Neutral Lipid Storage Leads to Acylceramide Deficiency, Likely Contributing to the Pathogenesis of Dorfman–Chanarin Syndrome

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TO THE EDITOR

Dorfman–Chanarin syndrome (DCS) is an autosomal recessive, neutral lipid storage disorder with ichthyosis (NLSDI) due to loss-of-function mutations in *CGI-58* (a/β-hydrolase domain-containing protein 5, *ABHD5*). *CGI-58* encodes a 39 kDa protein, a widely expressed cofactor in mammalian tissues including epidermis that activates adipose triglyceride (TG) lipase (reviewed in Schweiger *et al.*, 2009; Yamaguchi and Osumi, 2009), as well as other still unidentified TG lipases (Radner *et al.*, 2009). CGI-58 expression increases during keratinocyte differentiation; and conversely, knockdown of this cofactor reduces keratinocyte differentiation (Akiyama *et al.*, 2008). Epidermal permeability barrier defects due in part to lamellar/nonlamellar phase separation of secreted lipids, within extracellular domains of the stratum corneum (SC) have been proposed to account for the barrier abnormalities in NLSDI (Elias and Williams, 1985; Demerjian *et al.*, 2006). Although defective extracellular lipid organization clearly is one contributor (Demerjian *et al.*, 2006), we assessed whether diversion of free fatty acid (FA) into esterified lipids causes lipid abnormalities that further impact barrier formation.

We recently reported a DCS patient with a, to our knowledge, previously unreported *CGI-58* missense mutation (Ujihara *et al.*, 2010), exhibiting abnormal barrier-related structures resembling other NLSDI patients (Demerjian *et al.*, 2006). Both mild and severely affected ichthyotic SCs revealed increased TG and decreased FA levels in comparison with SC fraction from normal subjects (Ujihara *et al.*, 2010). Pertinently, the extent of the increase in TG levels correlated with site-specific differences in the severity of the dermatosis (Ujihara *et al.*, 2010). These observations suggest that divergence of FA to TG contributes to disease phenotype in NLSDI.

We previously showed that ω-*O*-acylceramides (or acylCer) that have only been identified in differentiated layers of epidermis in terrestrial mammals are essential constituents of the epidermal permeability barrier; that is, lack of acylCer formation results in neonatal death due to abnormal epidermal permeability barrier function (Vasireddy *et al.*, 2007). AcylCer and the de-ω-*O*-esterified form (ω-hydroxy [ω-OH] ceramide [Cer]) are present either as free (unbound) or bound species (Uchida and Holleran, 2008). The latter form a continuous lipid monolayer, the corneocyte-bound lipid envelope (CLE); that is, a pool of ω-OH Cer, which is covalently bound to the external surface of the cornified envelope (Uchida and Holleran, 2008). Although free acylCer are critical for the formation of the lamellar membranes (Bouwstra *et al.*, 1998), our previous studies suggest the CLE is also important for normal permeability barrier function (Behne *et al.*, 2000). Prior studies suggest that FAs derived from TG are used in the ω-*O*-esterification step to form acylCer (Wertz and Downing, 1990). Moreover, TG, linoleate, and acylCer, but not phosphoglycerolipid content, decline in acyl-CoA: diacylglycerol acyltransferase-2-deficient mice, which also show a permeability barrier abnormality (Stone *et al.*, 2004). In addition, linoleate, which is the predominant FA that is used for ω-*O*-esterification, is enriched in TG in mouse skin (Stone *et al.*, 2004). Thus, we hypothesized that a failure of TG hydrolysis, due to abnormal CGI-58 function, could attenuate permeability barrier formation in NLSDI by decreasing acylCer content of affected SC.

Therefore, we first investigated the lipid profiles in solvent extracts of SC from this DCS patient (Ujihara *et al.*, 2010). Neither cholesterol (Ujihara *et al.*, 2010) nor total (bulk) Cer (Figure 1a) content was altered. Yet, Cer comprises a family of at least 10 species in humans (Uchida and Holleran, 2008). Because not only bulk Cer amount but also each individual Cer species contributes to the formation of competent lamellar structures required for barrier function (Bouwstra *et al.*, 1998), we subfractionated Cer into individual Cer species. Whereas the major Cer subfractions, NS (Cer 2), NP (Cer 3), and AS (Cer 5) were not significantly altered, acylCer were present at only trace levels in the patient sample (Figure 1b; because sample amounts were limited, other minor Cer species; that is, EOH (Cer 4), AP (Cer 6) (AP), AH (Cer 7) could not be quantitated) (abbreviations for Cer structures are according to Motta. *et al.* and Robson K. *et al.*, details reviewed in Uchida and Holleran, 2008).

Abbreviations: acylCer, acylceramide; Cer, ceramide; CGI-58, Comparative Gene Identification-58; CLE, bound lipid envelope; DCS, Dorfman–Chanarin syndrome; FA, fatty acid; KC, keratinocyte; NLSDI, neutral lipid storage disorder with ichthyosis; ω-OH, omega hydroxy; SC, stratum corneum; TG, triacylglyceride

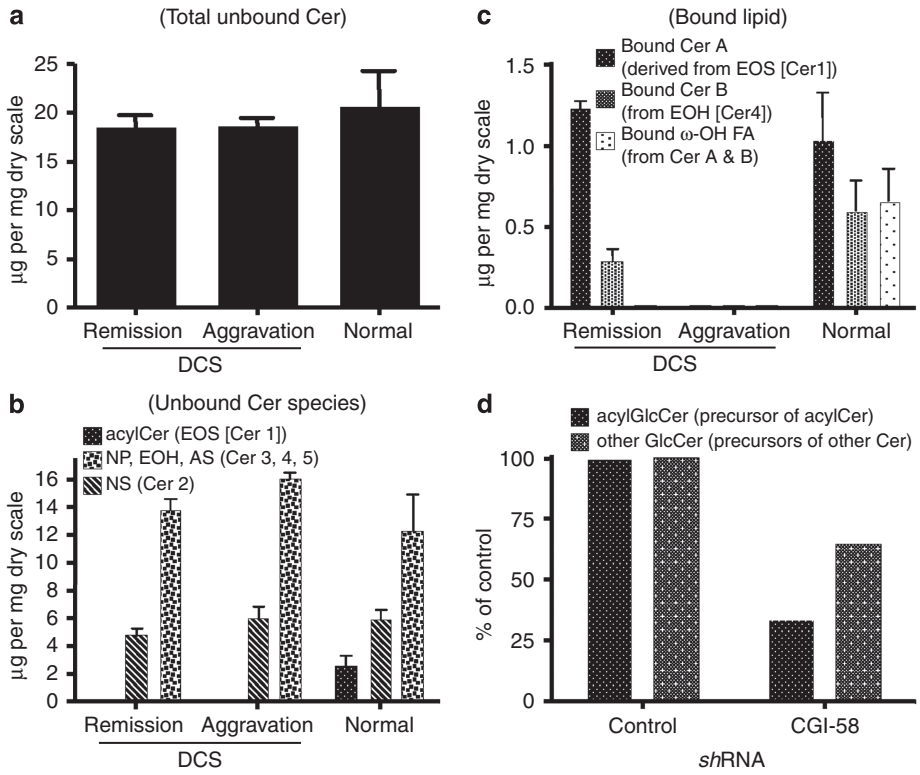

Figure 1. Lipid profile in the stratum corneum. Both unbound acylCer and bound ω-OH Cer deficiencies occur in a Dorfman–Chanarin syndrome (DCS) stratum corneum (SC) (**a–c**), whereas diminished CGI-58 expression decreases acylglucosylCer production (**d**). Normal SC from sunburn lesion as controls. CHK transfected with lentivirus-expressed shRNA (CGI-58 or control vector) were cultured in differentiation-inducing medium (Uchida *et al.*, 2001). Lipids were isolated from SC or cells and quantitated using thin-layer chromatography-scanning densitometry as described previously (Uchida *et al.*, 2001). $n=1$ (DC) and $n=3$ (normal). All studies were approved by the institutional ethics review boards (Kochi University and University of California, San Francisco) and were performed according to the Declaration of Helsinki Principles.

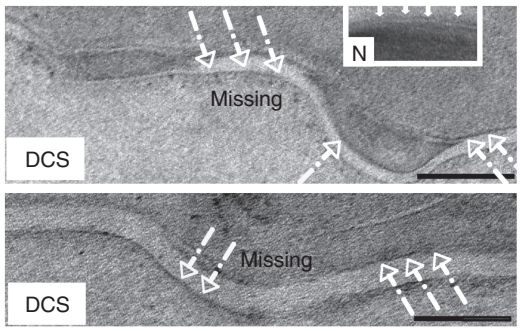

Figure 2. Electron micrographs display lack of continuous lipid monolayer, CLE, in the SC from two DCS patients (Demerjian *et al.*, 2006) vs. normal subject (inset, N). Skin samples were fixed in Karnovsky's fixative, and post-fixed with ruthenium tetroxide or osmium tetroxide, as previously described (Behne *et al.*, 2000). Ultrathin sections were examined, after further contrasted with lead citrate, with a Zeiss 10A (Carl Zeiss, Thornwood, NY) (Behne *et al.*, 2000). Arrows (with solid line, presence and with dotted line, absence) indicate CLE structures. Bars = 100 nm.

Not only bound ω-OH Cer, but also bound ω-OH FA (resulting from the subsequent hydrolysis of some bound ω-OH Cer by ceramidase) decline significantly in DCS (Figure 1c). As with TG accumulation (Ujihara *et al.*, 2010), the decrease in these bound lipids reflects disease severity. Accordingly, this patient, as well as in two additional DCS patients (Demerjian *et al.*, 2006), lacked CLE on ultrastructural analysis of affected SC (Figure 2).

Finally, we investigated whether the decreased acylCer in DCS is due to a gain-of-function of mutation in *CGI-58*, rather than a deficiency of TG-derived FA. A substantial decrease in acylglucosylCer (= acylCer precursor), but not in other glucosylCer species, was evident in lentivirus-expressed CGI-58 shRNA-treated cultured human keratinocytes (Figure 1d). Hence, by facilitating the lipolysis of TG, CGI-58 provides FA for ω-*O*-esterification leading to acylCer formation. The recent demonstration of a lethal, postnatal permeability barrier defect and deficiency of both acylCer and bound ω-OH Cer in *cgi-58*-null mice (Radner *et al.*, 2009) further supports this conclusion.

We conclude from these and previous studies that CGI-58 not only facilitates TG lipolysis but also provides FA for the ω-*O*-esterification of Cer leading to acylCer production, as well as bound ω-OH Cer generation leading to CLE formation (see Supplementary Figure S1 online). These studies highlight that the deficiency of an essential barrier constitute, acylCer, likely contributes to the permeability barrier

abnormality in DCS. Although the function of the CLE is still unclear, a role as a necessary scaffold for the lamellar bilayer organization is likely (Uchida and Holleran, 2008). Thus, CLE deficiency, coupled with disorganization of extracellular lamellar bilayers, likely merge to provoke the barrier abnormality in NLSDI (see Supplementary Figure S2 online). Finally, to overcome this metabolic disadvantage in forming the epidermal permeability barrier, epidermal proliferation likely increases, which in turn results in hyperkeratosis, phenotypic features common to virtually all of the ichthyoses (Demerjian *et al.*, 2006; Akiyama *et al.*, 2008), that is, 'A compromised permeability barrier 'drives' the hyperproliferative epidermis in NLSDI and other ichthyoses' (Elias *et al.*, 2008).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Detection of Human Papillomavirus DNA in Plucked Eyebrow Hair from HIV-Infected Patients

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TO THE EDITOR

The risk of developing human papillomavirus (HPV)-related benign and malignant cutaneous lesions is markedly increased in immunosuppressed people such as organ-transplant recipients (Harwood *et al.*, 2000) and HIV-infected patients (Grulich *et al.*, 2007; Stier and Baranoski, 2008). Although HPV DNA in plucked eyebrow hair has been well investigated (Boxman *et al.*, 1997) in renal transplant recipients and immunocompetent patients (ICPs) and correlated with both benign and malignant cutaneous lesions (Struijk *et al.*, 2003; Plasmeijer *et al.*, 2009), very little is known about HPV prevalence in eyebrow hair from HIV patients.

The study design was approved by the research ethics committee and all

Abbreviations: HPV, human papillomavirus; ICP, immunocompetent patient