ORIGINAL ARTICLE

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Psoriasis-Associated Late Cornified Envelope (LCE) Proteins Have Antibacterial Activity

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Terminally differentiating epidermal keratinocytes express a large number of structural and antimicrobial proteins that are involved in the physical barrier function of the stratum corneum and provide innate cutaneous host defense. Late cornified envelope (*LCE*) genes, located in the epidermal differentiation complex on chromosome 1, encode a family of 18 proteins of unknown function, whose expression is largely restricted to epidermis. Deletion of two members, *LCE3B* and *LCE3C* (*LCE3B/C-del*), is a widely-replicated psoriasis risk factor that interacts with the major psoriasis-psoriasis risk gene *HLA-C*06*. Here we performed quantitative trait locus analysis, utilizing RNA-seq data from human skin and found that *LCE3B/C-del* was associated with a markedly increased expression of *LCE3A*, a gene directly adjacent to *LCE3B/C-del*. We confirmed these findings in a 3-dimensional skin model using primary keratinocytes from *LCE3B/C-del* genotyped donors. Functional analysis revealed that LCE3 proteins, and LCE3A in particular, have defensin-like antimicrobial activity against a variety of bacterial taxa at low micromolar concentrations. No genotype-dependent effect was observed for the inside-out or outside-in physical skin barrier function. Our findings identify an unknown biological function for LCE3 proteins and suggest a role in epidermal host defense and *LCE3B/C-del*-mediated psoriasis risk.

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INTRODUCTION

Psoriasis vulgaris is a common inflammatory skin disease determined by both genetic and environmental factors (Nestle et al., 2009). Based on a number of genome wide association studies and meta-analyses thereof, more than 60 susceptibility loci have been identified that account for 20–25% of psoriasis heritability (Tsoi et al., 2012; Tsoi et al., 2015b; Zuo et al., 2015). Psoriasis is characterized by dysregulated cutaneous immune responses involving innate immunity (tumor necrosis factor-α and NF-κB pathways) and exaggerated T helper type 1 (Th1) and T helper type 1 (Th17) lymphocyte activation (Johnston et al., 2013; Jordan et al., 2012; Lowes et al., 2014). In addition, psoriasis-associated genes expressed by keratinocytes and with a presumed role in epidermal homeostasis have also been genetically implicated in psoriasis, including the late cornified envelope (*LCE*) genes (de Cid et al., 2009; Huffmeier et al., 2010) and the beta-defensins (Hollox et al., 2008). Despite the identification of many candidate genes, functional studies that explain the contribution of genetic polymorphism to psoriasis risk are largely lacking. A number of studies, however, have shown a plausible link between genes from susceptibility loci and immunobiological features of psoriasis such as an association between variation at *TNFAIP3* and response to tumor necrosis factor-α blockade (Tejasvi et al., 2012), and the association between the *IL12B* risk allele and increased Th1-cytokine levels (Johnston et al., 2013). In addition, only very few risk loci involve coding variants that are amenable to experimental verification in animal models or in vitro cellular models of skin biology or inflammation (Jordan et al., 2012; Tsoi et al., 2012). Among the psoriasis susceptibility regions, the major histocompatibility complex class I gene *HLA-C*06:02* (PSORS1) and the LCE region harboring a deletion of the *LCE3B* and *LCE3C* genes (originally designated PSORS4) (Capon et al., 2001) provide

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Abbreviations: AMP, antimicrobial peptide; EDC, epidermal differentiation complex; LCE, late cornified envelope; LCE3B/C-del, the 32 kb deleted fragment comprising LCE3B and 3C; NN, normal skin; PN, nonlesional psoriatic skin; PP, lesional psoriatic skin; Th1, T helper type 1; Th17, T helper type 17; SC, stratum corneum; wt, wild type

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plausible candidates to investigate at the functional level. *HLA-C*06:02* is by far the strongest psoriasis risk factor, with an odds ratio estimated to be between 2.6 and 5 in Caucasians (Genetic Analysis of Psoriasis Consortium & the Wellcome Trust Case Control Consortium 2 et al., 2010; Nair et al., 2009), and the odds ratio for the *LCE* deletion (odds ratio ~ 1.3) (Huffmeier et al., 2010) is one of the highest among the remaining psoriasis-associated loci (Tsoi et al., 2012). *LCE* genes are expressed only in epidermis and oral epithelia (Bergboer et al., 2011; Jackson et al., 2005) and are assumed to encode structural proteins with a role in epithelial barrier formation; however, no functional studies supporting this contention have been published so far. Remarkably, the *LCE3* group, which encompasses the *LCE3B* and *LCE3C* genes, is under regulation of psoriasis-associated Th1 and Th17 cytokines (Bergboer et al., 2011; Niehues et al., 2016). *LCE3B/C-del* affects a 32 kb fragment in the epidermal differentiation complex (EDC) on chromosome 1, which is commonly deleted in the non-African population (allele frequency of *LCE3B/C-del*: 60–70%) (de Cid et al., 2009). In addition to the loss of the protein coding genes *LCE3B* and *LCE3C*, it also removes three intergenic fragments that harbor potential regulatory sequences (de Guzman Strong et al., 2010). In this study, we show that *LCE3B/C-del* causes an upregulation of the flanking *LCE3A* gene. Our hypothesis-driven functional studies have revealed that these three proteins are unlikely to be involved in skin barrier function and rather represent antimicrobial proteins.

RESULTS

Expression quantitative trait loci analysis of genes in the EDC

The association results between the deletion surrogate rs4112788 and expression traits are shown in Figure 1a. Within the EDC region, only the expression levels of *LCE3A*, *LCE3B*, and *LCE3C* are significantly (false discovery rate < 0.1) associated with the marker in both normal (NN) and psoriatic (PP) skin (Table 1). As expected, expression of both LCE3B and LCE3C are decreased in PP skin in the presence of the G allele of rs4112788, which is in linkage disequilibrium with *LCE3B/C del*. We observed the same result for *LCE3C* expression in NN, whereas *LCE3B* could not be assessed as it was not expressed in more than 20% NN skin samples. In fact, of the 80 NN samples, we detected expression of *LCE3A* and *LCE3C* in 43 and 40 samples, respectively, but *LCE3B* was expressed only in one sample. In contrast, of the 92 PP samples, we detected expression of *LCE3A*, *LCE3B*, and *LCE3C* in 92, 48, and 55 samples, respectively. Figure 1b shows that *LCE3A* expression levels were elevated in individuals with the GG genotype (surrogate for del/del) compared with individuals with the AA genotype, in both NN and PP samples. *LCE3A* is strongly and significantly ($P = 7.7 \times 10^{-30}$) upregulated in PP (mean expression level $= 5.8 \times 10^{3}$) compared with NN skin (mean expression level $= 20$). The fold change difference in expression between GG/AA genotypes is 2.1 in PP samples, and the contrast is even higher in NN samples (mean expression level is 35 for GG and 0 for AA). We did not observe a significant genotype difference for *LCE3A* expression in nonlesional psoriatic (PN) skin samples, likely due to the smaller sample size. RNA-seq data showed that expression of *LCE3A* behaves similarly between NN and PN skin (NN = 4.6 vs. 4.5 in PN skin). See Supplementary Table S1 online for the normalized RNAseq expression data of all individuals.

Genotype-dependent expression of LCE genes in 3-dimensional (3D) reconstructed epidermis

To substantiate these findings in vitro, and to determine *LCE3B/C-del*-dependent epidermal morphology and differentiation, we generated 3D reconstructed epidermis from wild type/wild type (wt/wt) (N = 6) and del/del (N = 6) keratinocytes. We have previously shown that in such a 3D reconstructed epidermis model, the spatiotemporal expression of LCE

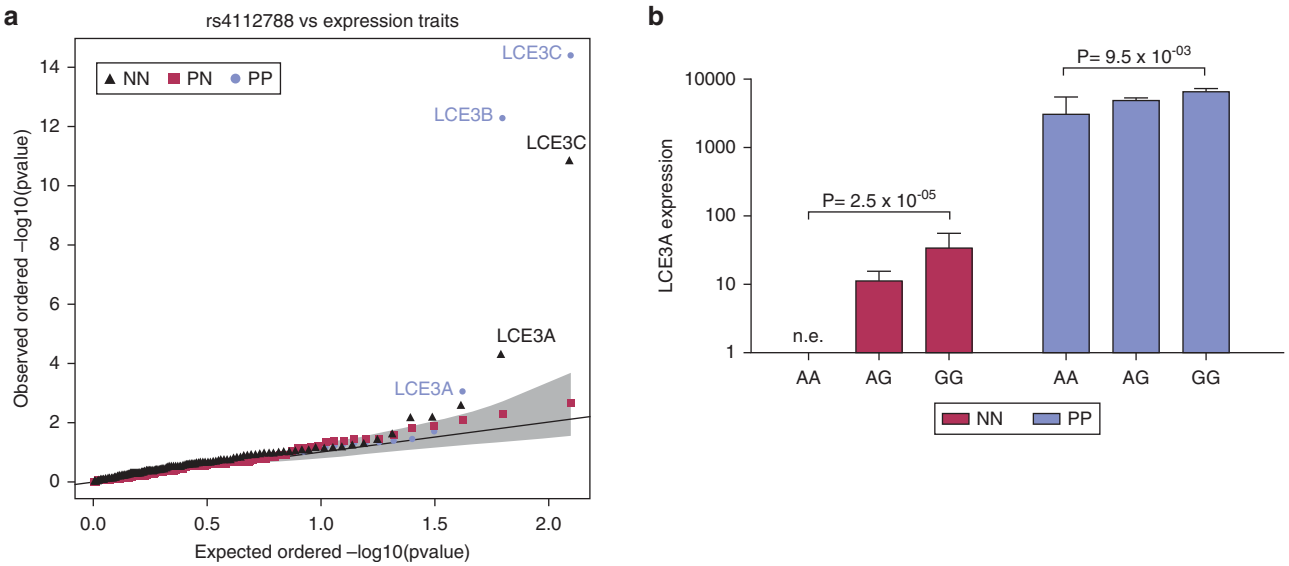

Figure 1. In vivo eQTL analysis. (**a**) Quantile-quantile plot of rs4112788 versus expression traits in the epidermal differentiation complex region. The shaded area indicates 95% confidence interval of statistical significance. (**b**) Estimated effect size of LCE3B/C-del on in vivo LCE3A expression in the eQTL data set. Error bars = standard error of the mean. eQTL, expression quantitative trait loci; LCE, late cornified envelope; n.e., no expression; NN, normal skin; PN; nonlesional psoriatic skin; PP, lesional psoriatic skin.

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