations at these sites.[1-3] PC affects males and females equally with an estimated prevalence of 0.9 cases per million.[4]

Clinically, PC features a triad of nail dystrophy, plantar keratoderma and plantar pain presenting at birth or in early childhood. The pain is severe and debilitating, especially on weight-bearing areas and a significant limiting factor with regard to activity of daily living. Plantar pain specifically has the most profound impact on quality of life. Other common clinical manifestations include: cysts, follicular hyperkeratosis, oral leucokeratosis, hyperhidrosis and palmar keratoderma.[5,6] The pain reported by PC patients has not been formally characterized and therefore its pathophysiology remains elusive. As no specific treatment or cure is known for PC, therapy is generally directed towards symptomatic improvement.[6,7] A systematic evaluation of the chronic pain and sensory profile of patients with PC is necessary to delineate the pathophysiology of the pain and design efficient pain management.

To the best of our knowledge only two studies conducted sensory testing among patients with PC. Wallis et al.[8] reported alterations in mechanical detection and pain thresholds in the feet of PC patients yet the nature of the alteration was not reported probably due to lack of a control group. Pan et al.[9] reported a lower pressure-pain thresholds in PC-affected plantar skin of 10 patients compared to intact skin along with less sweat gland innervation and Meissner corpuscles, and more Merkel cells and blood vessels in PC-affected skin as reveled by skin biopsies. Consequently, the function of non-nociceptive nerve fibers has not been evaluated in PC and the function of nociceptive fibers was evaluated on a small sample size and requires additional testing. Furthermore, pain modulation capacity of patients with PC, has never been evaluated previously and is essential for the understanding of chronic pain pathology.

Due to the dearth of information regarding the sensory profile of PC patients and the possibility that such information can shed light on the pathophysiology PC and lead to better pain management, the aims were to evaluate, among PC patients vs. controls: 1) the somatosensory function and pain modulation capacity, and 2) the association between these factors and the chronic pain characteristics.

## Material and Methods

### *Subjects*

107 individuals participated, comprising two groups: (1) 62 individuals diagnosed as having genetically confirmed Pachyonychia Congenita (PC group; 29 males and 33 females, average age 44.8±16.4 years) and (2) 45 sex- and age-matched healthy controls (HC group; 18 males and 27 females, average age 39.2±15.5 years). PC patients with mutations in *KRT6A* or *KRT16* were recruited through the International Pachyonychia Congenita Research Registry (IPCRR). Healthy subjects were recruited among employees of the Tel-Aviv Medical Center as well as among members of the PC Project personnel.

The study was approved by the Western Institutional Review Board (WIRB 20111060). A written informed consent was obtained from all subjects, according to the Declaration of Helsinki guidelines after they received a full explanation of the study protocol and goals.

***Equipment***

*1. Thermal Stimulator*

Heat stimuli were delivered using a Peltier-based computerized thermal stimulator (TSA II, Medoc Ltd., Ramat-Ishay, Israel), with a 3 x 3 cm contact probe. The probe was attached to the testing site by means of a Velcro band. In case the testing site was too large, the examiner held the probe.

*2. Water bath*

Cold stimuli were delivered using a 4 liter, cylindrical water bath filled with ice water and maintained at a temperature of 10°C.

*3. Pressure algometer*

Pressure stimuli were delivered, using a hand-held pressure algometer (Somedic Sales AB, Algometer type II, Sweden) with a 1cm diameter contact probe that is pressed against the skin. The exertion of a constantly increasing rate of pressure is monitored on the display.

*4. Semmes-Weinstein monofilaments*

Mechanical stimuli were applied with Semmes-Weinstein Monofilaments (North Coast Medical Inc., Morgan Hill, California) comprised of 20 calibrated monofilaments, with sizes ranging between 1.65-6.65, inducing a calibrated force ranging between 0.008-300 gm, respectively.

*5. Visual analog scale (VAS)*

Chronic and experimental pain intensity was measured with a VAS consisting of a horizontal red bar (the visual side) exposed to the subject, while the side facing the experimenter displays an analogue scale with values between 0="no pain sensation" and 10="the most intense pain sensation imaginable." Subject moved the inner slider according to their pain experience.

***Sensory testing***

*1. Sensory thresholds*

Sensory detection thresholds were measured to evaluate the sensitivity of the nervous system to innocuous stimuli and to evaluate conduction in C fibers (warmth threshold), A-delta fibers (cold threshold) and A-beta fibers (touch threshold).[11] Warm and cold detection thresholds were measured with the computerized thermal stimulator .[12] Mechanical detection threshold was measured with the Semmes-Weinstein monofilaments.[13] Pain thresholds were measured in order to evaluate the sensitivity of the nervous system to noxious stimuli and to evaluate conduction in nociceptive C fibers and A-delta fibers. Heat-pain threshold (HPT) was measured with the computerized thermal stimulator .[12] Pressure pain threshold (PPT) was measured with the pressure algometer.[14] All thresholds were measured in the feet (painful regions) and forearms (painfree regions), with the method of Limits in which a series of stimuli are presented at a gradually increasing magnitude and subjects are instructed to inform of the tested sensation.

*2. Conditioned pain modulation (CPM)*

CPM is an experimental paradigm representing the spinal-bulbar-spinal inhibitory circuit termed diffuse noxious inhibitory control (DNIC), mediated by the brain stem subnucleus reticularis dorsalis.[15,16] DNIC refers to inhibition of pain by neurons outside the area from which the nociceptive input originated and it was measured as an index of pain inhibition capacity. CPM was measured by administering a noxious stimulus to one arm (the test stimulus [TS]) and evaluating its perceived

intensity alone and in the presence of another noxious stimulus applied to the contralateral hand (conditioning stimulus [CS]).[17]

*3. Temporal summation of pain (TSP)*

TSP refers to a phenomenon in which perceived pain gradually increases in response to repeated/constant, moderately painful stimuli of fixed intensity. TSP is centrally mediated by frequency-dependent increments in spinal nociceptive neurons and was measured as an index of nociceptive excitability.[18,19] Subjects immersed their hand (up to the wrist line) in a cold water bath (10°C) for 30 seconds and rated their pain at immersion, after 15 seconds and at the end of hand immersion using the VAS. The magnitude of TSP was calculated by subtracting the last from the first VAS rating.[17]

*4. Abnormal sensations*

Allodynia is pain evoked by a normally non-noxious stimulus [20] associated with hyperexcitability of the nervous system and often present in neuropathic pain patients. [21,22] Static and dynamic allodynia were measured with monofilaments .[13] Hyperpathia is the emergence of a sudden, strong painful sensation, which persists after stimulation is turned off,[20] and is also associated with hypereactivity of the pain system especially in neuropathic pain.[23] Hyperpathia was measured with the thermal stimulator .[13]

***Questionnaires***

*1. Mc'Gill pain questionnaire*

Chronic pain was quantified with the short form of the Mc'Gill pain questionnaire (MPQ) [24] and with VAS.

*2. DN4*

Chronic pain patients completed the DN4 (Douleur Neuropathique-4) questionnaire, a clinician-administered questionnaire developed and validated for the purpose of discriminating neuropathic pain from non-neuropathic pain.[25]

***Experimental design***

Testing took place in a quiet room with ambient temperature maintained constant. Subjects were examined on a single testing session that was preceded by a training session. Prior to testing, each PC patient was interviewed regarding the location of painful and pain-free body regions and the chronic pain characteristics (intensity, duration, quality, ameliorating and aggravating factors, spontaneous or evoked abnormal sensation in painful regions, etc). Patients rated chronic pain on a VAS and completed the MPQ. Then sensory testing commenced and included the evaluation of abnormal sensations and measurement of thresholds (in the pain-free volar surface of the forearm and in the painful distal third of the plantar surface of the foot), CPM and TSP (both tested in the forearms and hands only), in random order. If blisters and/or bandages prevented from testing the designated foot region, testing was done on an adjacent region (the mid third of the foot in most occasions). PPT specifically was measured in the foot due to reports of pain during weight bearing/walking. Testing of controls was performed at comparable body locations. The order of the testing with thermal and mechanical stimuli was also randomized.

***Statistical analysis***

Data were processed with IBM SPSS statistics software (version 23). Between-group (patients/controls, K6a/K16) comparisons were conducted with t-tests (age, time-durations, pain intensity, detection/pain thresholds, CPM), Man-Whitney tests (DN4) and Chi-square tests (sex, pain at rest/weight bearing, abnormal sensations). Repeated-measure analysis of variance (rANOVA) was used to analyze the time trend and group effect on TSP. Correlations between pairs of variables were calculated with Pearson's or Spearman's r. Multiple testing problems were addressed by using the False Discovery Rate procedure[26] that controls for the expected proportion of falsely rejected

hypotheses, which is the desirable control against errors originating from multiplicity. A 2-sided p-value <0.05 was considered statistically significant.

**Results**

***Characteristics of the study groups and of the chronic pain***

Table 1 describes the group and chronic pain characteristics. Age and sex distributions were similar across groups. Most of the PC patients (86%) reported suffering from chronic pain, localized mostly in the palmar and plantar regions. Pain intensity was moderate-severe ranging between 2.7±2.3 at its least, to 8.7±1.3 at its worst. The two mutation PC subtypes (K6a/K16) did not differ in chronic pain intensity. The quality of chronic pain based on the MPQ was mostly aching (87%), throbbing (83%), sharp (83%), stabbing (79%) and shooting (79%). The most frequent affective pain descriptor was tiring. Almost all the chronic pain patients (93%) reported that the pain is felt during weight bearing and only the minority reported pain during rest. Out of the 53 patients with chronic pain, 29 (55%) reported taking analgesic medications regularly and 17 (32%) reported them to efficiently reduce the pain. Figure 1 presents the abnormal sensations in the painful body regions, with allodynia, tingling and electric shocks being the most frequent.

***Sensory testing***

***1. Detection and pain thresholds***

Table 2 presents the sensory thresholds. PC patients had higher thresholds for thermal sensation than controls in both the upper and lower limbs and also a higher touch detection threshold in the lower limbs, suggesting thermal and mechanical hypoesthesia. The two groups did not differ in heat-pain threshold; however PC patients had lower pressure-pain threshold in the lower limb indicative of mechanical hyperalgesia. Importantly, there were no differences in any of the thresholds tested between the two PC gene subtypes (not shown).

***2. Pain modulation***

PC patients had a significantly lower ability to inhibit pain than controls ($p<0.01$) (Figure 2 left bars). Pain ratings decreased from 4.43±3.8 to 3.8±2 among PC patients (delta of -0.75 VAS units) and from 4.87±1.7 to 3.1±1.5 among controls (delta of -1.65). Both groups exhibited similar TSP as indicated by a significant effect of time ($F(2,164)=163.5$, $p<0.001$), but lack of group effect ($F(1,82)=3.0$, $p=0.087$) and group by time interaction ($F(2,164)=0.20$, $p=0.81$) (Figure 2 right bars). Pain ratings increased from 1.94±1.8 to 4.9±3 among PC patients (delta of 3.0 VAS units) and from 2.98±2.0 to 5.9±2.5 among controls (delta of 2.9). No differences were observed in CPM or TSP between the two PC gene subtypes.

***3. Correlations between variables***

Neither pain intensity nor DN4 score correlated significantly with any of the sensory testing (Table 3). A longer chronic pain duration was associated with less intense pain, higher thermal detection thresholds (i.e. more pronounced hypoesthesia) but higher pressure pain threshold (i.e. less pronounced hyperalgesia). Pressure pain threshold correlated negatively with CPM; patients with more enhanced mechanical hyperalgesia in the foot had less efficient CPM.

***4. DN4 questionnaire***

The average DN4 score was 4.0±2.6 (ranging from 0 to 10) (Table 1). The majority of PC patients with chronic pain (62%) had DN4 score$\geq$4 suggesting positive diagnosis for neuropathic pain. Although sensory testing was similar for patients with DN4 score above and below a score of 4, the former exhibited significantly higher rates of tingling (18/33 vs. 8/28, $p<0.05$) and numbness (12/33 vs. 3/28, $p<0.05$) and higher chronic pain intensity (MPQ 25.1±9 vs. 17.9±9, $p<0.05$) than the latter.

## Discussion

The results show that 86% of PC patients report chronic pain that was especially prominent in the feet and during weight bearing. In order to ascertain the nature and origin of the pain, the chronic pain and sensory profile were characterized and the diagnostic DN4 questionnaire was completed.

### *Chronic pain characteristics*

The majority of patients reported moderate-strong pain of aching quality, a feature more typical to pain of musculoskeletal origin.[27] Yet, at the same time, similarly frequent were the descriptors; throbbing, sharp and shooting characteristic of neuropathic pain. [22,28,29] Almost all chronic pain patients reported that the pain was felt mainly during weight bearing, a complaint not typical to neuropathic pain in which pain is usually spontaneous but may worsen during activity.[22] Some PC patients also reported that the pain is evoked or exacerbated when the affected skin is wet, a complaint that seems specific to PC.

### *Sensory profile*

PC patients exhibited increased thermal thresholds in both the feet and forearms than controls indicating reduced thermal sensitivity (hypoesthesia). The thick skin of the feet due to hyperkeratosis may be the reason for the feet thermal hypoesthesia requiring more stimulation energy in order to activate the cutaneous thermo-receptors. However, the thermal hypoesthesia in the intact forearm is more difficult to explain. Perhaps PC patients are generally hyposensitive to thermal stimuli because skin thickness is increased throughout the body. Alternatively, perhaps PC patients are less attentive than controls to external, innocuous stimuli because of constant background pain.

Despite higher thermal detection thresholds, heat-pain thresholds in the feet were similar for patients and controls. This apparent discrepancy may result from a combination of thick skin and sensitized thermal nociceptors (pain receptors) due to PC pathology. Namely, thicker skin may require higher stimulation energy to produce heat-pain among PC patients than controls however if the nociceptors are hypersensitive in PC they require lower stimulation energy to signal pain than normal. Consequently the stimulation temperatures are counterbalanced leading to an apparent "normal" heat-pain threshold among the patients. When using pressure instead of heat for testing pain threshold, a modality that is not influenced by skin thickness, decreased pain threshold (hyperalgesia) in the feet of PC patients is revealed. This local mechanical hyperalgesia is consistent with the patients' reports of pain during weight bearing and may suggest that underneath the hyperkeratosis the nociceptors are indeed sensitized. Wallis et al.[8] reported abnormal detection of touch and pressure-pain among PC patients but they did not specify whether these were increased or decreased, information that is essential for diagnosis. Mechanical hyperalgesia, as found herein, is a common sign of neuropathic pain[22,29,30] and its presence in the feet of PC patients indicates peripheral sensitization, most probably of C and A delta fiber nociceptors.[31]

The normal TSP indicates that PC patients did not present generalized hyperexcitability. Yet, the patients did have reduced CPM compared with controls indicative of dysfunctional pain inhibition capacity. Such a dysfunction is common in chronic pain patients of various etiologies,[15-16] including neuropathic pain.[12,32,33] It is not clear whether deficient CPM preceded the development of the chronic pain in PC (and thus contributed to its emergence) or resulted from it. Although the magnitude of CPM did not correlate with neither the intensity nor the duration of chronic pain it did correlate with the mechanical hyperalgesia in the feet; the more enhanced mechanical hyperalgesia the less efficient CPM. This correlation suggests that the reduced ability to inhibit pain may contribute to one of the major features of the chronic pain in PC which is pain during weight bearing.

***Pathological sensations***

Along with hyperalgesia, allodynia which was highly prevalent among the PC patients also indicate sensitization as often observed in neuropathic pain.[22,31] The presence of static allodynia in particular, which is mediated by unmyelinated C fibers[21,34] suggest that pain in PC is associated with nociceptive fiber sensitization. The PC patients exhibited several additional "positive signs" characteristic of neuropathic pain including tingling and electric-shock like sensations.[22,29] Paradoxical sensations and hyperpathia, revealed herein during thermal testing, are also reported among neuropathic patients,[12,23,31] although these features are less frequent than allodynia.

***The DN4 questionnaire***

A score of 4 is the cut-off value for the diagnosis of neuropathic pain using the DN4 questionnaire[25] as recently validated in e.g. diabetic polyneuropathy [35], back pain [36] and peripheral neuropathies.[37] About 62% of PC patients received a score $\geq$4, which is in agreement with Wallis et al.[8] who used a different diagnostic tool (painDETECT). Interestingly, sensory testing was similar among patients with DN4 above and below a score of 4; however the latter show somewhat reduced frequencies of tingling and less severe chronic pain.

***Conclusions and possible mechanisms***

Neuropathic pain develops as a result of lesions or disease affecting the peripheral or central somatosensory nervous system.[22,38] Neuropathic pain may encompass a broad spectrum of signs and symptoms and combinations of gain ("positive signs") and loss ("negative signs") of sensibility are shared across the major neuropathic pain syndromes[22,23] as also found herein. The "positive" neuropathic symptoms characteristic of PC patients included: spontaneous electric-shock like sensations and tingling, allodynia and mechanical hyperalgesia. The "negative" neuropathic signs and symptoms included thermal and mechanical hypoesthesia although spontaneous signs (e.g., numbness and weakness) were not frequent among PC patients. The aforementioned signs along

with the pain complaints and the DN4 scores all support the possibility that the chronic pain in PC is- at least partly- of neuropathic origin. This does not preclude the possibility that in some patients the pain is of nociceptive origin, or has started as nociceptive but with the progression of the diseases elements of the nervous system were affected as well.

The mechanism of chronic pain in PC is yet unclear. Pan et al.[9] have recently found that painful PC-affected skin demonstrated multiple alterations, including increased density of Merkel Cells and blood vessels and reduced numbers of Meissner corpuscles and densities of small unmyelinated nerve fibers compared to intact skin. These findings support the possibility that damage to peripheral nerve fibers may be involved in the mechanism of pain in PC. Damage to sensory nerve fibers can generate a chain of events leading to peripheral sensitization.[29] The observed hyperalgesia and allodynia in the PC-affected regions herein may well be manifestations of peripheral sensitization. In addition, changes in the function of ion channels, specifically that of transient receptor potential channels,[39] may possibly contribute to peripheral sensitization in neuropathic conditions, and in the case of PC as well owing to their expression by keratinocytes.[40,41] The events leading to peripheral sensitization often involve activation of pro-inflammatory substances and such were already introduced in association with PC pathology; keratinocytes from PC-affected skin might express higher levels of neurotrophic factors such as interleukin-18, that in turn can increase neuronal excitability.[42,43] Peripheral sensitization can, in turn, lead to spontaneous and evoked pain as well as hypersensitivity to painful stimuli as reported by PC patients.

It should be pointed out that the aforementioned positive and negative signs and symptoms are suggestive, but not pathognomonic for neuropathic pain. These signs and symptom fulfill two out of three levels of certainty in the diagnosis of neuropathic pain according to a recently revised grading system.[22] The alterations in small unmyelinated nerve fibers in PC and the location of pain in the same regions seem to fulfill the *possible neuropathic pain* criteria. The presence of both negative and positive signs in the PC-affected regions (although the former to a lesser extent than the latter)

partly fulfill the next level of certainty- *probable neuropathic pain.* The level "probable" is suggested to be sufficient to initiate treatment according to neuropathic pain guidelines. The final level of certainty- *definite neuropathic pain*- requires that an objective diagnostic test confirming the lesion/disease of the somatosensory nervous system.[22] While the sensory testing herein and skin biopsy analysis by Pan et al. may partly comply with such requirement a definite diagnosis is uncertain.

Several limitations should be considered. First, the patients were not assessed at the time of PC diagnosis and therefore the results represent both primary and secondary consequences of any peripheral pathology that may have initiated the pain. Second, some features of the PC patients suggested that they may also experience chronic pain other than neuropathic. Third, the analgesic medications taken by about half of the patients may have affected the results although sensory testing of these patients were similar to those not taking such medications. In summary, the clinical examination and the sensory testing support the possibility that the chronic pain reported in the PC-affected regions is- at least in part- of neuropathic origin. Medications aimed to reduce neuropathic pain may be useful for patients with PC. These may include, but not restricted to, tricyclic antidepressants, serotonin- noradrenaline reuptake inhibitors (SNRIs) and GABAergic agonists. [44]

**Acknowledgement**

We thank all the individuals involved in the study and to Holly Evans of Pachyonychia Congenita Project for coordinating the study.

This work was supported by the Pachyonychia Congenita Project.

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**Figure legends**

Figure 1: **Pathological sensations among the PC patients**. Static allodynia, tingling and electric shock-like sensations were highly prevalent (95% confidence intervals of 42-67%, 39-68% and 32-60%, respectively) whereas dynamic allodynia, paradoxical pain and hyperpathia were less frequent (95% confidence intervals of 11-44%, 5-37% and 2-32%, respectively). Bars denote percentages.

Figure 2: **Pain modulation among the PC patients**. The magnitude of CPM (delta VAS) was significantly lower among patients with PC compared to healthy controls (**$p<0.01$). The magnitude of TSP (delta VAS) was similar across groups. Bars denote group mean ± SEM.

**Table 1: Characteristics of the study groups and the chronic pain of PC patients**

| | PC (n=62) | Controls (n=45) | P-value |
|---|---|---|---|
| Age (mean±SD, years) | 44.8 (16.4) | 39.2 (15.5) | 0.09 |
| Sex (males/females) | 29/33 | 18/27 | 0.48 |
| Gene mutation (K6a/K16) | 29/33 | - | - |
| Duration of PC (mean±SD, years) | 42.8 (17.8) | - | - |
| Prevalence of chronic pain (yes, %) | 53 (86%) | - | - |
| Duration of chronic pain (mean±SD, years) | 33.6 (22.3) | - | - |
| Pain intensity by VAS (mean±SD, range 0-10) | 5.7 (1.6) | - | - |
| Pain intensity by MPQ (mean±SD) | 22.3 (10.3) | - | - |
| Pain during rest (yes, %)* | 9 (17%) | - | - |
| Pain during weight bearing (yes, %)* | 50 (94.3) | - | - |
| DN4 (mean±SD, range 0-10) | 4.0 (2.6) | - | - |

PC= Pachyonychia Congenita, SD= standard deviation, VAS= visual analog scale, MPQ= McGill pain questionnaire, DN4= Douleur Neuropathique- 4 questionnaire, p-value: comparison between the groups (2-tailed). *Calculation out of out of the 53 patients with chronic pain.

**Table 2: Average (±SD) of sensory and pain thresholds among the study groups**

| | PC (n=62) | Controls (n=45) | P-value |
|---|---|---|---|
| Upper limbs | | | |
| Warm detection threshold (°C) | 34.9 (0.9) | 34.5 (1.1) | **<0.05** |
| Cold detection threshold (°C) | 29.9 (1.3) | 30.7 (0.5) | **<0.001** |
| Touch detection threshold (gf) | 3.3 (0.5) | 3.2 (0.6) | 0.6 |
| Heat-pain threshold (°C) | 42.9 (3.2) | 43.5 (3.0) | 0.28 |
| Lower limb | | | |
| Warm detection threshold (°C) | 42.4 (4.5) | 39.5 (3.3) | **<0.001** |
| Cold detection threshold (°C) | 25.8 (4.3) | 29.2 (1.4) | **<0.0001** |
| Touch detection threshold (gf) | 3.6 (0.5) | 3.4 (0.4) | **<0.05** |
| Heat-pain threshold (°C) | 47.4 (2.9) | 46.5 (1.9) | 0.11 |
| Pressure-pain threshold (kPa) | 195.3 (138) | 429.6 (211) | **<0.0001** |

PC= Pachyonychia Congenita, SD= standard deviation, p-value: comparison between the groups (2-tailed), kPa=kilopascal, gf=gram force

**Table 3: Correlations coefficients within the PC group**

| | Pain duration | PC duration | Pain intensity | DN4 | Age | WDT | CDT | MDT | HPT | PPT |
|---|---|---|---|---|---|---|---|---|---|---|
| PC duration | **.65**$^{***}$ (0.39-0.81) | | | | | | | | | |
| Pain intensity | **-.44**$^{**}$ (-0.61--0.27) | -.03 | | | | | | | | |
| DN4 | -.1 | .019 | .08 | | | | | | | |
| Age | **.65**$^{***}$ (0.35-0.83) | **.98**$^{***}$ (0.84-0.97) | -.10 | .03 | | | | | | |
| WDT | **.36**$^{*}$ (0.02-0.67) | .22 | -.07 | .15 | .22 | | | | | |
| CDT | **-.49**$^{**}$ (-0.68--0.13) | .32^ | .30^ | -.00 | -.18 | **-.55**$^{***}$ (-0.69--0.29) | | | | |
| MDT | .07 | .27 | -.09 | -.19 | .17 | .21 | -.05 | | | |
| HPT | .06 | .10 | -.20 | .28 | -.00 | **.65**$^{***}$ (0.29-0.83) | **-.49**$^{**}$ -(0.92--0.13) | .35 | | |
| PPT | **.34**$^{*}$ (0.10-0.93) | .21 | -.33$^{\wedge}$ | -.07 | .25 | -.04 | -.12 | -.17 | .14 | |
| CPM | -0.1 | -.03 | .13 | .00 | .04 | .05 | .11 | .04 | -.25 | **-0.31**$^{*}$ (-0.40--0.1) |
| TSP | .204 | -.01 | -.01 | -.07 | -.05 | -.09 | -.04 | **-.46**$^{**}$ (-0.82--0.10) | -.18 | .04 |

**PC= Pachyonychia Congenita, DN4= Douleur Neuropathique-4, WDT=warm detection threshold, CDT=cold detection threshold, MDT=mechanical detection threshold, HPT=heat pain threshold, PPT=pressure pain threshold, CPM=conditioned pain modulation, TSP=temporal summation of pain. ^p=0.055-0.065, *p<0.05, **p<0.01, ***p<0.0001 (2-tailed). In parentheses are 95% confidence intervals for significant, standardized coefficients.**

Appendix 1: detailed study recruitment &v sample sizer calculations, inclusion criteria, sensory testing methods, statistical methods

**Recruitment and sample size:** PC patients with mutations in *KRT6A* or *KRT16* were recruited through the International Pachyonychia Congenita Research Registry (IPCRR). Healthy subjects were recruited among employees of the Tel-Aviv Medical Center as well as among members of the PC Project personnel. The sample size was calculated for specific power and α based on expected means and standard deviations (2-samples, 2-sided) separately for CPM and TSP and the larger sample size out of the two calculations was taken. The values were based on preliminary data and on our previous studies. For $\alpha=0.05$ and statistical power of 80% the calculation yielded a sample size of 15 subjects and for $\alpha=0.01$, 19 subjects (effect size 0.79-0.87). Considering that the PC groups included people with two different mutations that required comparison, and considering that these tests were not previously done among patients with PC, the actual sample size was about two-fold that of the calculated one.

**Inclusion criteria** for patients with PC were: 1) Age above 18 years, 2) Enrolled in the International Pachyonychia Congenita Research Registry (IPCRR) (WIRB 20040468), 3) Completion of genetic testing to confirm the diagnosis of PC [10], 4) Ability to understand and comply with the requirements of the investigators. Exclusion criteria for all subjects were: 1) Skin infections of the feet and forearms, 2) Diseases (other than PC for the patients) that may cause sensory abnormalities (e.g., diabetic peripheral neuropathy, 3) Pain complaints (other than those related to PC in the patients) that may confound the assessment (e.g., peripheral or central neuropathic pain conditions), 4) History of any unstable medical disease (e.g., cardiovascular or renal insufficiency), 5) History of significant psychiatric disease/disorder that could preclude reliable information as judged by the investigators.

**Methods for threshold measurements**: Warm and cold detection thresholds were measured with the computerized thermal stimulator. Subjects received four successive stimuli of gradually increasing or decreasing temperatures, respectively, starting from a baseline temperature of 32°C (rate of 2°C/s), with an inter-stimulus-interval of 10 sec. The subjects were asked to press a switch when a thermal sensation (either warm or cold) was first perceived; thus, defining warm detection threshold and cold detection threshold and resetting the probe temperature to baseline values. Warm and cold thresholds were calculated as the average of four successive stimuli of each thermal sensation separately [12]. Mechanical detection threshold was measured with the Semmes-Weinstein monofilaments. The examiner applied the filaments in an increasing order, starting from the thinnest one. The subjects were asked to report when touch sensation was first perceived and at that point they were asked to localize the stimulus perceived. The threshold for touch was the calibrated force of the monofilament first perceived [13]. Heat-pain threshold was measured with the computerized thermal stimulator. Subjects received four successive stimuli of gradually increasing temperatures, starting from a baseline temperature of 32°C (rate of 2°C/s), with an inter-stimulus-interval of 30 sec. The subjects were asked to press a switch when a pain sensation was first perceived; thus, defining the HPT and resetting the probe temperature to baseline values. HPT was the average reading of the four successive stimuli [12]. Pressure pain threshold (PPT) was measured with the pressure algometer using the modified method of

limits [14]. The tip of the pressure algometer probe was placed perpendicular over the skin. Gradual pressure was applied from a baseline intensity of 0 kPa at a rate of 30kpa/sec, with an inter-stimulus interval of 30 seconds. Subjects were instructed to press the switch when the first pain sensation was perceived, thus "freezing" the display with the corresponding pressure reading and recording it. PPT was the averaged reading of three successive stimuli of increasing pressure [14].

**Methods for pain modulation measurement:** For the CPM test, the TS was administered with the computerized thermal stimulator at an intensity equivalent to 2°C above the subject's individual HPT, for duration of 30 seconds, applied to the volar aspect of the forearm. The CS was immersing the contralateral hand in the cold water bath (10°C) for 40 seconds. First, the subjects gave pain ratings for the TS alone using the VAS, every 10 seconds. After 2 minutes break, the contralateral hand was immersed in the water up to the wrist level, and after 10 seconds of hand immersion, the TS was applied a second time and VAS ratings were obtained. The magnitude of CPM was calculated by subtracting the average VAS rating of the TS in the presence of the CS from the average VAS rating of the TS alone [17].

**Methods for evaluating pathological sensations:** Static allodynia was examined by a single application of a monofilament no. 4.74 perpendicular on the skin and dynamic allodynia was tested by gently dragging the same monofilament along the subjects' skin for 3 cm/1 sec. In both instances the subjects were asked to report the quality of sensation evoked by the stimulus [13]. Hyperpathia was tested in a sample of 30 PC patients with the thermal stimulator by gradually heating the skin from an adaptation temperature of $35^0$C at a rate of 2°/sec [13].

**Questionnaires:** The main component of the short form of the Mc'Gill pain questionnaire (SF-MPQ) consists of 15 descriptors divided into 11 sensory and 4 affective descriptors, which are rated on an intensity scale of 0 = none, 1 = mild, 2 = moderate or 3 = severe. Three pain scores are derived from the sum of the intensity rank values of the words chosen for sensory, affective and total descriptors. The SF-MPQ also includes the Present Pain Intensity (PPI) index of the standard MPQ and a visual analogue scale (VAS) [24]. The DN4 (Douleur Neuropathique-4) questionnaire includes a series of four questions consisting of both sensory descriptors and signs related to bedside sensory examination (total of 10 items). A score of 1 is given to each positive item and a score of 0 to each negative item. The total score is calculated as the sum of the 10 items and the cut-off value for the diagnosis of neuropathic pain is a total score of 4/10.