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Loss of Papillary Dermal Calcitonin Gene Related Peptide-Expressing Neurons Significantly Correlates with Uremic Pruritus

Journal of Investigative Dermatology (2016) **136,** 2323–2325; doi:10.1016/j.jid.2016.06.629

TO THE EDITOR

Uremic pruritus (UP) arises in the setting of advanced kidney disease. Numerous confounding metabolic abnormalities can contribute to UP. We defined UP as chronic itch of greater than 6 weeks' duration that persisted (i) despite adequate dialysis and resolution of hyperparathyroidism or any electrolyte abnormalities and (ii) in the absence of peripheral neuropathy requiring treatment or of systemic or autoimmune diseases contributing to pruritus. Chronic itch can arise from decreased activation threshold and increased basal activity of cutaneous itch-signaling nerve fibers, defined as peripheral sensitization (Dhand and Aminoff, 2013; Schmelz, 2010). Chronic itch may also arise from neuroplasticity in the central nervous system, called central sensitization, such as augmentation of afferent itch sensation after loss of spinal cord inhibition (Ikoma et al., 2006; Ross, 2011).

UP arises without skin inflammation, suggesting a role for central sensitization. Human epidermal innervation often, but not always, is reduced in small-fiber neuropathy with pain central sensitization (Joint Task Force of the EFNS and the PNS, 2010). Loss of epidermal and papillary dermal nerves has been documented, using a pan-neuronal marker, in some human pruritic skin diseases (Maddison et al., 2008; Maddison et al., 2011). To our knowledge, quantitative neuroanatomic changes in putative itch- and/or pain-sensing innervation in UP skin have not been reported to date. Human cutaneous pain- and/or itch-sensing nerves include peptidergic Aδ- and C-fibers that terminate at or near the dermal-epidermal junction and express TRPV-1, calcitonin gene related peptide (CGRP), and substance P (SP) (Timmes et al., 2013). In rodent skin, itch specificity may further be conferred by expression of natriuretic polypeptide b (NPPB) (Mishra and Hoon, 2013). We hypothesized that UP results in part from preservation of cutaneous itch nerve fiber signaling in the setting of decreased cutaneous itch-inhibiting pain fibers.

Twenty-five subjects with end-stage renal disease (ESRD) receiving hemodialysis (see Supplementary Table S1 online) provided written informed consent. This study was approved by the Boston University institutional review board and adhered to the Declaration of Helsinki (see Supplementary Materials online). In 12 uremic

Abbreviations: CGRP, calcitonin gene related peptide; ESRD, end-stage renal disease; NPPB, natriuretic polypeptide B; PD, papillary dermal; PDNL/mm, papillary dermal nerve length per mm of epidermis; SP, substance P; UP, uremic pruritus; UPS, uremic pruritus subjects; VAS, visual analog scale; VAS-week, visual analog scale score for average itch over the preceding week

Accepted manuscript published online 21 July 2016

Table 1. Quantitation of epidermal and papillary dermal innervation (median, 25–75% quantiles)

	UPS	Control	*P*-Value
IENF density, mean ± SD	13.30 ± 7.90	17.70 ± 3.80	0.10
IENL/mm in µm/mm, mean ± SD	854.10 ± 572.50	963.00 ± 294.80	0.31
PDNL/mm in µm/mm, mean ± SD	2,241.60 ± 917.14	2,952.60 ± 855.90	0.0009*
%PD-CGRP$^+$	7.20 (1.90–17.90)	28.10 (19.70–36.20)	<0.0001*
%PD-CGRP$^+$/NPPB$^-$	6.80 (1.70–16.60)	22.80 (12.50–31.40)	<0.0001*
%PD-SP$^+$	2.70 (0.80–4.70)	3.60 (2.00–5.90)	0.10
%PD-NPPB$^+$	0.80 (0–2.90)	1.80 (0.20–5.20)	0.01*
%PD-NPPB$^+$/CGRP$^-$	0.60 (0–1.20)	0.40 (0–0.90)	0.30
%PD-NPPB$^+$/CGRP$^+$	0 (0–0.80)	3.00 (0–8.20)	0.0003*

Abbreviations: CGRP, calcitonin gene related peptide; IENF, intraepidermal nerve fiber; IENL/mm, intraepidermal nerve length/mm epidermis; NPPB, natriuretic polypeptide precursor B; PD, papillary dermis; PDNL/mm, papillary dermal nerve length/mm epidermis; SP, substance P.
*$P < 0.05$.

pruritus subjects (UPS), the median (78/100) of the visual analog scale (VAS) score for average itch over the preceding week (VAS-week) was significantly greater than the 13 age- and sex-matched ESRD control subjects without UP (0/100) ($P = 0.0001$). VAS-week was selected because UP can be paroxysmal and can show day-to-day variation (Yosipovitch et al., 2001). VAS-week did not differ significantly by sex within each group (UPS, $P = 0.75$; control subjects, $P = 0.06$). UPS and ESRD control subjects did not differ significantly by race, sex, age, or duration of hemodialysis (see Supplementary Table S2 online). In UPS, the symptoms, total functioning, and emotions scores of the ItchyQoL questionnaire (frequency version) were all significantly higher ($P < 0.0001$ for all scores) versus control subjects (see Supplementary Table S3 online) and indicated quality of life reductions comparable to those from atopic dermatitis, urticaria, and idiopathic causes (Desai et al., 2008).

A biopsy sample was taken from normal-appearing and nonscratched back skin with intact sensation to pinprick and light touch that was only pruritic in UPS. Immunohistochemistry was performed on 50-µm–thick, free-floating skin sections to quantitate total and peptidergic innervation of the epidermis and papillary dermis (see Supplementary Materials and Supplementary Figures S1–S3 online). Quantitation of TRPV-1 antibody staining showed no significant difference between UPS and controls for intraepidermal nerve fiber density ($P = 0.1$) or epidermal nerve length in µm/mm of epidermis ($P = 0.31$) (Table 1). In all ESRD subjects, CGRP-, SP-, or NPPB-expressing intraepidermal nerves were virtually absent (see Supplementary Figures S1–S3), as were intraepidermal CGRP$^+$ and SP$^+$ nerves in normal human skin (Timmes et al., 2013).

Papillary dermal nerve length (PDNL)/mm was significantly reduced (~24%) in UPS ($P = 0.0009$). In ESRD control subjects, of total papillary dermal (PD)-protein gene product 9.5$^+$ nerves, CGRP-expressing nerves accounted for approximately 28% (%PD-CGRP$^+$), NPPB-expressing nerves approximate 1.8% (%PD-NPPB$^+$), and SP-expressing nerves approximately 3% (%PD-SP$^+$). This %PD-CGRP$^+$ was similar to that measured of back (~26%) and trunk (~21%) in normal human skin without pruritus or skin disease (Timmes et al., 2013) (see Supplementary Tables S4 and S5 online). %PD-CGRP$^+$ was significantly reduced by approximately 81% in UPS ($P < 0.0001$), with the absolute loss of PD-CGRP$^+$ nerves (median, = ~670 µm/mm epidermis) similar to the absolute decrease in PDNL/mm (median = ~711 µm/mm epidermis). %PD-CGRP$^+$/NPPB$^-$ accounts for greater than 80% of PD-CGRP$^+$ population and was also significantly decreased in UPS ($P < 0.0001$). %PD-NPPB$^+$ was significantly decreased ($P = 0.01$), resulting from loss of PD-NPPB$^+$/CGRP$^+$ population ($P = 0.0003$). PD-NPPB$^+$/CGRP$-$ nerves ($P = 0.3$) were preserved, as were %PD-SP$^+$ ($P = 0.1$). Table 1 and Supplementary Tables S4 and S5 report

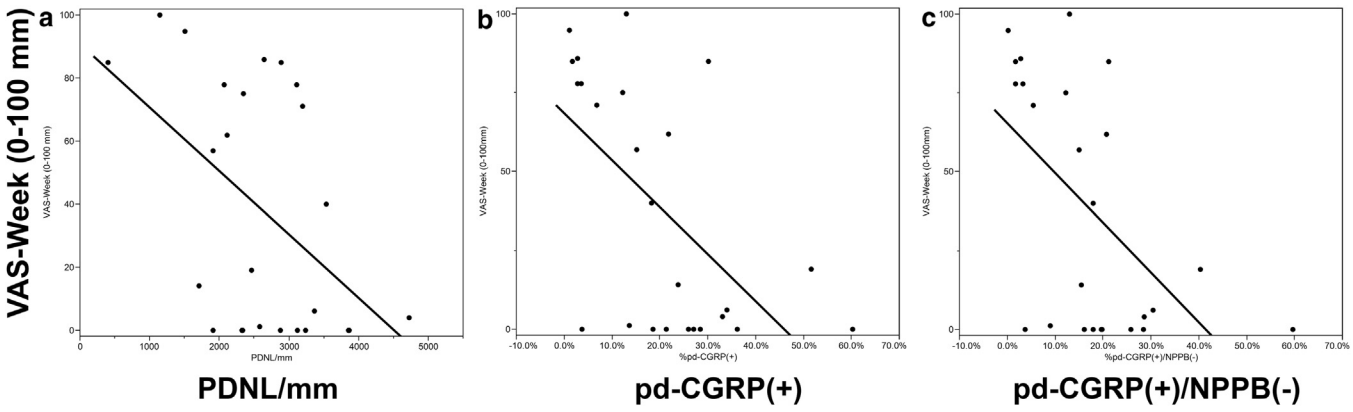

Figure 1. Negative correlations of VAS-week with papillary dermal nerve length measurements. VAS-Week was significantly negatively correlated with PDNL/mm epidermis, %pd-CGRP+, and %pd-CGRP+NPPB-. (**a**) PDNL/mm epidermis (CC = −0.49, $P = 0.006$). (**b**) %PD-CGRP$^+$ (CC = −0.59, $P = 0.0007$). (**c**) %PD-CGRP$^+$/NPPB$^-$ (CC = −0.56, $P = 0.0006$). CC, correlation coefficient; CGRP, calcitonin gene related peptide; NPPB, natriuretic polypeptide B; PD, papillary dermal; PDNL, papillary dermal nerve length; VAS-week, visual analog scale score for average itch over the preceding week.

the medians and 25–75% quartiles for these nerve measurements.

Permutation statistical analysis was performed to correlate VAS-week itch scores with nerve length measurements (see Supplementary Materials). VAS-week significantly and negatively correlated with PDNL/mm ($P = 0.006$; $P = 0.003$), %PD-CGRP$^+$ ($P = 0.0007$; $P = 0.03$) and %PD-CGRP$^+$/NPPB$^-$ ($P = 0.0006$; $P = 0.004$) in all subjects (Figure 1) or in UPS, respectively. VAS-week also significantly and negatively correlated with intraepidermal nerve fiber density ($P = 0.02$) in all subjects and with intraepidermal nerve fiber density ($P = 0.01$) and intraepidermal nerve length/mm of epidermis ($P = 0.0004$) in UPS. The %PD-SP$^+$ ($P = 0.12$; $P = 0.17$) and %PD-NPPB ($P = 0.14$; $P = 0.14$) did not significantly correlate with VAS-week in all subjects or UPS, respectively. Hemodialysis duration did not correlate with VAS-week in all subjects ($P = 0.14$) or UPS ($P = 0.22$). Supplementary Table S6 online provides detailed correlation coefficients and *P*-values.

UPS skin did not show epidermal neuropathy compared with ESRD controls or non-ESRD back skin, and sensation to light touch and pinprick was intact in all subjects, arguing against small fiber neuropathy causing pruritus in this study population (Brenaut et al., 2015). We further mitigated small fiber neuropathy by selecting back skin to avoid length-dependent neuropathy and excluding subjects with pruritus restricted to their lower legs or distal arms. Epidermal nerves are at least nociceptors, because their loss paralleled hypoalgesia to heat and pinprick pain in normal human skin (Nolano et al., 1999). Epidermal nociceptor innervation correlated negatively with UP. Pain can inhibit itch (Ross, 2011), and these data suggest preservation of epidermal itch inhibition in UPS sufficient to modulate UP severity but insufficient to prevent UP.

UPS skin showed a papillary dermal neuropathy, resulting from reduced PD-CGRP+ nerves. Further studies are required to determine if this dermal neuropathy contributes to UPS by peripheral and/or central sensitization. Human CGRP-expressing cutaneous sensory afferents are reported to mediate both pain and itch (Handwerker, 2010), although CGRP itself did not cause pain, itch, or axon reflex erythema in human microdialysis experiments (Weidner et al., 2000). Because this dermal neuropathy correlated negatively with pruritus, we postulate preferential loss from the pain-sensing PD-CGRP population. NPPB expression alone was insufficient for itch specificity, because PD-NPPB$^+$/CGRP$^+$ nerves were decreased in UPS. Preservation of SP$^+$ and NPPB$^+$/CGRP$^-$ papillary dermal nerves in UPS makes these nerve groups putative itch-sensing candidates. Our findings are limited in that they do not establish causal relationships between the described neuroanatomic changes in human skin and UP. In addition, the spinal and supraspinal effects of CGRP are likely complex. Nevertheless, these data establish scientific rationale for future studies in humans using CGRP-based therapeutic agents to offer treatments for debilitating uremic pruritus, possibly by stimulating itch-inhibitory pathways.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Thomas Ruenger for advice with experimental design. We thank Michael Kirber for assistance with image acquisition and analysis. This study was supported in part by Clinical and Translational Science Awards grant UL1-TR000157 from the National Institutes of Health.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.06.629.

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