Lipidomics analysis of facial lipid biomarkers in females with self-perceived skin sensitivity

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Abstract

Background and Aims: Self-perception of sensitive skin (SPSS) has several consequences, including skin barrier damage, which is prevented by barrier sebum. We analyzed lipidome profiles of skin surface lipids (SSLs) in patients with SPSS and healthy controls and explored the mechanism of action of potential lipid markers on the repair of damaged barrier cells to better understand SSL abnormality in these patients.

Methods: Ultrapercision liquid chromatography–quadrupole time-of-flight mass spectrometry was used to investigate SSL variations in major lipid classes, subclasses, and species. Reverse-transcription polymerase chain reaction (RT-PCR) was used to examine changes in intracellular gene expression following cell barrier damage repair by potential lipid markers.

Results: There were significant differences in the lipidomes of individuals between groups. Individuals with SPSS showed significantly increased levels of two diacylglycerols and one very-short-chain free fatty acid and significantly decreased levels of three ceramides (Cers), four glycerophospholipids, and one very-long-chain free fatty acid. RT-PCR revealed that after damage repair by Cer/Glucosylceramide (GlcCer), the expression of two genes in the sterol regulatory element-binding protein and three in the peroxisome proliferator-activated receptor pathway significantly increased. Causes of skin barrier damage in patients with SPSS are related to the amount and type of lipids.

Conclusion: Cer/GlcCer can promote lipid synthesis and secretion by upregulating lipid-related gene expression to repair barrier damage.

Keywords
lipidomics, skin sensitivity, skin barrier, skin surface lipid, ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry
1 | INTRODUCTION

Sensitive skin is a condition of subjective cutaneous hyperreactivity to environmental factors. Self-perceived sensitive skin (SPSS) is a type of sensitive skin that typically affects the face, with the cheeks and cheekbones being the most commonly affected areas, followed by the forehead, chin, and eyebrows. It is characterized by subjective symptoms such as burning, tingling, itching, and tightness when the skin is irritated by physical, chemical, and psychological factors, sometimes accompanied by objective signs such as erythema, scaling, and dilated capillaries. Damage to the skin barrier is one of the major causes of SPSS. Variations in the skin surface lipid (SSL) content are directly associated with the quality of skin barrier function. Several proteases are involved in the process of lipid metabolism and the most studied lipid metabolism pathways include the sterol regulatory element-binding protein (SREBP) and peroxisome proliferator-activated receptor (PPAR) signal transduction pathways.

Lipidomics analysis of the skin can provide a vast amount of information from limited tissue samples, including bioactive lipid mediators, structural lipids, and lipid imaging. This approach enables us to study lipids by quantifying the changes in individual lipid classes, subclasses, and molecular species that reflect lipid differences. Cutaneous lipid metabolism results in a continuous flux of bioactive lipid species and the increasing appreciation of the translational role of skin lipidomics have driven developments in lipidomic technologies. In the present study, ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) was used to investigate the facial lipids of women with SPSS and normal nonsensitive skin (NS), in terms of the main lipid classes, subclasses, and individual species.

In this study, the SSL collection methods, UPLC-QTOF-MS parameters, and statistical methods are based on our previous work on acne in young men. Potential markers of lipid differences between SPSS and NS women were screened and the mechanism of action of potential lipid markers on the repair of damaged barrier cells was elucidated. This study provides a basis for the development of cosmetic products targeting the SPSS population.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Ammonium formate, formic acid, acetonitrile, isopropyl alcohol, and methanol of LC-MS grade, were purchased from Thermo Fisher Scientific. SSL-adsorbent tapes Sebutape® were purchased from CuDerm Corporation. HaCaT cells were purchased from the National Infrastructure of Cell line Resource; DMEM (Dulbecco's modified Eagle's medium) culture medium and phosphate-buffered saline were purchased from HyClone. Trypsin, fetal bovine serum, nonessential amino acids, double resistance, and dimethyl sulfoxide were purchased from Gibco (Thermo Fisher Scientific). Lipid standards were purchased from Avanti polar lipid. Fast Quant RT Kit (with gDNase) and SuperReal PreMix (SYBR Green) were purchased from Beijing Tiangen Biochemical Technology Co.

2.2 | Participants

A total of 58 female participants aged 18–25 were recruited in Beijing. Based on the volunteers' judgment regarding their own skin conditions, those who considered themselves to have sensitive facial skin were classified as the SPSS group (n = 24) and those who considered their facial skin to be insensitive were classified as the NS group (n = 34). We used a four-point scale for problems with sensitive skin. Participants were asked about each facial skin condition and were asked to rate the extent to which they suffered from these conditions as follows: 1: never, 2: rarely, 3: often, and 4: always. The conditions included acne, dryness, dullness, redness, sweating, sensitivity, roughness, and swelling. Exclusion criteria were as follows: Patients with other comorbidities such as severe heart, lung, liver, or kidney diseases; mental illness; diabetes; autoimmune disease; and significant endocrine disorders; as well as patients who were uncooperative or had incomplete information were excluded from the study. The purpose of this study was disclosed to all volunteers, who signed an informed consent form. This study was noninvasive, using the facial lipid-adsorbent tape, Sebutape, to adhere to facial lipids, ensuring respect for the human subjects and the protection of their health. This study followed the principles of the Declaration of Helsinki and was reviewed by the Medical Ethics Committee of the Chinese People's Liberation Army Air Force Specialized Medical Center (2021-161-PJ01).

2.3 | Sample collection and storage

Before sample collection, participants washed their faces with water and sat in a room under constant temperature and humidity (24°C and 50% relative humidity) during which they filled out a questionnaire on sensitive skin. The Sebutape® test strip was stuck to the right cheek of the volunteer, removed after 3 min, and stored in a sample tube at −80°C.

2.4 | Sample preparation

The samples were removed from the refrigerator at −80°C and a mixture of chloroform and methanol was added. Three minutes later, an equal volume of acetone was added and the mixture was left to stand for 10 min. The lipids were collected from the sebum sampling paper via the modified Bligh and Dyer method and the lipid extracts were dried using nitrogen gas. Finally, the lipids were redissolved in a mixture of reagent methanol and isopropanol and assayed on the UPLC-QTOF-MS.
2.5 | UPLC and mass spectrometry analysis

A Waters ACQUITY UPLC-I-Class chromatographic apparatus was employed in this study. High-resolution mass measurements were performed with a Waters Xevo G2-XS QTOF-MS equipped with an electrospray ionization interface operating in the positive ion mode. Leucine encephalin (m/z 554.2771) was used as an external standard for accurate mass calibration and nitrogen was used as the nebulizing and desolvation gas. The target retention time tolerance was 0.100 min and the tolerance level for m/z error was 5 ppm. UPLC-QTOF-MS data were collected as centroided raw data by Masslynx 4.1. The UPLC elution program and parameters of QTOF-MS have been previously described. The above instruments were from Waters Corporation.

2.6 | Data extraction and analysis

The data were collected using MassLynx4.1 software and processed using Progenesis QI V2.0 and Ezinfo 3.0 software (Waters Corporation). The raw data were first imported into QI software for peak alignment. Then, the samples were divided into SPSS and NS groups and peak extraction was performed to obtain compound information, which was then imported into Ezinfo software (Waters Corporation). In combination with partial least squares discriminant analysis (PLS-DA), the differences between the groups were observed using score plots, before the compounds with a strong influence on the groups (a variable influence on projection [VIP] > 1) were screened using S-plots and imported back into Progenesis QI V2.0 software, where other screening conditions were set to screen out compounds that met the conditions (p ≤ 0.05, fold change > 2). Finally, the compounds were compared with the LIPID MAPS structure database (http://www.lipidmaps.org/) to obtain specific information on the characteristic lipid components.

2.7 | Cell survival rate analysis

Cell survival rate was determined using a cell counting kit-8 (CCK-8). HaCaT cells were inoculated at a density of 1 × 10⁶ cells/ml in 96-well plates, followed by the addition of 10 µl of CCK-8 reagent into each well, which was incubated for 4 h before the optical density was measured at 450 nm to determine cell survival rates.

2.8 | RNA isolation and quantitative real-time PCR

Total RNA was extracted with 1 ml Trizol reagent per sample, according to the manufacturer’s instructions (Transgene). The RNA was first reverse-transcribed into complementary DNA using a Fast Quant RT Kit (with gDNAse) and then subjected to qPCR with SuperReal PreMix (SYBR Green). The used primers are described in Table 1.

Statistical significance was calculated using Student's t test in SPSS 26.0 (IBM) and a statistical probability of p < 0.05 was considered significant.

### Table 1 | Reverse-transcription polymerase chain reaction primer sequences

| No. | Protein name | Primer sequence |
|-----|-------------|-----------------|
| 1   | SREBP-1c    | 5'-GGAGCCATGGTGCCTT-3' |
|     |             | 5'-TCAATAGGCAAGGATCA-3' |
| 2   | FFA         | 5'-CAGGCAACACAGTGGAC-3' |
|     |             | 5'-CGGAGTGAATCTGGGTGAT-3' |
| 3   | SCD         | 5'-CCGGGGAGATATCTCGTGT-3' |
|     |             | 5'-GGCTAGTCACTGGCAGAG-3' |
| 4   | PPARα       | 5'-GCGAGATTCAGCTACGAAG-3' |
|     |             | 5'-ATCCCCGACAGAAGGCACT-3' |
| 5   | PPARβ       | 5'-CACCAACGAGACCTTCGGC-3' |
|     |             | 5'-ACCCCTACATGCAACA-3' |
| 6   | PPARγ       | 5'-TGGAATTAGATGACCGACCTTGG-3' |
|     |             | 5'-AGGACTCAGGTGGTTCAGC-3' |
| 7   | GCase       | 5'-ACATGACCCATCCACATCGG-3' |
|     |             | 5'-GAGGAGACCAAGGGGTTC-3' |
| 8   | β-actin     | 5'-CCAACCCGGGAAGAGATGA-3' |
|     |             | 5'-CCAGAGGCTGACAGGATG-3' |

Abbreviations: FFA, fatty acid synthase; GCase, β-glucocerebrosidase; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein.

3 | RESULTS

3.1 | Multivariate data analysis showed significant differences between SPSS and NS women

Multivariate data analysis was performed and assessed using PLS-DA. The separation between the lipid components within the two groups was observed according to the resulting score plot (Figure 1). There were both significant and nonsignificant differences found between the women in the two groups. This may be owing to two factors: first, the participants did not judge their own skin condition accurately; second, the factors that cause sensitive skin such as skin barrier disorders, increased neuroactivity, or enhanced inflammatory responses are complex, and mildly inflammatory or mildly neurological sensitive skin is not necessarily accompanied by significant skin barrier disorders.

3.2 | Correction of grouping methods

To reduce errors owing to inaccurate personal judgment or the complexity of the causes of sensitive skin, the grouping results were corrected using the Dempster–Shafer evidence theory with reference to a questionnaire. The grouping results were the very insensitive group (NS1), insensitive group (NS2), mildly sensitive group (SS1), and severely sensitive group (SS2).
To verify the rationality of this grouping method, the NS1 and NS2 groups and SS1 and SS2 groups were grouped separately to verify the rationality of the grouping. First, five samples were selected from each of the NS1 and NS2 groups (Figure 2A); then, the remaining samples in each of the two groups were set as new groups and joined the first two groups for analysis (Figure 2B). Using the same validation method, significant differences were demonstrated between the SS1 and SS2 groups (Figure 2C, D).

The results showed that this grouping method could effectively separate the insensitive women into NS1 and NS2 groups and the sensitive women into heavily sensitive and lightly sensitive groups, that is, the grouping method is reasonable and feasible.

3.3 Eleven individual lipid species were responsible for the differences between NS1 and SS2

To make the results of the screening of potential facial lipid markers between sensitive and normal populations more accurate, the NS1 and SS2 groups were selected as the experimental groups for the experiment and significant differences were observed between the women in these two groups (Figure 3).

Based on the well-established stability of UPLC-QTOF-MS and the significant differences between the NS1 and SS2 women, the entities that were responsible for differences in the lipidome profiles were analyzed.

After the data were imported into Waters Progenesis QI 2.0, data alignment, peak-picking, normalization, and assignment were performed to identify each distinct entity by matching its exact mass with those in the Basic Lipid database. As multivariate data analysis indicated significant differences between NS1 and SS2 women, OPLS-DA was performed to select the most important individual lipid species of these subclasses responsible for this discrimination. The selection criteria were VIP > 1, p < 0.05, and fold change > 2. The screened potential lipid markers were matched to the human lipid database Basic Lipid and 11 significantly different lipid components were identified, of which three ceramides (Cers), four glycerophospholipids, and one very-long-chain free fatty acid (FFA) showed their highest values in women in the NS1 group and two diacylglycerols (DGs) and one very-short-chain FFA showed their highest values in women in the SS2 group (Table 2).

3.4 Results of screening for potential lipid markers

The lipid information between the four groups (NS1, NS2, SS1, and SS2) was compared between groups to obtain six sets of comparison results and a total of 62 potential lipid markers with significant differences were identified (Table 3). These 62 potential lipid markers were then statistically analyzed to obtain the repetition frequency of each potential lipid marker in the comparison results between the groups.

Based on the information on the 62 potential lipid markers listed in Table 3 and the current status of lipid standards on sale, two potential lipid markers, glucosylceramides (GlcCer; d18:1/16:0) and Cer (d18:0/16:0) were finally selected as samples to perform subsequent experiments (hereafter referred to as GlcCer and Cer, respectively).

GlcCer is a glycosyl ester, consisting of a glucose glycosyl group bound to Cer by a β-glycosidic bond. GlcCer is hydrolyzed to Cer by β-glucocerebrosidase (GCase), a precursor substance for the...
production of Cer. Studies have shown that all Cers on the human skin surface can be generated by the hydrolysis of GlcCer and that GlcCer on the human skin surface is generally composed of long-chain FFAs and phytosphingosine. Specific information on the two lipid standards can be found in Table 4.

### 3.5 Experimental results on the repair/protective effects of potential lipid markers on barrier-damaged HaCaT cells

A concentration gradient of sodium dodecyl sulfate (SDS) was applied to HaCaT cells for 24 h and a CCK-8 assay was performed to obtain the absorbance value at 450 nm using an SDS concentration curve (Figure 4A). The final concentration of SDS at 80% cell viability was 50 μg/ml and was considered the optimum stimulation concentration to cause cell barrier damage.

A concentration gradient of Cer and GlcCer was applied to cells for 24 h and a CCK-8 assay was performed to obtain the absorbance value at 450 nm across the various concentrations of Cer and GlcCer (Figures 4B,C). The absorbance values were all <100%, that is, Cer/GlcCer had a toxic effect on the cells, but the cell survival rates showed an upward trend with the increase of Cer/GlcCer concentration, demonstrating that their toxic effects gradually weaken with an increase in concentration. The results showed that when the concentration of Cer was higher than 50 μg/ml and the concentration of GlcCer was higher than 4.3625 μg/ml, the cytotoxic effect of both was at a minimum.

The repair effect of Cer showed a parabolic variation in the concentration range. When the concentration of Cer was low, the cell survival rate tended to decrease with the increase of sample
concentration and after reaching the lowest point, the cell survival rate tended to increase with the increase of Cer and finally stabilize. This result indicates that, when the sample concentration was low, the repair effect on the barrier-damaged HaCaT cells was weak and there was a superimposed toxic effect with SDS; however, when Cer was higher than the lowest point, the repair effect on the barrier-damaged HaCaT cells was enhanced and the cell damage caused by SDS could be repaired. The final optimal concentration of Cer was 50 μg/ml (Figure 5A). The optimal concentration of GlcCer was 4.3625 µg/ml (Figure 5B) and it was found that when the cell survival rate was low, the effect of GlcCer concentration was much lower than that of Cer. GlcCer was more effective than Cer in repairing barrier-damaged HaCaT cells.

3.6 | Cer/GlcCer can repair barrier-damaged cells by upregulating the expression of some genes related to lipid metabolism

The expression of the seven target genes relative to the housekeeping gene (β-actin) after sample action was calculated using Roche Cycler 480 SW 1.5.1 companion software. The expression of the blank group was set

![FIGURE 3](https://example.com/figure3.png) Scores of lipid differences between samples from NS1 and SS2 groups. Results show obvious separation between SS2 (red squares) and NS1 (black squares) women. NS1, very insensitive group; NS2, insensitive group; SS1, mildly sensitive group; SS2, severely sensitive group.

**TABLE 2** Information on potential lipid markers between the NS1 and SS groups

| Description             | Formula       | M/Z       | Retention time (min) | Highest mean | Lipid type |
|-------------------------|---------------|-----------|----------------------|--------------|------------|
| Cer(d18:1/26:1(17Z))    | C<sub>44</sub>H<sub>85</sub>N0<sub>3</sub> | 676.65    | 10.91                | NS1          | Cer        |
| Cer(d18:0/h26:0)        | C<sub>44</sub>H<sub>89</sub>N0<sub>4</sub> | 696.68    | 11.96                |              |            |
| Cer(d18:0/h24:0)        | C<sub>42</sub>H<sub>85</sub>N0<sub>4</sub> | 668.65    | 10.96                |              |            |
| PC(4:0/18:1)            | C<sub>30</sub>H<sub>58</sub>N0<sub>2</sub>P | 614.39    | 0.54                 | GP           |
| PA(13:0/15:0)           | C<sub>31</sub>H<sub>61</sub>O<sub>8</sub>P | 593.42    | 6.23                 |              |
| PA(17:0/19:0)           | C<sub>39</sub>H<sub>77</sub>O<sub>8</sub>P | 705.54    | 11.51                |              |
| PA(12:0/18:0)           | C<sub>33</sub>H<sub>65</sub>O<sub>8</sub>P | 621.45    | 7.99                 |              |
| Ceriporic acid B        | C<sub>21</sub>H<sub>38</sub>O<sub>4</sub> | 355.28    | 3.93                 | FFA          |
| 5-amino-pentanoic acid  | C<sub>3</sub>H<sub>12</sub>NO<sub>2</sub> | 118.09    | 0.55                 | SS2          |
| DG(14:1/20:1/0:0)       | C<sub>37</sub>H<sub>68</sub>O<sub>5</sub> | 592.51    | 615.49               | DG           |
| DG(12:0/18:0/0:0)       | C<sub>33</sub>H<sub>64</sub>O<sub>5</sub> | 563.46    | 7.38                 |              |

Abbreviations: DG, diacylglycerol; FFA, free fatty acid; GP, glycerophospholipid; NS1, very insensitive group; PA, phosphatidic acid; PC, phosphatidylcholine; SS2, severely sensitive group.
| Group description                              | NS1 | NS1 | NS1 | NS2 | NS2 | SS1 | Repetition frequency |
|-----------------------------------------------|-----|-----|-----|-----|-----|-----|----------------------|
| Cer(d18:0/h24:0)                              | 0   | 0   | 1   | 0   | 1   | 1   | 3                    |
| 9R,10S-epoxy-stearic acid                     | 1   | 1   | 0   | 0   | 0   | 0   | 2                    |
| PA(12:0/18:0)                                 | 0   | 0   | 1   | 0   | 1   | 0   | 2                    |
| GlcCer(d18:1/16:0)                            | 0   | 0   | 0   | 1   | 0   | 1   | 2                    |
| TG(19:1(9Z)/22:0/22:1(11Z)][iso6]             | 0   | 1   | 0   | 0   | 0   | 1   | 2                    |
| PE(15:1(9Z)/12:0)                             | 0   | 0   | 0   | 1   | 0   | 0   | 2                    |
| DG(17:2(9Z,12Z)/21:0/0:0)[iso2]               | 0   | 1   | 0   | 1   | 0   | 0   | 2                    |
| PA(O-16:0/15:0)                               | 0   | 1   | 0   | 1   | 0   | 0   | 2                    |
| 11-keto palmitic acid                         | 1   | 0   | 0   | 0   | 0   | 0   | 1                    |
| 11-methoxy-12,13-epoxy-9-octadecenoic acid    | 1   | 0   | 0   | 0   | 0   | 0   | 1                    |
| PS(20:5(5Z,8Z,11Z,14Z,17Z)/13:0)              | 1   | 0   | 0   | 0   | 0   | 0   | 1                    |
| PE(14:1(9Z)/12:0)                             | 1   | 0   | 0   | 0   | 0   | 0   | 1                    |
| PA(12:0/12:0)                                 | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| PC(22:4(7Z,7Z,10Z,13Z,16Z,19Z)/21:0)          | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| PC(P-18:1(9Z)/0:0)                            | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| PE(O-20:0/22:4(7Z,10Z,13Z,16Z))               | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| DG(18:4(6Z,9Z,12Z,15Z)/16:1(9Z)/0:0)[iso2]    | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| TG(17:1(9Z)/21:0/21:0)[iso3]                  | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| TG(20:2(11Z,14Z)/22:0/22:0)[iso3]             | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| TG(21:0/22:0/22:2(13Z,16Z))[iso6]             | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| Type IV cyanolipid eicosanoyl ester           | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| Ceriporic acid A                              | 0   | 1   | 0   | 0   | 0   | 0   | 1                    |
| Cer(d18:1/26:1(17Z))                          | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| Cer(d18:0/h26:0)                              | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| DG(14:1/20:1/0:0)                             | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| DG(12:0/18:0/0:0)                             | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| PC(4:0/18:1)                                  | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| PA(13:0/15:0)                                 | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| PA(17:0/19:0)                                 | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| 5-amino-pentanoic acid                        | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| Ceriporic acid B                              | 0   | 0   | 1   | 0   | 0   | 0   | 1                    |
| PE(17:2(9Z,12Z)/18:0)                         | 0   | 0   | 0   | 1   | 0   | 0   | 1                    |
| DG(16:1(9Z)/22:0/0:0)[iso2]                   | 0   | 0   | 0   | 1   | 0   | 0   | 1                    |
| DG(15:1(9Z)/21:0/0:0)[iso2]                   | 0   | 0   | 0   | 1   | 0   | 0   | 1                    |
| Phthioceranic acid (C45)                      | 0   | 0   | 0   | 1   | 0   | 0   | 1                    |
| Docosanedioic acid                            | 0   | 0   | 0   | 0   | 1   | 0   | 1                    |
| PI(O-18:0/12:0)                               | 0   | 0   | 0   | 0   | 1   | 0   | 1                    |

(Continues)
The data was then normalized to obtain the relative expression results of the target genes. The results of this experiment showed that the expression of six lipid metabolism‐related genes (sterol regulatory element‐binding protein [SREBP]‐1c, fatty acid synthase [FAS], stearoyl‐CoA desaturase [SCD], peroxisome proliferator‐activated receptors [PPARα and PPARγ], and GCase) significantly decreased after SDS induced cell barrier damage and the change in PPARβ/δ expression was not significant. This indicates that the skin suppressed the expression of some lipid metabolism‐related genes when barrier function was impaired (Figure 6) and that barrier damage was closely associated with a low expression of certain lipid metabolism‐related genes.

In the SREBP-1c pathway, after repair of barrier‐damaged HaCaT cells by Cer/GlcCer, the expression of the two target genes SREBP-1c and SCD significantly increased compared with that in the SDS group, with no significant change in FAS expression (Figures 7A,B). It is hypothesized that GlcCer and Cer have the same metabolic regulation mechanism on the SREBP-1c pathway and both can achieve cell barrier‐damage repair by activating intracellular SREBP-1c pathway proteins and the high expression of its target gene SCD; the effect of GlcCer is superior to that of Cer.

**TABLE 3** (Continued)

| Group description | NS1 NS2 | NS1 SS1 | NS1 SS2 | NS2 SS1 | NS2 SS2 | SS1 SS2 | Repetition frequency |
|-------------------|---------|---------|---------|---------|---------|---------|----------------------|
| PE-NMe(O-14:0/O-14:0) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| DG(16:0/22:0/0:0)[iso2] | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| Eicosanedioic acid | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| N-docosahexaenoyl histidine | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| PG(O-18:0/16:0) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| PA(17:2[9Z,12Z]/12:0) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| 1-(14-methyl‐pentadecanoyl‐2-(8-[3]‐ladderane‐octanyl)‐sn‐glycerol | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| PC(22:6[4Z,7Z,10Z,13Z,16Z,19Z]/18:2[9Z,12Z]) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| Cer(d18:2/23:0) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| 9E,11Z‐Pentadecadienal | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| Cer(d18:0/16:0) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| Cer(d18:0/14:0) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| Cer(d18:1/24:1[15Z]) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| TG(18:1[9Z]/22:0/22:1[13Z])[iso6] | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| TG(19:1[9Z]/20:0/22:1[11Z])[iso6] | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| PC(21:4[6Z,9Z,12Z,15Z]/0:0) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| PC(22:6[4Z,7Z,10Z,13Z,16Z,19Z]/22:4[7Z,10Z,13Z,16Z]) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| PS(18:0/19:1[9Z]) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| PE(18:0/O‐18:0) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| PI(22:2[13Z,16Z]/20:1[11Z]) | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| N‐stearoyl proline | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| 17‐methyl‐nonadecanoic acid | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| cis‐cetoleic acid | 0       | 0       | 0       | 0       | 1       | 0       | 1 |
| N'2'‐(4‐benzenesulfonamide)‐ethyl arachidonoyl amine | 0       | 0       | 0       | 0       | 1       | 0       | 1 |

Abbreviations: Cer, ceramide; DG, diacylglycerol; GP, glycerophospholipid; NS1, very insensitive group; NS2, insensitive group; PA, phosphatidic acid; PC, phosphatidylincholine; SS1, mildly sensitive group; SS2, severely sensitive group; TG, triglycerid; PE, phosphatidyl ethanolamine; PI, phosphatidylinositol; PS, phosphatidylinerse.

The results of this experiment showed that the expression of six lipid metabolism‐related genes (sterol regulatory element‐binding protein [SREBP]-1c, fatty acid synthase [FAS], stearoyl‐CoA desaturase [SCD], peroxisome proliferator‐activated receptors [PPARα and PPARγ], and GCase) significantly decreased after SDS induced cell barrier damage and the change in PPARβ/δ expression was not significant. This indicates that the skin suppressed the expression of some lipid metabolism‐related genes when barrier function was impaired (Figure 6) and that barrier damage was closely associated with a low expression of certain lipid metabolism‐related genes.

In the SREBP-1c pathway, after repair of barrier‐damaged HaCaT cells by Cer/GlcCer, the expression of the two target genes SREBP-1c and SCD significantly increased compared with that in the SDS group, with no significant change in FAS expression (Figures 7A,B). It is hypothesized that GlcCer and Cer have the same metabolic regulation mechanism on the SREBP-1c pathway and both can achieve cell barrier‐damage repair by activating intracellular SREBP-1c pathway proteins and the high expression of its target gene SCD; the effect of GlcCer is superior to that of Cer.
In the PPAR pathway, compared with that in cells in the SDS group, damaged cells repaired by Cer/GlcCer upregulated the expression of three lipid metabolism-related genes (PPARα, PPARγ, and GCase) within this pathway (Figures 7C,D). It is hypothesized that the repair effect of Cer/GlcCer on barrier damage can be achieved by activating PPARα, PPARγ, and their target gene GCase expression to enhance lipid secretion; the repair effect of GlcCer is superior to that of Cer.

4 | DISCUSSION

The equimolar concentration of Cers, FFAs, and cholesterol, which are the main lipid components of the human stratum corneum, is the best ratio for maintaining skin barrier function. When the ratio of these three components is altered by external stimuli or metabolic imbalance, it can lead to abnormal skin barrier function.
Using UPLC-QTOF-MS, a total of 11 potential lipid markers were identified between the most significantly different lipids in the NS1 and SS2 groups. The identified lipid markers GlcCer(d18:1/16:0) and Cer(d18:0/16:0) were then employed in a barrier damage cell model and both were able to significantly repair cell damage without conferring protection. Reverse-transcription polymerase chain reaction was used to observe changes in the expression of seven genes involved in two lipid-related metabolic pathways in cells after barrier injury, as well as after barrier repair by GlcCer and Cer.

A total of three Cers, four glycerophospholipids, and one very-long-chain FFA were screened for significant differences between NS1 and SS2 groups and two DGs and one very-short-chain FFA were found in higher levels in SS2 women. Ceramides were notable because they are required for a functional permeability barrier.9 The decrease in very-long-chain Cers (C > 22) and increase in short-chain Cers lead to a decrease in the denseness of the skin adipose tissue, which in turn affects the skin barrier function.10 The results of this experiment showed that three very-long-chain Cers were significantly different between groups and all of them were more abundant in women in the NS1 group. Also, by constructing lipid bilayer models of normal and atopic skin, as well as lipid bilayers containing ceramide only, Jung et al.11 found that there was a high correlation between atopic dermatitis and reduced ceramide content in the lipid bilayer of the skin,
with the thickness of the lipid bilayer decreasing and it's structure weakening when the ceramide ratio was lower. This is consistent with our study. Phosphatidylcholine (PC), one of the main components of cell membranes, has barrier-protective effects.\textsuperscript{12} Phosphatidic acid (PA), a glycerophospholipid, is a cell-signaling molecule and a component of cell membranes, maintaining their integrity and stability.\textsuperscript{13} In animal cells, PA can also improve cell survival, promote cell proliferation, especially the proliferation of T-lymphocytes, and improve the body's anti-inflammatory ability.\textsuperscript{14} Notably, all three PAs listed in Table 2 are in saturated form and the study found that unstimulated CTLL-2 cells contained more saturated PAs than after IL-2 stimulation.\textsuperscript{15} Our results may be attributed to the fact that T cells are more active in sensitive skin. In our study, two of the three PAs with odd-numbered fatty chains were higher in the NS1 group. Odd-chain fatty acids are the major fatty acid group in the lipids of human skin\textsuperscript{16}; PAs with odd-numbered fatty chains warrant further investigation. Glycerophospholipids can maintain the stability of the skin barrier function through the action of maintenance. In the results of this experiment, the highest values of one PC and three PAs were found in women in the NS1 group and their lowest values were found in women in the SS2 group, resulting in low skin water content and reduced skin resistance to external damage, which is consistent with the skin sensitivity state exhibited by the SPSS population on a daily basis. FFAs are the structural lipids that constitute the skin barrier. The stability of the skin barrier is not only influenced by its content but changes in its structure can also affect the degree of barrier stability.\textsuperscript{17} Short-chain FFAs have a destructive effect on the skin barrier,\textsuperscript{18} while long-chain FFAs can reduce moisture loss from the skin surface, block the entry of harmful substances, and maintain normal skin barrier function.\textsuperscript{19} The results of this study showed a significant increase in a very-short-chain FFA (5-amino-pentanoic acid) and a decrease in a very-long-chain FFA (ceriporinic acid B) in women of the SS2 group compared to women of the NS1 group. The combination of the biological functions of the two FFAs and their distribution between groups more accurately reflects the altered structure of FFAs and the imbalance in the distribution of long and short-chain FFAs, resulting in a deficiency of barrier function in the SPSS population. Triglyceride (TG) on the skin surface can be degraded by skin resident bacteria to produce DG and FFA\textsuperscript{20}; DG is also a second messenger involved in the inflammatory response.\textsuperscript{21} The two types of DG obtained from the present screening were both high in women in the SS2 group, showing that DG could cause skin barrier dysfunction in the SPSS population by enhancing the skin inflammatory response. Uehara et al\textsuperscript{22} analyzed the RNA in skin surface lipids, obtained noninvasively by wiping the skin surface with an oil-absorbing film, to compare participants with questionnaire-based "sensitive" ($n=11$) and "nonsensitive" ($n=10$) skin. The skin surface lipid RNA profiles also indicated a mild inflammatory state in the sensitive skin group and, overall, olfactory receptor gene expression may be a potential indicator of sensitive skin.\textsuperscript{22}

Combined with the current production status of lipid standards, GlcCer(d18:1/16:0) and Cer(d18:0/16:0) were finally selected after screening and these two lipid markers were used as samples for subsequent experiments. A comparison of the repair/protective effects of GlcCer and Cer on barrier-damaged HaCaT cells revealed that both had a restorative effect on barrier-damaged HaCaT cells yet did not confer protection. The repair effect of GlcCer on damaged cells was superior to that of Cer.

The pathways related to skin lipid metabolism mainly include the SREBP-1c and PPAR pathways, among which SREBP-1c can regulate the expression of FAS and SCD.\textsuperscript{23} It has been suggested that SREBP-1c affects skin barrier function in two ways: first, SREBP-1c can regulate the synthesis and secretion of lipids and then affect the barrier function;\textsuperscript{24} second, SREBP-1c participates in the regulation of epidermal cell proliferation and differentiation, that is, the formation of stratum corneum to maintain a healthy barrier function. FAS is a key enzyme involved in the synthesis of long-chain fatty acids and SCD is a key enzyme that catalyzes the formation of saturated lipid coenzyme A into monounsaturated lipid coenzyme A. Its catalytic product is an important component of TG and cholesterol esters and is also involved in cellular stress and inflammatory responses.\textsuperscript{25} PPARs include PPARα, PPARβ/δ, and PPARγ,\textsuperscript{26} which can regulate the expression of GCase. PPARs maintain the homeostasis of the skin barrier via two mechanisms, promoting skin lipid secretion (including promoting the formation and secretion of epidermal lipids and lamellar bodies) and enhancing the activity of lipid-related metabolic enzymes.\textsuperscript{27} PPARs can also inhibit skin inflammation and studies have confirmed that activated PPARs can inhibit the release of inflammatory factors and the differentiation of inflammatory cells and reduce the skin inflammation induced by ultraviolet radiation.

Immunohistochemical methods and enzyme activity detection methods have shown that, after the topical application of a PPAR activator, the activity of GCase in the epidermis increases, accompanied by an increase in GCase messenger RNA expression. GCase is the key enzyme that catalyzes the production of Cers from glucose ceramide.\textsuperscript{28} In the present study, upon cell barrier damage, the expression of six genes related to the SREBP-1c and PPARs lipid metabolism pathways significantly decreased. It was hypothesized that the barrier damage inhibited the expression of some lipid-related genes, resulting in insufficient intracellular lipid synthesis and secretion, which in turn aggravated the barrier damage.

The expression of two genes involved in the SREBP-1c pathway, SREBP-1c and SCD, and three genes involved in the PPAR pathway, PPARα, PPARγ, and GCase, was significantly upregulated in damaged cells after repair by Cer/GlcCer and it was hypothesized that Cer/GlcCer could promote the expression of some lipid-related genes. The expression of all three genes involved in the PPAR pathway, PPARα, PPARγ, and GCase, was significantly upregulated. We propose that Cer/GlcCer can promote lipid synthesis and secretion to repair barrier damage by upregulating the expression of certain lipid-related genes.

5 | CONCLUSION

SPSS has a complex etiology. Here, UPLC-QTOF-MS was used to screen for differences in lipid profiles between women with severe SPSS and nonsensitive women. Two lipid markers, GlcCer
and Cer, were then used in a barrier-damage cell model and were found to achieve repair but not convey protection. Lipid-related gene expression was investigated and significant differences in expression were identified in seven genes involved in two different lipid metabolism pathways. This study contributes to an improved understanding of the role of lipid profile composition in SPSS.

**AUTHOR CONTRIBUTIONS**

Yuchen Ma: writing – original draft; writing – review & editing. Le Cui: formal analysis. Yan Tian: conceptualization. Congfen He: conceptualization; writing – original draft; writing – review & editing.

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**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

**TRANSPARENCY STATEMENT**

All authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies related to the study have been explained.

**DATA AVAILABILITY STATEMENT**

The authors confirm that the data supporting the findings of this study are available within the article.
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