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Palmoplantar keratoderma in *Slurp1*/*Slurp2* double-knockout mice

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Mutations in *SLURP1*, encoding a secreted protein of keratinocytes, cause a palmoplantar keratoderma (PPK) known as *mal de Meleda* [1]. When the link between *SLURP1* mutations and *mal de Meleda* was uncovered, there was speculation that SLURP1 might be a ligand for a cell-surface receptor of keratinocytes [1]. Also, because the predicted structure of SLURP1 resembled cobra neurotoxins, there was speculation that SLURP1 might influence acetylcholine signaling [1]. Pharmacologic studies have supported that concept [2–4], but there is no clear evidence that SLURP1 binds to acetylcholine receptors, nor is it clear why perturbations in acetylcholine signaling would cause PPK. We showed that *Slurp1*-deficient mice on a mixed C57/129 genetic background have PPK [5]. Interestingly, the knockout mice also exhibit hind-limb clasping [5], a phenotype that is often observed with neuropathy. Because neurological disease is not thought to be a feature of *mal de Meleda*, the hind-limb clasping phenotype in mice was perplexing. It was unclear whether it was a *bona fide* phenotype of *Slurp1* deficiency or whether it was caused by a strain 129 "passenger gene" [6] that segregated with the targeted *Slurp1* mutation [5]. Addressing that issue in a definitive fashion would require determining whether hind-limb clasping occurs in *Slurp1* knockout mice created on an inbred genetic background.

Recently, we found that inactivation of *Slurp2*, which encodes another secreted protein of keratinocytes, causes disease phenotypes that are indistinguishable from those observed in *Slurp1* knockout mice (*i.e.*, PPK, hind-limb clasping) [7]. These findings were documented in two lines of *Slurp2* knockout mice, including one with a simple nonsense mutation in exon 2 of *Slurp2* [7]. SLURP1 and SLURP2 are members of the Ly6 superfamily and are

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Conflict of interest

The authors have no conflict of interest to declare.

predicted to share a three-fingered structural motif, but amino acid sequence identity is minimal [7]. Why deficiencies in two distinct family members would produce virtually identical disease phenotypes is unclear.

We sought to define disease phenotypes in mice with a deficiency of both SLURP1 and SLURP2. One possibility was that the two keratinocyte proteins work in different pathways and that the loss of both proteins would result in extremely severe disease (*e.g.*, early-onset diffuse disease involving all skin and adnexa, perhaps even causing embryonic lethality). Another possibility is that the two proteins function in the same pathway and that the disease phenotypes associated with the loss of both proteins would be no different than the loss of SLURP1 alone.

Because *Slurp1* and *Slurp2* are located only ~14.7 kb apart on the same chromosome, it was not possible to generate double-knockout mice by breeding *Slurp1* and *Slurp2* knockout mice. We therefore used CRISPR/Cas9 genome editing [8] to create double-knockout mice (*Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$). As a control, we generated mice lacking only SLURP1 (*Slurp1*$^{-/-}$). Both lines were generated and maintained on an inbred background (FVB/NJ). *Slurp1*$^{-/-}$ mice had a premature stop codon in exon 1; *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice had a large deletion in *Slurp1* (including exon 1) and had a premature stop codon in *Slurp2* (Fig. S1). Mice were genotyped by PCR (Fig. S2). Studies were approved by UCLA's Animal Research Committee. Both *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice manifested PPK. In both lines of mice, the PPK was apparent at 8 weeks of age, and the severity of the PPK in the two lines was indistinguishable (Fig. 1). Apart from the paws, the skin and adnexa in both lines were normal. Both *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice exhibited hind-limb clasping at 8 weeks of age, coinciding with the appearance of PPK (Fig. 1).

The histopathology of the paw skin of *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice, as judged by H&E–stained sections, was indistinguishable (Fig. 2a), but the thickness of the epidermis and stratum corneum was slightly greater in *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice (Fig. 2b–c). In an earlier study, we found that *Krt16*, *Lce3a*, and *Lce3f* were expressed at higher levels in the paw skin of *Slurp1*$^{-/-}$ mice, while levels of *Krt24* and *Lce1m* expression were reduced [7]. In the current study, we confirmed those gene-expression perturbations, but we found no evidence that the changes were exaggerated in *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice. Indeed, the levels of *Krt16* and *Lce3a* expression in paw skin were slightly higher in the *Slurp1*$^{-/-}$ mice than in *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice (Fig. 2d).

Given the small but significant increase in epidermal thickness in adult *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice, it is conceivable that the PPK was slightly more severe in those mice. However, the PPK in *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice was not evident at an earlier time point, nor did these mice have more generalized involvement of the skin. Also, the gene-expression perturbations in the paw skin of *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice were not exaggerated. The fact that the disease phenotypes in *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice were quite similar implies that SLURP1 and SLURP2 likely function together or that they work sequentially within the same pathway.

Both *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice, which were created and maintained on an inbred background, developed hind-limb clasping. These studies indicate that this phenotype, typically found in the setting of neuropathy and other forms of neurological disease, is a *bona fide* phenotype of *Slurp1* deficiency. We suspect that the hind-limb clasping could be a direct consequence of PPK and impaired sensory input from the skin of the paw.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

PPK palmoplantar keratoderma

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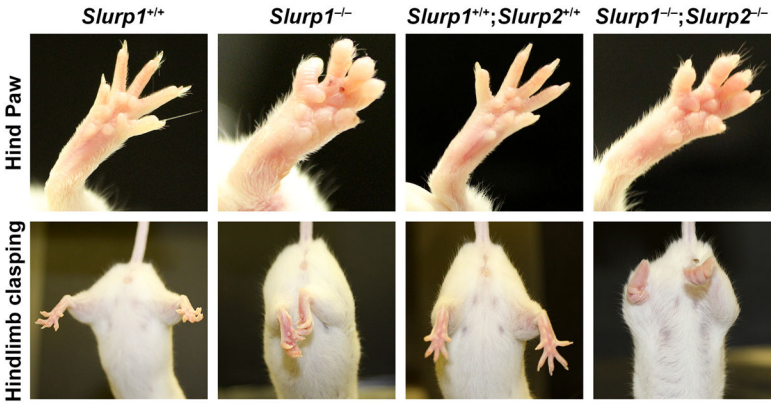

Fig. 1. PPK and hind-limb clasping phenotypes in *Slurp1*$^{+/+}$, *Slurp1*$^{-/-}$, *Slurp1*$^{+/+}$;*Slurp2*$^{+/+}$, and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice.

PPK and hind-limb clasping were invariably present in *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice. The corresponding wild-types were maintained and assessed for each knockout line (*Slurp1*$^{+/+}$ and *Slurp1*$^{+/+}$;*Slurp2*$^{+/+}$, respectively). Mice were photographed at 18 weeks of age. The severity of the PPK and hind-limb clasping phenotypes in *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice were not noticeably different.

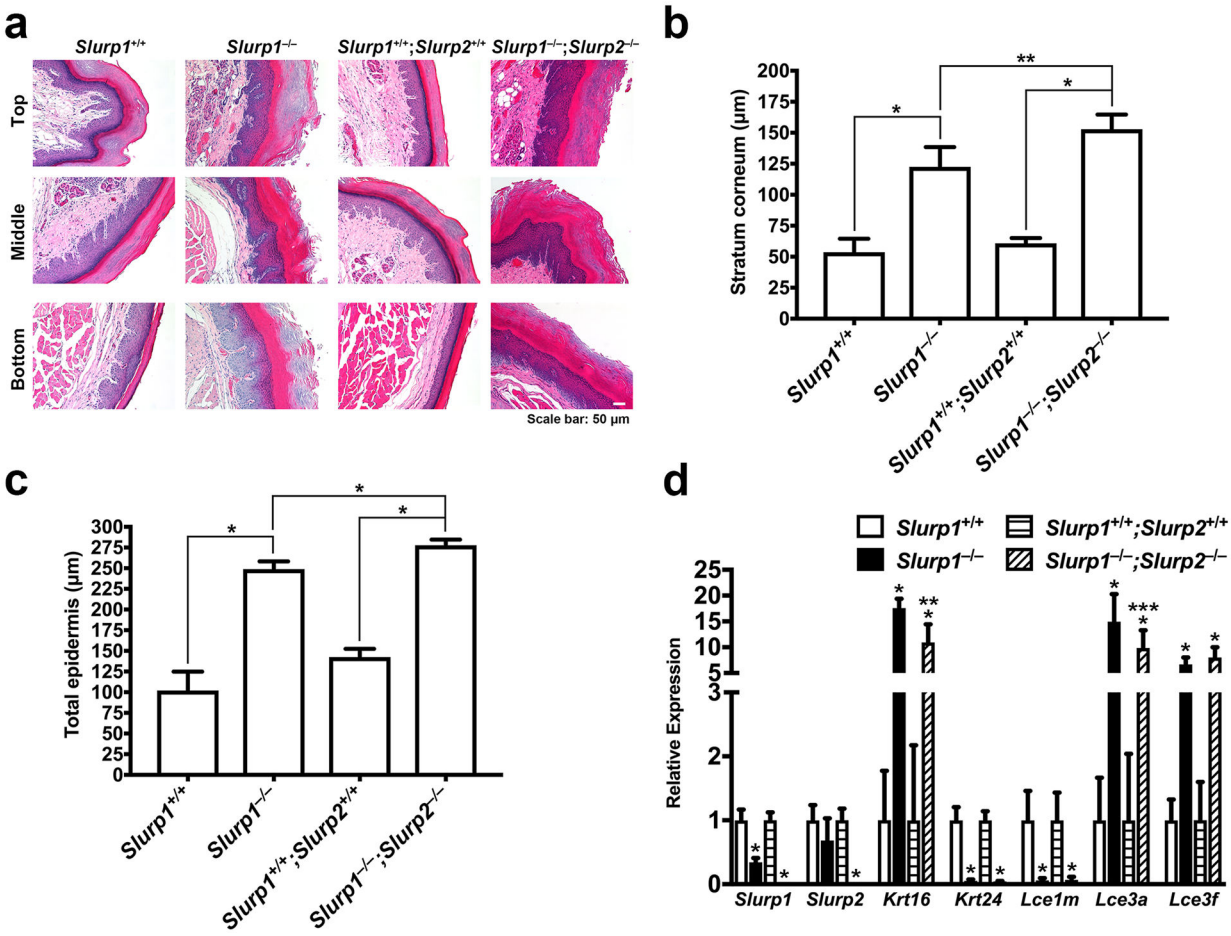

Fig. 2. Influence of *Slurp1* deficiency and combined *Slurp1*/*Slurp2* deficiency on PPK.

The epidermis was harvested from the volar surface of the hind paw and examined at three levels in the paw skin [Top (distal, closest to digits), Middle, Bottom (proximal, closest to the ankle)]. (a) Hematoxylin and eosin–stained sections; all sections were made perpendicular to the surface of the skin. Scale bar, 50 μm. (b–c) Measurements of the stratum corneum thickness (b) and epidermal thickness (c) in *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice (n = 7/group) and in littermate *Slurp1*$^{+/+}$ and *Slurp1*$^{+/+}$;*Slurp2*$^{+/+}$ mice (n = 4/group). The thickness of the epidermis and stratum corneum was measured in three successive levels separated by 50 μm each (two locations/level; six locations total) on the hind paw on each mouse, and then averaged to obtain a single value for each mouse. The stratum corneum was thicker in *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice than in wild-type mice (*Slurp1*$^{+/+}$ and *Slurp1*$^{+/+}$;*Slurp2*$^{+/+}$) (*$p < 0.0001$). A small but significant difference in stratum corneum and epidermal thickness was observed between *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice (**$p = 0.0016$ and *$p < 0.0001$). (d) Gene expression, as judged by qRT-PCR, in *Slurp1*$^{+/+}$ (n = 6), *Slurp1*$^{-/-}$ (n = 8), *Slurp1*$^{+/+}$;*Slurp2*$^{+/+}$ (n =7), *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ (n = 9) mice. Gene expression was normalized to cyclophilin A and compared to expression levels in wild-type mice (set at 1.0). The expression of five genes (*Krt16, Lce3a, Krt24, Lce1m, Lce3f*) was significantly perturbed in *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice compared to *Slurp1*$^{+/+}$ or *Slurp1*$^{+/+}$;*Slurp2*$^{+/+}$ mice (*$p < 0.0001$);

levels of *Krt16* and *Lce3a* expression were higher in *Slurp1*$^{-/-}$ mice than in *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice (**$p = 0.002$ and ***$p = 0.0322$, respectively). As expected from the nature of the mutations (see Fig. S1), the level of *Slurp1* was reduced in both *Slurp1*$^{-/-}$ and *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice. *Slurp1* transcripts were eliminated in the *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice, and the transcripts in the *Slurp1*$^{-/-}$ mice contained a nonsense mutation. *Slurp2* expression was eliminated in *Slurp1*$^{-/-}$;*Slurp2*$^{-/-}$ mice (*$p < 0.0001$). All statistical analyses were performed with an unpaired Student's *t*-test.