Lipidomics Profiling of Skin Surface Lipids in Senile Pruritus

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Study design:

Senile pruritus is common, yet its etiology remains unknown. We examined the lipidomics profiles of skin surface lipids (SSL) in the elderly to better understand potential causes for senile pruritus. Firstly, we used transepidermal water loss (TEWL) to assess skin barrier function in the senile pruritus. Then we used Ameliorated Kawashima itch scale to measure the pruritus score. At last ,Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and multivariate data analysis were used to investigate SSL alternations in the senile pruritus in the compared with healthy controls.

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Abstract

Background: Senile pruritus is common, yet its etiology remains unknown. We examined the lipidomics profiles of skin surface lipids (SSL) in the elderly to better understand potential causes for senile pruritus.

Methods:Transepidermal water loss (TEWL) was used to assess skin barrier function. Ameliorated Kawashima itch scale were used to measure the pruritus score. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and multivariate data analysis were used to investigate SSL alternations.

Results:The results showed that the senile pruritus have higher TEWL values than controls (13.13±4.28 versus 6.71±2.45, $p<0.01$). LC-MS/MS showed significant differences in lipidomics and identified 81 species of SSL that differ between two groups. Compared to controls, the levels of ceramides, diacylcerols, fatty acids, phosphatidylcholines, phosphatidylethar, phytosphingsines, sphingosines, diacylceryl-3-O-carboxyhydroxymethylcholine, diacylglyceryl trimethylhomoserine, unsaturated free fatty acids increased, whereas triacylglycerol decreased. CER-EOS, CER-NDS and CER-NS were positively correlated with TEWL values ($p<0.05$). Sphingomyelin, Cer-NP, Cer-AS, Cer-NDS, Cer-NS were positively correlated with pruritus severity scores, while Cer-BS, Cer-EODS, Cer-EOS, Cer-AP were negatively correlated.

Conclusions:Our study indicated that the senile pruritus have impaired skin barrier function and altered SSL composition. Select SSL species identified in this study may be potential target for future studies on the pathogenesis of idiopathic senile pruritus.

Trial registration: Peking University International Hospital. Num:YN2018QN04, Date: 2019.1

Key words: Lipidomics; the senile pruritus; skin surface lipids

Background:

Senile Pruritus is defined as a chronic itching in elderly patients with unknown etiology, and is a diagnosis of exclusion given after all other causes of pruritus have been ruled out(1). With prolonged life expectancies throughout the world in the setting of rapid medical advancements, ageing-related skin diseases including skin pruritus are becoming increasingly relevant(2). Pruritus is the most frequently reported dermatological complaint in the elderly population(3). The severe itching in the elderly may not be self-limited, and may lead to sleep disorders and even psychiatric complications such as depression in certain cases, which significantly decrease the patient's quality of life(4). The pathophysiology of senile pruritus remains unclear, though several causes have been hypothesized, including age-related changes in the skin, neurological disorders and abnormal immunological responses(5). Hence, research into pruritus in the elderly population is urgently needed.

Skin surface lipids (SSL) are a mixture of sebaceous gland lipids and intercellular lipids located in the outer layer of epidermal cells. SSL plays an important role in maintaining the skin's barrier function and helps regulate various aspects of the integumentary system, including cell proliferation, cell apoptosis, immunity, inflammation (6). Abnormal SSL composition is involved in the pathogenesis of common skin diseases such as atopic dermatitis, acne, psoriasis and seborrheic dermatitis(7). Lipidomics is an emerging field of study that involves large-scale, comprehensive studies of the end of products of lipid metabolism (8). LC-MS/MS is a new analytical technique employed in lipidomics that utilizes liquid chromatography

coupled to mass spectrometry, and allows the identification and quantification of cellular lipid species(9).

As little is known about the SSL profiles of the senile pruritus, we conducted the current study using LC-MS/MS to compare the SSL profiles in patients with senile pruritus versus controls without pruritus in order to evaluate the role of SSL in the pathogenesis of senile pruritus.

Methods

Participants

The study was reviewed and approved by the ethics committee of Peking University International Hospital. Informed consent to participate in the study was obtained from each patient (the senile pruritus) and healthy individuals (the elderly without pruritus) before enrolling in the study. Individuals above the age of 60 years were eligible for inclusion. A total of 40 participants from Beijing area were enrolled in this study, including 20 senile pruritus patients with the duration of pruritus for more than 6 months and 20 healthy persons (controls). All patients had physician-confirmed diagnosis of senile pruritus and were able to complete this study. All patients must follow a complete examination and check-up to excluded diabetes, liver or kidney dysfunction, malignant tumor, HIV infection, thyroid disorder, anxiety or depression disorder, psoriasis, ichthyosis, scabies, eczema, bullous pemphigoid and other

dermatoses that can cause skin prutitus. All the participants didn't receive any treatments or drugs (including drugs against cardiovascular, antiepileptic, antibiotic, antipsychotic drugs and so on) that could interfere with assessment of study. All the participants did not bathe or apply topical moisturizer 24 hours before the test. The study ensured that all participants were equal for demographic characteristic of sex and age.

TEWL measurement

Transepidermal water loss (TEWL) measurement was performed at the site of 1cm below the right knee using a portable VapoMeter (TM300, CK, Germany). Measuring time is between 8 to 10 seconds. Briefly, the VapoMeter was left at standard ambient conditions in a cool air-conditioned room at temperature 23℃ and humidity 50%. The detecting probe was put on the target area and 3 consecutive readings were collected from the same site and averaged for each tested person.

Skin lipid sampling

Before sample collection, participants were instructed to stay at standard ambient conditions (room temperature for 23℃ and humidity for 50%) in a cool air-conditioned room for 30 minutes. Sebutape (CuDerm, Dallas) was used to obtain the SSL sample at the same site 1 cm below the right knee for 10 minutes. Samples were immediately stored at -80℃ prior to further analysis.

Itch intensity scales to assess the pruritus severity

Pruritus severity of each patient was evaluated by the Ameliorated Kawashima Itch Scale (supplementary table 1) (10), which rated itch severity on a five-point scale (0, 1, 2, 3, 4) separately for diurnal and nocturnal assessments. The pruritus score was calculated by adding the diurnal score to the nocturnal score (range 0~8).

LC-MS/MS analysis and identification

LC (liquid chromatography) separation was performed using Phenomenex Kinetex 1.7u EVO C18, column (2.1 X 50 mm, 100A, Agilent, USA). The column was maintained at 40℃. The injected sample volume was 3 μL for each run in the full loop injection mode. The flow rate of the mobile phase was 0.5 mL/min. In RPLC mode, gradient elution was performed with the following solvent system: (A) 50% acetonitrile (Optima^{TM} LS/MS grade, Fisher) –water and 10mM NH_4COOH, (B) IPA with 10% formic acid and 10mM NH_4COOH. The gradient started with 90% A and decreased to 0% A in 11 min, holding at 100% B for 6 min, then turned to 90% A immediately, and holding at 90% A for 3 min. Mass spectrometry was performed using Triple TOF 5600+, an orthogonal accelerated time of flight mass spectrometer (AB Sciex, USA) equipped with an electrospray ion source. Data was acquired in positive and negative-V-geometry mode for each chromatography separation technique LC-MS analysis. The capillary voltages were set to 5500V(+) and -4500 V(-), cone gas 50 L/h, desolvation gas 600 L/h, source temperature 550℃. The scan

range was from m/z 50 to 1200 in the full scan mode and data were collected in IDA mode. An independent reference Lock-mass ion via the MS-Dial (ver.3.70, April 17, 2019) was used to ensure mass accuracy during data acquisition.

The assigned modified metabolite ions were identified by database searches in the MS-Dial Lipidomics MSP databases. The chromatographic retention behavior was also considered to reduce false-positive matches.

Statistical analysis

The multivariate analysis, partial least squares discrimination analysis (PLS-DA), was constructed to determine the distributions and find the metabolic difference between two groups using the MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/MetaboAnalyst/). The PLS-DA models were cross-validated using a 10-fold method with unit variance scaling. R^2 was used to evaluate the fitting condition of the PLS-DA models, and Q^2 was used to assess the predictive ability. Negative or very low Q^2 values indicate that the differences between groups are not statistically significant. The PLS-DA model removes variation in the X matrix that is not correlated to the Y matrix. Thus, normally only one predictive component is used for the discrimination between two classes.

Comparisons of two groups related to the intensities of integrated regions were made by the two-tailed Welch's t-test, which was performed within MetaboAnalyst4.0, and *p*-values < 0.05 were considered statistically significant.

Results

Clinical data of patients and controls

Clinical data of patients and controls enrolled in the study are listed in table 1 （Table 1). All the participants were enrolled in this study from January to March in 2019. Twenty patients with senile pruritus include 9 male and 11 female, with the average of age at 69.05±6.38y.All patients complained of pruritus for more than half a year Dermatology examination showed , cutaneous xerosis , decrustation and or excoration in the lower limb. Twenty control subjects include 8 male and 12 female, with the average of age at 67.4±6.35y. Both group was equal for age (p=0.208) and sex(p=0.584). All participants were confirmed without any systemic diseases such as diabetes, liver or kidney dysfunction, malignant tumor, HIV infection, thyroid disorder, anxiety or depression disorder, psoriasis, ichthyosis, scabies, eczema, bullous pemphigoid and other dermatoses that can cause skin prutitus, or receiving any treatments that could interfere with assessment of study. All participants did not bathe or apply topical moisturizer 24 hours before the study.

Skin Barrier Function

TEWL is commonly used for assessing human skin barrier function. Increase of TEWL values usually represents damage of the skin barrier(9). In this study, senile pruritus patients had significantly higher TEWL values than controls (Table 1,13.13±4.28 versus 6.71±2.45, p<0.01). These results suggest that senile pruritus is

associated with dysfunction of the skin barrier, which is consistent with symptoms of xerosis commonly observed in the senile pruritus.

SSL Profiles

To understand if SSL changes occur in the idiopathic senile pruritus, we analyzed the SSL profile in the skin of the senile pruritus and healthy controls using LC-MS/MS technique. A partial least squares discriminant analysis (PLS-DA), which is a supervised multivariate data analysis method, was used to analyze the data from the two groups. Based on the analysis of the lipid dataset, SSL profiles in the patient group appeared to be distinct from that in the control group (Fig.1, R^2=0.9469, Q^2=0.12092). The result indicated that alteration of SSL may be related to the development of the senile pruritus.

Differences in lipids

We detected a total of 796 SSL lipids by LC-MS/MS, which were classified into 25 categories. We also compared the two groups by lipid species. Based on the PLS-DA analysis and Q-value evaluation, several parameters were used to identify the lipid species with significant difference between patients and controls. These parameters included VIP > 1 (Variables importance for projection), FC (fold change) > 1.2 or <0.83 and Q-value < 0.05. Based on the above criteria, the volcano plot and heat map were created (Fig.2a,b). 81 lipids with significant change were identified based on

their difference between two groups (supplementary table 2). These 81 SSL lipids belong to 17 categories based on biochemical features. The relative amount of each class of lipid was calculated and compared between the two groups (Fig.2c). Several lipids including diacylcerols (DAGs), fatty acids (FA), phosphatidylcholines (PCs), phosphatidylethar (PE), phytosphingsines, sphingosines, diacylceryl-3-O-carboxyhydroxymethylcholine (DGCC), diacylglyceryl trimethylhomoserine (DGTS) were significantly increased in the stratum corneum (SC) of the skin in the senile pruritus, whereas TAG was significantly decreased.

Association between SSL alteration and skin barrier damage

To evaluate if certain SSL components are related to impaired skin barrier function in the senile pruritus, we analyzed the correlation of TEWL with categories of lipids. Out of all lipids compared, only several Cers species resulted in positive correlations with TEWL (table 2). Specifically, the level of Cer-EOS, Cer-NDS and Cer-NS had significant positive correlations with the TEWL value.

Correlation of SSL alteration and itch severity in the idiopathic senile pruritus

Severe itching in the senile pruritus significantly affect the quality of life. We then evaluate the relationship of itch severity with Cers level. The correlation of SSL alteration with pruritus severity score rated by the ameliorated Kawashima itch scale has been evaluated respectively. The comparative results are summarized in table 3 (table 3). Generally, sphingomyelin, Cer-NP, Cer-AS, Cer-NDS and Cer-NS showed positive relationship with pruritus scores, while Cer-BS, Cer-EODS, Cer-EOS,

Cer-AP have negative relationship, although statistical difference has not been observed. As sphingolipids have been identified as the important pruritogenic substance(11), the results suggest that the significance of sphingomyelin in the senile pruritus needs to be further studied

Discussion:

The pathophysiology of the senile pruritus is unclear. Cutaneous xerosis is most common clinical feature in senile pruritus and is thought to be the consequence of skin aging due to decrease of sweat and sebum production(3, 12). Furthermore, dry skin is considered to be an important factor in triggering pruritus through altering the barrier function of the SC(13). In humans, SSL comes from sebum secreted by sebaceous glands and cornified keratinocytes, which cover the skin surface and protect the skin and play a role in maintaining skin moisture. As a result, variations in SSL composition can damage the skin barrier function. In turn, skin barrier damage could further alter SSL components and accelerate cutaneous xerosis. Due to the complex interplay between components of the skin barrier, any changes in skin surface such as with the SSL composition may be related to pruritus development. In the current study, we used LC-MS/MS to elucidate the lipid composition in the senile pruritus and asymptomatic controls.

Epidermal barrier dysfunction is strongly linked to chronic pruritus(14). TEWL leads to activation of serine proteases such as SC chymotryptic enzyme by increasing the

pH on the skin surface(15). TEWL is also correlated to xerosis(16). In the current study, we found that the senile pruritus has a higher value of TEWL compared to that in controls, and the value of TEWL is positively correlated to the score of pruritus severity (data not shown). Hence, our results indicate an impaired skin barrier function in the senile pruritus compared to asymptomatic elderly controls. As atopic dermatitis (AD) is an inflammatory skin disease characterized by severe itching and skin barrier damage, AD is a good example to understand the physiological role of the epidermal barrier. When the epidermal barrier function is damaged, external stimulants can enter the skin, cause inflammation and elicit itch-inducing mediators(17). Although no significant inflammation exists in the senile pruritus, it is reasonable to conjecture that skin barrier damage in the senile pruritus plays a crucial role of inducing a similar itch-scratch cycle such as that seen in AD.

Alterations of SSL have previously been reported in several inflammatory diseases, such as AD and acne(18-20). In comparing the lipid profiles between the senile pruritus patients and healthy controls, the PLS-DA score plot showed distinct SSL compositions between two groups.

Triacylglycerol (TAG) is the only lipid significantly decreased in senile pruritus. TAG is a minor component of lamellar bodies and extracellular matrix in the SC of healthy skin(21, 22). Reduction of TAG synthesis or increased degradation of TAG can lead to severe skin defects in humans and mice, and epidermal TAG metabolism plays a key role in the maintenance of a proper permeability barrier in the skin(23, 24). Previous

studies also showed that certain groups of TAG are correlated with impaired barrier function in AD (25). Thus, TAG deficiency might be another contributing factor in the barrier dysfunction of senile pruritus.

Skin lipids not only play a structural role in maintaining skin barrier, but also have a variety of biological and pathophysiological functions in the skin. Sphingolipid, one of the important pruritogenic substances, is comprised of a complex set of lipids including sphingomyelin and ceramides. The activity of sphingomyelin deacylase, an enzyme that converts sphingomyelin into sphingosylphosphorylcholin (SPC) and free fatty acid, is elevated in atopic dermatitis patients(26). Intradermal injection of SPC elicits hind-paw scratching in mice(27). In our study, we observed that sphingomyelin and some Cers have a positive relationship with pruritus score. Therefore, further investigations into the pruritogenic function of certain SSL may be valuable.

Conclusions:

In summary, our study clearly displayed that the senile pruritus has distinct SSL profiles in their SC compared to normal healthy controls. Differences in expression of 81 individual lipids have been identified between patients and controls. Correlation analysis showed that some SSL components are correlated with skin barrier damage. Although the study can not conclude that the changes of SSL cause the skin itching, but the result indicates that certain lipid of SSL in skin surface might be the trigger or

the consequence of pruritus in the elderly. Therefore, further investigations into on the pruritogenic function of certain SSL may be valuable. Future studies focusing on certain SSL components may lead to novel therapy for senile pruritus.

List of abbreviations:

SSL: skin surface lipids;

TEWL: transepidermal water loss;

LC-MS/MS: liquid chromatography coupled tandem mass spectrometry;

Cers: ceramides: DAGs: diacylcerols; FA: fatty acids; PCs: phosphatidylcholines; PE: phosphatidylethar; DGCC: diacylceryl-3-O-carboxyhydroxymethylcholine; DGTS: diacylglyceryl trimethylhomoserine; TAG: triacylglycerol

SC：stratum corneum

AD: atopic dermatitis

SPC: sphingosylphosphorylcholin；

PLS-DA: partial least squares discrimination analysis.

Declarations:

1. **Ethic approval and consent to participate**: The study was reviewed and approved by the ethics committee of Peking University International Hospital. Informed consent to participate in the study was obtained from each patient and healthy person before enrolling in the study.

2. **Consent for publication**: All the participants had signed the consent for publication in the informed consent in our institutional consent form.

3. **Availability of data and materials**: All data generated or analysed during this study are available from the correspongding author on reasonable request.

4. **Competing interests**: No competing interest

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6. **Authors contribution**:

Xiaolei Ma: data collection and analysis

Lulu Lu, Na Gao: Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and multivariate data analysis

Zheng Zhao: Transepidermal water loss detection

Mingru Cai: Ameliorated Kawashima itch scale detection

Gangwen Han: Clinical cases collection and experimental guidance

7. **Acknowlement**: Not applicable.

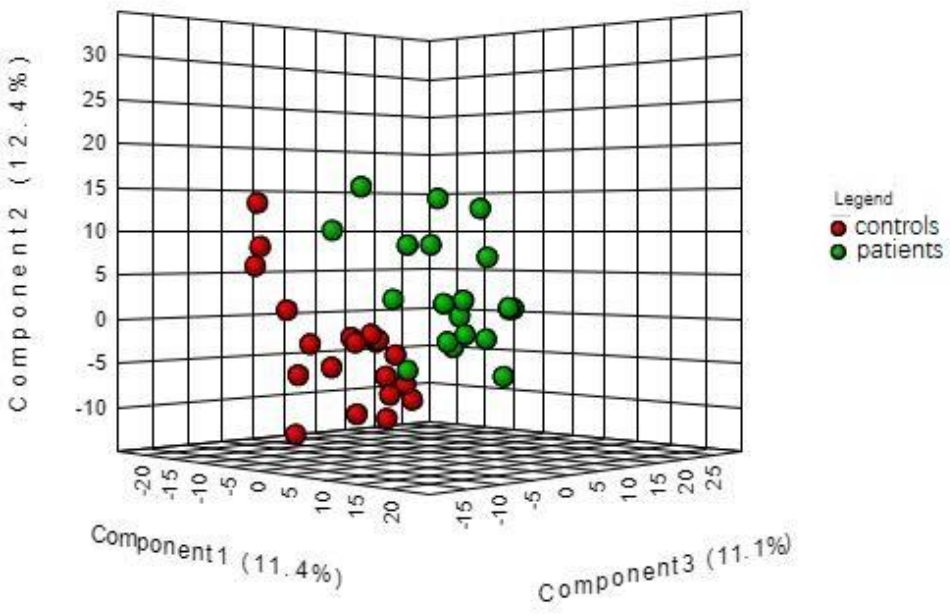
References

1. Lonsdale-Eccles A, Carmichael AJ. Treatment of pruritus associated with systemic disorders in the elderly: a review of the role of new therapies. Drugs Aging. 2003;20(3):197-208.
2. Stewart Williams J, Norström F, Ng N. Disability and ageing in China and India - decomposing the effects of gender and residence. Results from the WHO study on global AGEing and adult health (SAGE). BMC geriatrics. 2017;17(1):197.
3. Thaipisuttikul Y. Pruritic skin diseases in the elderly. The Journal of dermatology. 1998;25(3):153-7.

4. Garibyan L, Chiou AS, Elmariah SB. Advanced aging skin and itch: addressing an unmet need. Dermatologic therapy.26(2):92-103.
5. Clerc CJ, Misery L. A Literature Review of Senile Pruritus: From Diagnosis to Treatment. Acta dermato-venereologica. 2017;97(4):433-40.
6. Feingold KR, Elias PM. Role of lipids in the formation and maintenance of the cutaneous permeability barrier. Biochimica et biophysica acta. 2014;1841(3):280-94.
7. Gruber F, Kremslehner C, Narzt MS. The impact of recent advances in lipidomics and redox lipidomics on dermatological research. Free radical biology & medicine. 2019.
8. Lagarde M, Géloën A, Record M, Vance D, Spener F. Lipidomics is emerging. Biochimica et biophysica acta. 2003;1634(3):61.
9. Triebl A, Hartler J, Trötzmüller M, H CK. Lipidomics: Prospects from a technological perspective. Biochimica et biophysica acta Molecular and cell biology of lipids. 2017;1862(8):740-6.
10. Xie ZQ, Fu JZ, Chen G, Guan CJ, Liu WH, Wang LY, et al. [Correlation between Ameliorated Kawashima Itch Scale and Visual Analogue Scale]. Zhongguo yi xue ke xue yuan xue bao Acta Academiae Medicinae Sinicae. 2018;40(4):539-42.
11. Andoh T, Kuraishi Y. Lipid Mediators and Itch. In: Carstens E, Akiyama T, editors. Itch: Mechanisms and Treatment. Frontiers in Neuroscience. Boca Raton (FL)2014.
12. Farage MA, Miller KW, Elsner P, Maibach HI. Characteristics of the Aging Skin. Advances in wound care. 2013;2(1):5-10.
13. Yadgar RJ, Friedman AJ. Efficacy of a Skin Condition-Adapted Solution for Xerosis and Itch Relief Associated With Aging. Journal of drugs in dermatology : JDD. 2016;15(11):s91-s4.
14. Mollanazar NK, Smith PK, Yosipovitch G. Mediators of Chronic Pruritus in Atopic Dermatitis: Getting the Itch Out? Clinical reviews in allergy & immunology. 2016;51(3):263-92.
15. Ny A, Egelrud T. Epidermal hyperproliferation and decreased skin barrier function in mice overexpressing stratum corneum chymotryptic enzyme. Acta dermato-venereologica. 2004;84(1):18-22.
16. Choi JY, Kim EJ, Jang SI, Kim AR, Lee TJ, Lee HK. A new technique for evaluating heel xerosis grade and the effects of moisturizer on heel skin dryness. Skin research and technology : official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI). 2018;24(4):557-61.
17. Lee CH, Chuang HY, Shih CC, Jong SB, Chang CH, Yu HS. Transepidermal water loss, serum IgE and beta-endorphin as important and independent biological markers for development of itch intensity in atopic dermatitis. The British journal of dermatology. 2006;154(6):1100-7.
18. Li S, Ganguli-Indra G, Indra AK. Lipidomic analysis of epidermal lipids: a tool to predict progression of inflammatory skin disease in humans. Expert review of proteomics. 2016;13(5):451-6.
19. Zhou M, Gan Y, He C, Chen Z, Jia Y. Lipidomics reveals skin surface lipid abnormity in acne in young men. The British journal of dermatology. 2018;179(3):732-40.
20. Shen CP, Zhao MT, Jia ZX, Zhang JL, Jiao L, Ma L. Skin Ceramide Profile in Children With Atopic Dermatitis. Dermatitis : contact, atopic, occupational, drug.29(4):219-22.
21. Freinkel RK, Traczyk TN. Lipid composition and acid hydrolase content of lamellar granules of fetal rat epidermis. The Journal of investigative dermatology. 1985;85(4):295-8.
22. Grayson S, Johnson-Winegar AG, Wintroub BU, Isseroff RR, Epstein EH, Elias PM. Lamellar body-enriched fractions from neonatal mice: preparative techniques and partial characterization. The Journal of investigative dermatology. 1985;85(4):289-94.

23. Radner FP, Fischer J. The important role of epidermal triacylglycerol metabolism for maintenance of the skin permeability barrier function. Biochimica et biophysica acta. 2014;1841(3):409-15.
24. Stone SJ, Myers HM, Watkins SM, Brown BE, Feingold KR, Elias PM, et al. Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. The Journal of biological chemistry. 2004;279(12):11767-76.
25. Li S, Villarreal M, Stewart S, Choi J, Ganguli-Indra G, Babineau DC, et al. Altered composition of epidermal lipids correlates with Staphylococcus aureus colonization status in atopic dermatitis. The British journal of dermatology. 2017;177(4):e125-e7.
26. Andoh T, Saito A, Kuraishi Y. Leukotriene B(4) mediates sphingosylphosphorylcholine-induced itch-associated responses in mouse skin. The Journal of investigative dermatology. 2009;129(12):2854-60.
27. Andoh T, Katsube N, Maruyama M, Kuraishi Y. Involvement of leukotriene B(4) in substance P-induced itch-associated response in mice. The Journal of investigative dermatology. 2001;117(6):1621-6.

386 **Figure legends**



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388 **Fig 1. PLS-DA score plot of skin surface lipid (SSL) from idiopathic senile**

389 **pruritus patients and healthy person controls.** SSL profiles of **idiopathic senile**

390 **pruritus** (blue squares) and controls (red squares) are obvious separated. R2=0.9469,

391 Q2=0.12092.

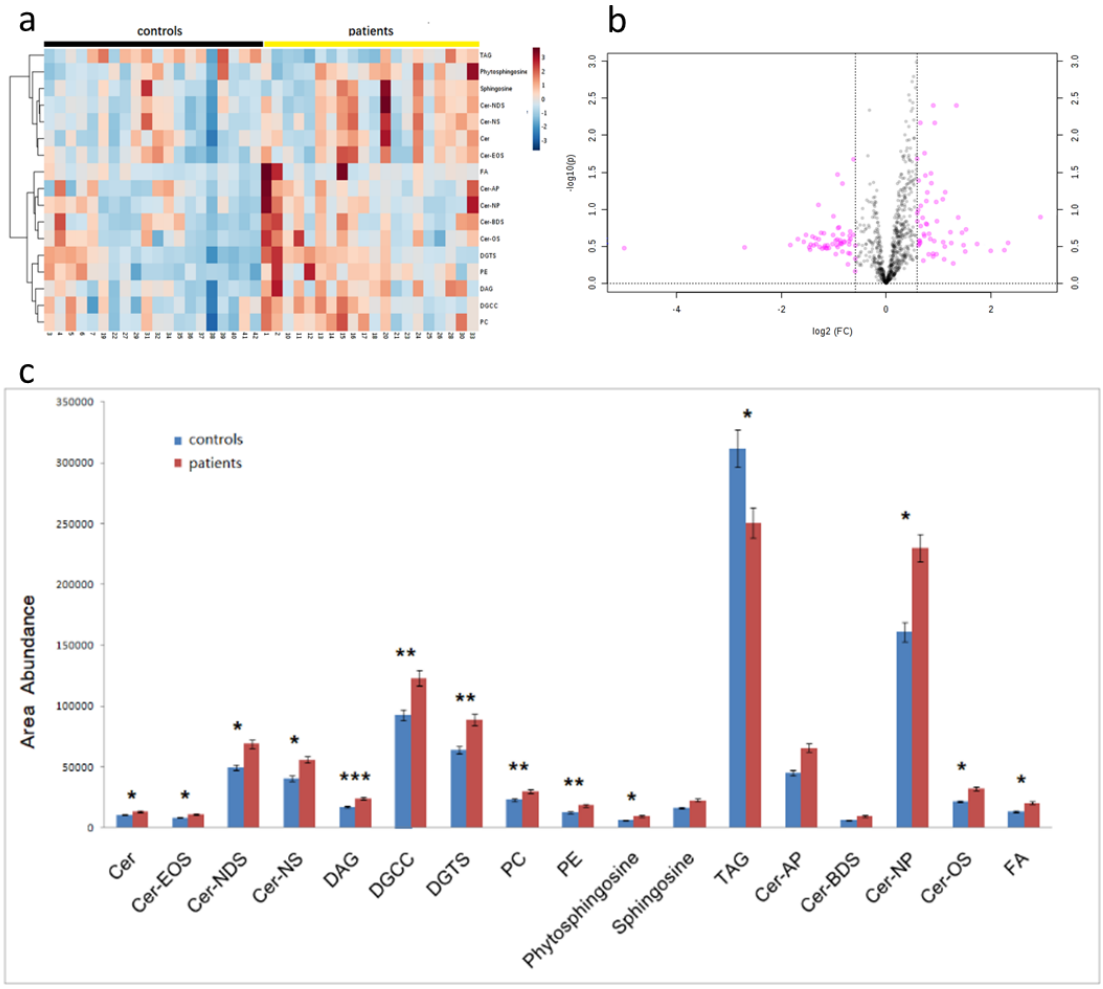

Fig 2. Identification of differential lipids and lipid metabolites between idiopathic senile pruritus patients and healthy person controls. (a) Heat map of SSL.The color is proportional to the intensity of SSL changes; red color represents upregulation, and blue represents down-regulation. (b)Volcano plot of SSL. The red dot represents 1.2 fold (right) and 0.83 fold (left) of variation and $p<0.05$. Total 81 lipids with significant change have been identified based on their difference between two groups. (c) The comparison of 17 main class of lipids between idiopathic senile pruritus and controls. Compaired to controls, 16 main class of lipids increased and only TAG decreased in senilepruritus. Results showed that there were significantly increased

402 levels of Cer, Cer-EOS, Cer-NS, DAG, DGCC, DGTS, PC, PE, Cer-NP, Cer-OS, FA
403 phytosphingsines and decreased level of TAG, while there were no significant change
404 of the relative amount of Cer-NP, Cer-BDS and sphingosines. ***p<0.001,
405 **p<0.01, *p<0.05.
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Table 1 Clinical data of patients and controls

No. of Patients	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**	**P13**	**P14**	**P15**	**P16**	**P17**	**P18**	**P19**	**P20**	**Average**	**SD**
Sex	M	M	M	M	F	M	F	M	M	F	F	F	F	F	F	M	M	F	F	F		
Age (y)	60	65	79	71	60	62	76	68	70	70	78	60	64	63	72	69	80	70	69	75	69.05	6.38
TEWL	13.02	10.4	10.29	14.62	11.38	24.66	17.56	9.84	10.55	15.38	7.59	21.46	8.22	10.05	12.76	11.28	13.13	12.89	16.37	11.07	13.13	4.28
Pruritus Score	1	4	6	4	2	3	7	5	2	2	5	3	2	6	3	2	2	8	6	8	4.05	2.19
No. Of controls	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**	**C13**	**C14**	**C15**	**C16**	**C17**	**C18**	**C19**	**C20**	**Average**	**SD**
Sex	F	M	F	F	F	F	F	M	M	F	F	F	F	M	F	M	F	M	M	M		
Age (y)	75	61	65	77	61	77	72	64	73	65	65	71	63	79	71	62	61	60	66	60	67.40	6.35
TEWL	7.41	4.83	5.11	5.35	4.71	7-93	10.46	7.83	9.12	10.86	5.34	4.55	5.26	4.76	5.35	4.66	10.12	5.38	4.94	11.43	6.71	2.45
Pruritus Score	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

TEWL：Transepidermal water loss； Pruritus Score：Rated by the Ameliorated Kawashima Itch Scale

Table 2. Correlation between TEWL and ceramide levels

Species of Cers	Total Cers	Cer-AP	Cer-BDS	Cer-EOS	Cer-NDS	Cer-NP	Cer-NS	Cer-OS
Correlation Coefficients with TEWL*	0.23336	0.061623	0.21673	0.34001	0.48352	0.08259	0.46858	0.27462
p value	0.14	0.7	0.17	0.03	0.002	0.61	0.002	0.08

*Pearson product-moment correlation coefficient was used to show the association between CER profiles and TEWL index. Note that Cer-EOS, Cer-NDS and Cer-NS in the table have significant positive correlations with the corresponding TEWL indexes ($p<0.05$)**.**

Table 3. Correlation between Pruritus Scores and SSL

Species of SSL	sphingomyelin	Cer-BS	Cer-EODS	Cer-NP	Cer-BDS	Cer-OS	Cer-ADS	Cer-AS	Cer-EOS	Cer-NDS	Cer-NS	Cer-AP
Correlation Coefficients with pruritus scores*	0.24624	-0.02536	-0.03254	0.13395	0.037961	0.11059	0.11935	0.16527	-0.06283	0.14215	0.17252	-0.00483
p value	0.13	0.88	0.84	0.41	0.82	0.49	0.46	0.31	0.7	0.38	0.29	0.98

*Pearson product-moment correlation coefficient was used to show the association between SSL and pruritus severity（scaled by pruritus score) in senilepruritus. Note that sphingomyelin, Cer-NP, Cer-AS, Cer-NDS, Cer-NS have positive correlations with pruritus scores, while Cer-BS, Cer-EODS, Cer-EOS, Cer-AP have negative correlations, but *p* value did not reach to statistically significant.