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Decreased Expression of Acetylcholine Esterase in Cholinergic Urticaria with Hypohidrosis or Anhidrosis

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TO THE EDITOR

Cholinergic urticaria (CholU) is a rare condition that is clinically characterized by pinpoint-sized, highly pruritic wheals. CholU is occasionally associated with depressed sweating, as reported under the name of hypohidrosis (incomplete lack of sweating) or anhidrosis (complete lack of sweating) (CholU with hypohidrosis or anhidrosis (CUHA) (Bito *et al.*, 2012)). We have recently shown that CUHA patients develop wheals exclusively on the hypohidrotic area where the expression of cholinergic receptor M3 (CHRM3) is incompletely decreased in sweat gland epithelial cells and mast cells, whereas the patients did not exhibit wheals in the anhidrotic area where CHRM3 expression is completely absent (Sawada *et al.*, 2010).

Acetylcholine esterase (AchE) is a processing enzyme of acetylcholine, and disordered AchE might contribute to the pathogenesis of CholU (Magnus and Thompson, 1956). However, the mechanism underlying the reduction of AchE release in CholU remains unelucidated. Moreover, there have been a considerable number of reports demonstrating that T lymphocytes infiltrate around eccrine glands in CholU patients and that the systemic corticosteroid therapy improves sweating, as well as urticaria (Nakazato *et al.*, 2004). We therefore investigated the eccrine gland expression of AchE and CHRM3 and characterized skin-infiltrating lymphocytes/mast cells and attracting chemokines.

Enrolled in this study were nine CUHA patients, three CholU patients, and five healthy controls (Table 1). CUHA and CholU were diagnosed on the basis of typical episodes of small pinpoint wheals following exercise and sweating (Black *et al.*, 1996), and were confirmed by our provocation test showing the development of numerous small wheals after exercise. Sweating was evaluated with starch-iodine test. The study design was approved by the review board of University of Occupational and Environmental Health and was conducted according to the Declaration of Helsinki guidelines. Measurements in this study were performed after written informed consent had been obtained from these patients.

Skin biopsy specimens were taken from the patients and healthy control subjects. Significantly higher numbers of lymphocytes (Figure 1a) and mast cells (Figure 1b) infiltrated around eccrine glands in CUHA patients than in controls and CholU patients. The lymphocytes consisted of a mixture of $CD4^+$ and $CD8^+$ T cells and a mixture of $CXCR3^+$ (mostly Th1 cells) and $CCR4^+$ T cells (mostly Th2 cells) in all cases. The deparaffinized sections were immunohistochemically stained with anti-AchE, anti-CHRM3, anti-CXCL9/MIG, anti-CXCR10/IP-10, anti-CXCL11/I-TAC, anti-CCL2/MCP-1, anti-CCL3/MIP-1α, anti-CCL5/RANTES, anti-CCL17/TARC, and anti-CCL22/MDC antibodies. Digitalized specimens were exported to JPG files by the NDP view software (Hamamatsu Photonics, Hamamatsu, Japan), and the staining intensity was expressed as ''red density'' (RD) (Hino *et al.*, 2010; Sawada *et al.*, 2010). The expression levels of CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC were highest in CUHA and

Abbreviations: AchE, acetylcholine esterase; AD, atopic dermatitis; CHRM3, cholinergic receptor M3; CholU, cholinergic urticaria; CUHA, cholinergic urticaria with hypohidrosis or anhidrosis; RD, red density

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Table 1. Characteristics of patients with CUHA (patients 1–9) or CholU (patients 10–12)

Patient	Age	Sex	Acetylcholine test[1]	Autologous serum skin test	Autologous sweat skin test	Accompany	Pain
1	23	Male	Weakly positive	Negative	Negative	Hypohidrosis and anhidrosis	+
2	50	Male	Weakly positive	Negative	Negative	Hypohidrosis and anhidrosis	+
3	24	Male	Weakly positive	Negative	Negative	Hypohidrosis and anhidrosis	+
4	36	Male	Weakly positive	Negative	Negative	Hypohidrosis and anhidrosis	+
5	20	Male	Weakly positive	Negative	Negative	Hypohidrosis and anhidrosis	+
6	21	Male	Weakly positive	Negative	Negative	Hypohidrosis and anhidrosis	+
7	25	Male	Weakly positive	Negative	Negative	Hypohidrosis and anhidrosis	+
8	35	Male	Weakly positive	Negative	Negative	Hypohidrosis and anhidrosis	+
9	41	Female	Weakly positive	Negative	Positive	Hypohidrosis and anhidrosis, AD	+
10	29	Female	Positive	Negative	Positive	AD	−
11	15	Male	Positive	Negative	Negative	—	−
12	25	Male	Positive	Negative	Negative	—	+

Abbreviations: AD, atopic dermatitis; CholU, cholinergic urticaria; CUHA, cholinergic urticaria with hypohidrosis or anhidrosis.
[1]Acetylcholine test was performed on the hypohidrotic area in CUHA.

moderate in CholU (Figure 1c). The AchE and CHRM3 expressions were lowest in CUHA and moderately abrogated in CholU patients (Figure 1d). The expression levels in all patients were assessed by RD with statistical significance (Supplementary Figure S1 online). Real-time PCR analyses of relative mRNA levels of these cytokines were performed as described previously (Sawada *et al.*, 2010). The mRNA expressions of CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC were elevated significantly in CUHA (Figure 1e), whereas AchE and CHRM3 were decreased in CUHA (Figure 1f), supporting the immunohistochemical data. Moreover, statistical analyses revealed that the expression levels of CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC positively correlated with the number of infiltrating lymphocytes and mast cells, and AchE and CHRM3 inversely correlated with them (Supplementary Figure S2 online), suggesting that the T cells exert a down-modulatory effect on the AchE and CHRM3 expressions.

The major finding of this study is the decreased expressions of AChE and CHRM3 in eccrine gland epithelial cells of CUHA patients. In all patients, the AchE and CHRM3 expressions were decreased concomitantly in association with the lymphocytic inflammation. This suggests that acetylcholine is incompletely degraded owing to paucity of AchE. Moreover, the overproduced acetylcholine is incapable of binding to CHRM3, whose expression is reduced in eccrine glands. Acetylcholine is then considered to overflow to adjacent mast cells, which are stimulated to degranulate by acetylcholine (Takahashi *et al.*, 1992). Thus, wheals, hypohidrosis, and pain seem to result from the low expression levels of AchE and CHRM3. It has been known that lymphocytes infiltrate around eccrine glands in CUHA (Sawada *et al.*, 2010). Our study showed the inverse correlation between the number of infiltrating lymphocytes and the expression levels of AchE and CHRM3 in eccrine glands. As the expression levels of CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC were increased in the eccrine gland epithelial cells of CUHA, it is likely that T cells are attracted by these chemokines and affect AChE and CHRM3 expressions.

CholU is frequently associated with atopic conditions, especially atopic dermatitis (AD) (Takahagi *et al.*, 2008), suggesting that Th2-skewing condition might be dominant in CUHA or CholU patients. In a former review, 43% cases of acquired idiopathic generalized anhidrosis displayed elevated serum IgE levels, suggesting an allergic etiology in CUHA (Nakazato *et al.*, 2004). We compared the chemokine expressions between AD and psoriasis vulgaris. Without CUHA or CholU, AD lesional skin expressed CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC at remarkably higher levels than psoriatic lesios and healthy control skin (Supplementary Figure S3 online). Histologically, epidermal keratinocytes in the urticarial skin of CUHA did not show apoptotic, dyskeratotic cells as a result of Tc1 attack of epidermal cells, suggesting that infiltration of Tc1 is minimal in CUHA. Instead, $CD4^+$ and $CD8^+$ T-cell populations chemoattracted by CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC might affect eccrine gland epithelial cells to abrogate the expression levels of AchE and CHRM3. It appears that mast cells also infiltrate around eccrine glands by virtue of the chemokines and promote the urticarial condition. CCL2/ MCP-1, CCL5/ RANTES, and CCL17/TARC have been shown as chemoattractants for mast cells (Conti *et al.*, 1997; Tsunemi *et al.*, 2006). Corticosteroid inhibits the expressions of CCL2/MCP-1, CCL5/ RANTES, and CCL17/TARC (Pype *et al.*, 1999; Hoshino *et al.*, 2005). These findings also support the notion that CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC have a crucial role in CUHA and CholU patients. Our study suggests that T cells infiltrating around eccrine glands are a possible pathogenetic trigger for CUHA.

CONFLICT OF INTEREST

The authors state no conflict of interest.

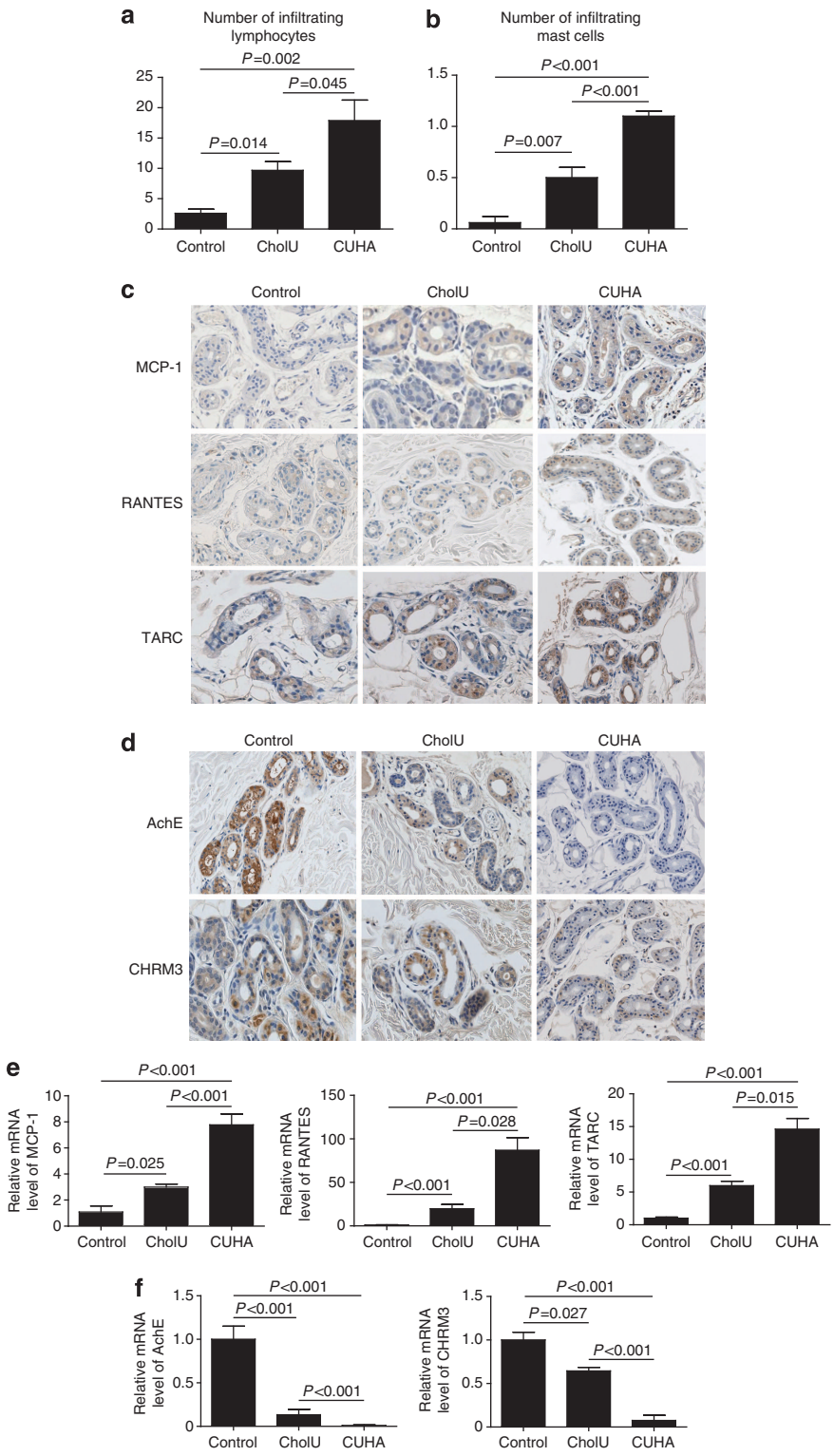

Figure 1. Histopathological and immunohistochemical examination of lymphocytes and mast cells infiltrating around sweat glands, and the expression of chemokines, acetylcholine esterase (AchE), and cholinergic receptor M3 (CHRM3) in eccrine glands. (**a**) The number of infiltrating lymphocytes around eccrine glands in high-power field (HPF; ×200). Bars = mean ± SD. (**b**) The number of mast cells around eccrine glands in HPF (×200). Bars = mean ± SD. (**c**) Representative immunohistochemical staining patterns of CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC. (**d**) Representative immunohistochemical staining patterns of AchE and CHRM3. (**e**) Relative mRNA levels of CCL2/MCP-1, CCL5/RANTES, and CCL17/TARC. (**f**) Relative mRNA levels of AchE and CHRM3. CholU, cholinergic urticaria; CUHA, CholU with hypohidrosis or anhidrosis.

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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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Mouse Alopecia Areata and Heart Disease: Know Your Mouse!

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TO THE EDITOR

The proceedings of a recent meeting on alopecia areata (AA) (Bertolini *et al.*, 2012) summarized work using the surgically induced C3H/HeJ mouse model for AA (McElwee *et al.*, 1998; Wang *et al.*, 2013), in which the investigators found enlarged hearts in affected mice, suggesting an association between AA and cardiac findings. However, the heart lesions described are a well-known strain-specific disease not limited to C3H substrains. These lesions have been described by a number of names including epicardial mineralization with fibrosis and dystrophic cardiac calcinosis (Eaton *et al.*, 1978; Frith and Ward, 1988). Crosses between C3H/HeJ and C57BL/6J mice have identified four quantitative trait loci (QTLs), designated as dystrophic cardiac calcinosis 1–4 (*Dyscalc1–4*; Ivandic *et al.*, 2001). Mapping to mouse Chromosome 7 (Ivandic *et al.*, 1996), *Dyscalc1* was subsequently identified as being due to non-synonymous single-nucleotide polymorphisms in the ATP-binding cassette, subfamily C (CFTR/MRP), member 6 (*Abcc6*) gene (Meng *et al.*, 2007; Aherrahrou *et al.*, 2008). Mutations in the human *ABCC6* gene and targeted mutations in the mouse *Abcc6* gene produce pseudoxanthoma elasticum (PXE) (Gorgels *et al.*, 2005; Klement *et al.*, 2005), a systemic metabolic disease with cutaneous features distinct from AA (Uitto *et al.*, 2010).

In a massive histopathological screening of all organ systems in 31 inbred strains of mice of both genders, dystrophic cardiac calcinosis was diagnosed in eight strains (Berndt *et al.*, in preparation; Sundberg *et al.*, 2011). C3H/HeJ and A/J strains were found to develop both heart lesions (Chase *et al.*, 2009) and AA (McElwee *et al.*, 1999) in the aging study, although in both cases more mice with normal skin had heart lesions than those with AA (Table 1a). Three strains were found to develop histologically confirmed AA (MRL/MpJ, SJL/J, and SWR/J), but none of these mice had any type of heart lesion. No correlation was found in a retired breeder study (Table 1b) (Berndt *et al.*, in preparation) or in a large mouse cross (C3H/HeJ × C57BL/6J, C3B6F2; Table 1c) generating F2 females for identifying AA eQTLs. Heart lesions varied in severity and location between the strains (Berndt *et al.*, in preparation). Genome-wide association mapping determined that none of the QTLs for dystrophic cardiac calcinosis corresponded to genomic regions identified to determine AA.

Abbreviations: AA, alopecia areata; Abcc6 (mouse gene), ABCC6 (human gene), ATP-binding cassette subfamily C, member 6, gene; Dyscalc1–4, dystrophic cardiac calcinosis 1–4; PXE, pseudoxanthoma elasticum; QTL, quantitative trait loci

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