

MARKER COMBINATION FOR SKIN TYPING AND USE THEREOF

Technical solution

The present invention relates to the field of biopharmaceuticals and
5 specifically to a combination of markers for skin typing and their applications.

Background

With the launch of the Microbiome Project, it is increasingly recognized that,
similar to the intestinal tract, a large number of microorganisms exist on the surface
10 of the human body. Intestinal tract studies have suggested that microorganism-host
interactions and strategies through fecal microbiota transplantation, probiotics and
prebiotics can alter intestinal microecology and thus improve intestinal homeostasis.
Research progress in skin microecology is relatively lagging behind, particularly in
the lack of metagenome data from large populations. Metagenome data from North
15 American populations suggest that a large individual differences in composition for
skin microorganisms but highly stable, i.e., individuals are relatively stable in their
microbial composition over time and space, with strong individual characteristics.
The present study reconfirms the existence of individual variability based on data
from the Chinese Han population and finds that microbial composition is
20 significantly associated with a range of skin phenotypes in the host, and that skin
microorganisms can be a potential new tool and target for improving skin
phenotypes.

However, the extreme individual variability poses a serious challenge for the
development and design of universal skin care products and pharmaceuticals.
25 Through further statistical analysis, we have found that despite the large individual
differences, there are still some key features and patterns at the population level,
the combination of these characteristics enables the population to be typed and thus
address the problem of large individual differences. Skin typing is a good way of
meeting the need for 'precision' / 'personalized' care at a low resolution. For
30 example, a commonly used skin typing - "dry", "normal" and "oily" skin –That is,
the host skin is typed according to its water and oil characteristics; Similar to this,

but different in that instead of water-oil characteristics, our study typed the population based on skin microbiome characteristics. This will help industry to develop skin care products and pharmaceuticals that are better suited to specific populations for different skin types and achieve a certain degree of
5 "precision"/"personalized" care to effectively improve skin homeostasis.

Therefore, there is an urgent need in the field to develop a new method for skin typing and determining skin aging, and to provide a new means of diagnosis and typing.

10 **Summary of the invention**

The purpose of the present invention is to type the population based on the microbiome characteristics of the skin, thus helping the industry to develop skin care products and drugs that are more suitable for a specific population based on different skin types. It provides a new method for skin typing and skin condition
15 determination, and provides a new means of diagnosis and typing.

The present invention targets attention to two strains of bacteria that dominate skin typing, *Moraxella osloensis* and *Cutibacterium acnes*, and on *Staphylococcus epidermidis*, one of the abundant bacterial colonization of the skin that plays an important role in the health and disease of the host. First, correlations between the
20 distribution abundance of three target skin symbiotic bacteria on the face and host skin phenotype were determined in metagenome data from a large-scale sample of the Chinese Han population, and then QPCR experiments were performed to verify and identify a strain of bacteria, *M. osloensis*, that promotes skin aging.

25 In a first aspect of the present invention, it provides a use of *Moraxella osloensis* (*M. osloensis*) or a detection reagent thereof for (a) skin typing; and/or (b) determining or characterizing skin conditions or for the preparation of a reagent or kit for (a) skin typing; and/or (b) determining or characterizing skin conditions.

In another preferred embodiment, the skin conditions includes: skin age, skin
30 hydration, skin elasticity, skin color, degree of skin aging.

In another preferred embodiment, the degree of skin aging is determined based

on one or more phenotypes selected from the group consisting of: porphyrin, oil, water content, gloss, pore area, skin yellow value, pore size, and colored patches.

In another preferred embodiment, the reagent or kit further comprises a reagent for detecting *Cutibacterium acnes* (*C. acnes*).

5 In another preferred embodiment, the reagent or kit further comprises a reagent for detecting *Moraxella bovoculi* and/or *Psychrobacter* sp.

In another preferred embodiment, the reagent or kit further comprises a reagent for detecting *Propionibacterium avidum*, *Propionibacterium granulosum*, *Staphylococcus*, *Propionibacterium acnes* phage and/or *Staphylococcus* phage.

10 In another preferred embodiment, the reagent or kit further comprises a reagent for detecting *Staphylococcus epidermidis* (*S. epidermidis*).

In a second aspect of the invention, it provides a combination of markers, comprising *Moraxella osloensis* (*M. osloensis*) and *Cutibacterium acnes* (*C. acnes*).

In another preferred embodiment, the combination of markers further
15 comprises *Moraxella bovoculi* and/or *Psychrobacter* sp.

In another preferred embodiment, the combination of markers further comprises *Propionibacterium avidum*, *Propionibacterium granulosum*, *Staphylococcus*, *Propionibacterium acnes* phage and/or *Staphylococcus* phage.

In another preferred embodiment, the combination of markers further
20 comprises *Staphylococcus epidermidis* (*S. epidermidis*).

In another preferred embodiment, the combination of markers is used for (a) skin typing; and/or (b) determining skin conditions.

In another preferred example, the skin conditions comprise: skin age, skin hydration, skin elasticity, skin color, and degree of skin aging.

25 In another preferred embodiment, the degree of skin aging is determined based on one or more phenotypes selected from the group consisting of: porphyrins, oil, water content, gloss, pore area, skin yellow value, pore size, and colored patches.

In another preferred example, the marker or the combination of markers is derived from a skin sample, preferably from the total skin or facial skin, more
30 preferably, from a cheek, forehead, wing of a nose.

In another preferred embodiment, the marker or the combination of markers is

derived from a skin sample of an Asian population.

In another preferred embodiment, the marker or the combination of markers is derived from a sample of a cheek, forehead, or wing of a nose.

In another preferred example, the level of each of the markers in the combination of markers is tested by one or more methods of the following group:
5 sequencing, PCR, protein quantitative detection.

In another preferred example, the method for detecting levels of the combination of markers further comprises one or more methods selected from the group consisting of: quantitative PCR of feature genes, qPCR, real-time
10 quantitative PCR, Metagenomics analysis, 16s RNA sequencing, mass spectrometry, Western blotting.

In another preferred example, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* in the combination of markers to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

15 $M/C \leq 1.3$, preferably, $M/C \leq 0.8$, more preferably $M/C \leq 0.4$, then the skin type is C type (or I type) and the phenotype includes: high oil content, high water content, good skin elasticity, low degree of the skin aging and bright skin colour.

In another preferred example, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* in the combination of markers to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:
20

$0.3 \leq M/C \leq 2.5$, preferably, $0.3 \leq M/C \leq 2.3$, more preferably, $0.8 \leq M/C \leq 1.8$, then the skin type is mixed type (or II type) and the phenotype includes: medium oil content, medium water content, average skin elasticity, medium degree of skin aging, medium skin colour.

25 In another preferred example, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* in the combination of markers to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

$M/C \geq 0.5$, preferably, $M/C \geq 1.8$, more preferably, $M/C \geq 2.2$, then the skin type is M type (or III type) and the phenotype includes: Low skin oil content,
30 low water content, poor skin elasticity, high degree of skin aging and dull skin colour.

In another preferred example, originated from a sample at the cheek, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

M/C $\leq$ 0.8, preferably, M/C $\leq$ 0.75, then the skin type is C type (or I type)
5 and the phenotype includes: high oil content, high water content, good skin elasticity, low degree of skin aging and bright skin colour.

In another preferred example, originated from a sample at the cheek, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

10 0.75 $\leq$ M/C $\leq$ 2, preferably 0.8 $\leq$ M/C $\leq$ 1.7, then the skin type is mixed type (or II type) and the phenotype includes: medium oil content, medium water content, average skin elasticity, medium degree of skin aging, medium skin colour.

In another preferred example, originated from a sample at the cheek, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

M/C $\geq$ 1.7, then the skin type is M type (or III type) and the phenotype includes: low skin oil content, low water content, poor skin elasticity, high degree of skin aging and dull skin colour.

20 In another preferred example, for a sample originating from the forehead, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

M/C $\leq$ 1.5, preferably M/C $\leq$ 1.25, then the skin type is C type (or I type)
25 and the phenotype includes: high oil content, high water content, good skin elasticity, low degree of skin aging and bright skin colour.

In another preferred example, originated from a sample at the forehead, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following
30 conditions:

1.25 $\leq$ M/C $\leq$ 2.5, preferably 1.3 $\leq$ M/C $\leq$ 2.2, then the skin type is

mixed type (or II type) and the phenotype includes: medium oil content, medium water content, average skin elasticity, medium degree of skin aging, medium skin colour.

5 In another preferred example, originated from a sample at the forehead, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

10 $M/C \geq 2$, preferably, ≥ 2.2 , then the skin type is M type (or III type) and the phenotype includes: low oil content of the skin, low water content, poor skin elasticity, high degree of skin aging, dull skin colour.

In another preferred example, for a sample originating from a wing of nose, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

15 $M/C \leq 0.5$ and, preferably, $M/C \leq 0.35$, then the skin type is C type (or I type) and the phenotype includes: high oil content, high water content, good skin elasticity, low degree of skin aging and bright skin colour.

20 In another preferred example, originated from a sample from a wing of nose, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

25 $0.35 \leq M/C \leq 0.7$, preferably, $0.35 \leq M/C \leq 0.6$, more preferably, $0.35 \leq M/C \leq 0.55$, then the skin type is mixed type (or II type) and the phenotype includes: medium oil content, medium water content, average skin elasticity, medium degree of skin aging, medium skin colour.

In another preferred example, originated from a sample from a wing of nose, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* meets with the following conditions:

30 $M/C \geq 0.5$ and, preferably, $M/C \geq 0.55$, then the skin type is M type (or III type) and the phenotype includes: low skin oil content, low water content, poor

skin elasticity, high degree of skin aging and dull skin colour.

In a third aspect of the present invention, it provides a method for skin typing or determining skin conditions, comprising:

- (1) providing a sample derived from the skin of a subject to be tested,
5 detecting the level (e.g. content) of each marker in a combination of sample markers, the combination comprising the following markers: the *Moraxella osloensis* and the *Cutibacterium acnes*, obtaining a level (M) (e.g. content) of *Moraxella osloensis* and a level (C) (e.g. content) of *Cutibacterium acnes*, respectively;
- 10 (2) typing the skin of the subject to be tested, and/or determining the skin conditions, based on the level (e.g. content) (M) of *Moraxella osloensis*, or comparing the level (e.g. content) (M) of *Moraxella osloensis* with the level (e.g. content) (C) of *Cutibacterium acnes* in the sample.

15 In another preferred example, the subject to be tested is derived from an Asian population.

In another preferred example, in step (2), the skin is typed or the skin conditions of a sample is determined based on the relative value (e.g. M/C) of the level (e.g. content) (M) of *Moraxella osloensis* to the level (e.g. content) (C) of *Cutibacterium acnes*.

20 In another preferred example, the skin conditions comprise: skin age, skin hydration, skin elasticity, skin color, and degree of skin aging.

In another preferred embodiment, the level (e.g. content) (M) of *Moraxella osloensis* and the level (e.g. content) (C) of *Cutibacterium acnes* in the sample of the subject to be tested is determined by one or more methods selected from the
25 group consisting of: sequencing, PCR, protein quantitative detection.

In another preferred example, the detection method of the level (e.g. content) (M) of *Moraxella osloensis* and the level (e.g. content) (C) of *Cutibacterium acnes* in the sample further comprises one or more methods selected from the group consisting of: quantitative PCR of feature genes, qPCR, real-time quantitative PCR,
30 Metagenomics analysis, 16s RNA sequencing, mass spectrometry, Western blotting.

In another preferred example, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

5 $M/C \leq 1.3$, preferably, $M/C \leq 0.8$, more preferably $M/C \leq 0.4$, then the skin type is C type (or I type) and the phenotype includes: high oil content, high water content, good skin elasticity, low degree of the skin aging and bright skin colour.

In another preferred example, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

10 $0.3 \leq M/C \leq 2.5$, preferably, $0.3 \leq M/C \leq 2.3$, more preferably, $0.8 \leq M/C \leq 1.8$, then the skin type is mixed type (or II type) and the phenotype includes: medium oil content, medium water content, average skin elasticity, medium degree of skin aging, medium skin colour.

15 In another preferred example, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

20 $M/C \geq 0.5$, preferably, $M/C \geq 1.8$, more preferably, $M/C \geq 2.2$, then the skin type is M type (or III type) and the phenotype includes: low skin oil content, low water content, poor skin elasticity, high degree of skin aging and dull skin colour.

In another preferred example, originated from a sample at a cheek, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

25 $M/C \leq 0.8$, preferably, $M/C \leq 0.75$, then the skin type is C type (or I type) and the phenotype includes: high oil content, high water content, good skin elasticity, low degree of skin aging and bright skin colour.

30 In another preferred example, originated from a sample at a cheek, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

$0.75 \leq M/C \leq 2$, preferably $0.8 \leq M/C \leq 1.7$, then the skin type is mixed type (or II type) and the phenotype includes: medium oil content, medium water content, average skin elasticity, medium degree of skin aging, medium skin colour.

5 In another preferred example, originated from a sample at a cheek, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

$M/C \geq 1.7$, then the skin type is M type (or III type) and the phenotype
10 includes: low skin oil content, low water content, poor skin elasticity, high degree of skin aging and dull skin colour.

In another preferred example, for a sample originating from a forehead, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the
15 following conditions:

$M/C \leq 1.5$, preferably $M/C \leq 1.25$, then the skin type is C type (or I type) and the phenotype includes: high oil content, high water content, good skin elasticity, low degree of skin aging and bright skin colour.

In another preferred example, originated from a sample at a forehead, when
20 the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

$1.25 \leq M/C \leq 2.5$, preferably $1.3 \leq M/C \leq 2.2$, then the skin type is mixed type (or II type) and the phenotype includes: medium oil content, medium
25 water content, average skin elasticity, medium degree of skin aging, medium skin colour.

In another preferred example, originated from a sample at a forehead, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the
30 following conditions:

$M/C \geq 2$, preferably, ≥ 2.2 , then the skin type is M type (or III type) and

the phenotype includes: low oil content of the skin, low water content, poor skin elasticity, high degree of skin aging, dull skin colour.

In another preferred example, for a sample originating from a wing of nose, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

$M/C \leq 0.5$ and, preferably, $M/C \leq 0.35$, then the skin type is C type (or I type) and the phenotype includes: high oil content, high water content, good skin elasticity, low degree of skin aging and bright skin colour.

In another preferred example, originated from a sample from a wing of nose, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

$0.35 \leq M/C \leq 0.7$, preferably, $0.35 \leq M/C \leq 0.6$, more preferably, $0.35 \leq M/C \leq 0.55$, then the skin type is mixed type (or II type) and the phenotype includes: medium oil content, medium water content, average skin elasticity, medium degree of skin aging, medium skin colour.

In another preferred example, originated from a sample from a wing of nose, when the ratio (M/C) of the level (M) (e.g. content) of the *Moraxella osloensis* to the level (C) (e.g. content) of the *Cutibacterium acnes* in the sample meets with the following conditions:

$M/C \geq 0.5$ and, preferably, $M/C \geq 0.55$, then the skin type is M type (or III type) and the phenotype includes: low skin oil content, low water content, poor skin elasticity, high degree of skin aging and dull skin colour.

In another preferred example, the relative value meets the following conditions: the skin is a type C when $M/C \leq 1.3$ and the skin is a type M when $M/C \geq 0.5$.

In another preferred embodiment, the relative value meets the following condition: the skin is a mixed type when $0.3 \leq M/C \leq 2.5$.

In another preferred example, when the level (e.g., content) (M) of the *Moraxella osloensis* is increased, it indicates a skin condition of increased skin age,

dark and yellow skin, reduced skin water content, decreased sebum and porphyrin content.

In a fourth aspect of the present invention, it provides a combination of the reagent for skin typing and/or detection of skin conditions, comprising a reagent for the
5 detection of each marker in the combination as described in the second aspect of the present invention.

In another preferred example, the reagent is used to detect the level (e.g., content) of each marker.

In another preferred embodiment, the reagent comprises a substance for detecting
10 the level of each marker in the combination described in the second aspect of the present invention by one or more methods selected from the group consisting of: sequencing, PCR, protein quantitative detection.

In another preferred example, the method for detecting the level of the marker further comprises: quantitative PCR of the feature gene, qPCR, real-time quantitative
15 PCR, Metagenomics analysis, 16s RNA sequencing, mass spectrometry, Western blotting.

In another preferred embodiment, the combination of the reagent comprises:
a first detection reagent for detecting the level (M) of *Moraxella osloensis*; and/or
a second detection reagent for detecting the level (C) of *Cutibacterium acnes*.

20 In a fifth aspect of the present invention, it provides a kit, comprising the combination of the reagent described in the second aspect of the present invention.

In another preferred embodiment, each marker in the combination described in the second aspect of the present invention is used as a standard.

In a sixth aspect of the present invention, it provides a system for typing the skin of
25 a subject to be tested and/or determining the skin conditions of a subject to be tested, comprising:

(a) a feature receiving module for receiving a skin sample feature data, which comprises: a quantitative information on each of *Moraxella osloensis* (M) and *Cutibacterium acnes* (C) in the skin sample;

30 (b) a computational processing module for calculating a feature data from the feature receiving module, thereby obtaining a respective ratio of each feature or the ratio

relationship between each feature; and comparing with a standard value for skin typing or feature characterization based on the respective ratio obtained or the ratio relationship between each feature, thereby obtaining a determination result of skin typing and/or skin conditions; and

5 (c) a result output module for receiving and outputting the determination result.

In another preferred example, the subject is a human.

In another preferred example, the subject is an Asian population.

In another preferred example, the subject comprises a man and a woman.

In another preferred example, the subject includes an infant, adolescent or adult.

10 In another preferred example, the quantitative information includes the respective level (e.g., content) of *Moraxella osloensis* (M) and *Cutibacterium acnes* (C).

In another preferred embodiment, the ratio relationship comprises a relative value, such as M/C, of the respective level (e.g., content) of *Moraxella osloensis* (M) and *Cutibacterium acnes* (C) in the skin sample.

15 In another preferred example, the system may be classified into at least two types for the skin conditions.

In another preferred example, the method of obtaining quantitative information comprises: sequencing, PCR, protein quantitative detection.

In another preferred embodiment, the method of obtaining quantitative information
20 further comprises: quantitative PCR of feature genes, qPCR, real-time quantitative PCR, Metagenomics analysis, 16s RNA sequencing, mass spectrometry, Western blotting.

In another preferred example, the feature receiving module comprises a sample collector, and a feature signal input.

In another preferred example, the computational processing module comprises a
25 processor, and a storage, wherein the storage stores a threshold information for skin type and/or skin conditions.

In another preferred example, the output module comprises any terminal, preferably a display, printer, tablet computer (PAD), smartphone.

In another preferred example, each module is connected by wired or wireless
30 means.

In a seventh aspect of the present invention, it provides a method for screening a

substance or composition for improving skin conditions, comprising:

(a) providing a screening bacteria, which is *Moraxella osloensis* (M), *Cutibacterium acnes* (C), or a screening bacteria (a mixed bacteria) comprising *Moraxella osloensis* and/or *Cutibacterium acnes*;

5 (b) Co-culturing the substance or composition to be screened with the screening bacteria and detecting the respective level (e.g. content) of *Moraxella osloensis* or *Cutibacterium acnes*; or the relative level (e.g. relative content) (M/C) between *Moraxella osloensis* and *Cutibacterium acnes*;

(c) based on (b) the respective level (e.g., content) of *Moraxella osloensis* or
10 *Cutibacterium acnes* after the culture; or the relative level (e.g., relative content) (M/C) between *Moraxella osloensis* and *Cutibacterium acnes*, thereby determining that the substance or composition to be screened is a skin conditions-improving substance or composition.

In another preferred example, when the content of *Moraxella osloensis* or the
15 relative level (e.g., relative content) (M/C) between *Moraxella osloensis* and *Cutibacterium acnes* is increased, it is indicated that the substance or composition to be screened is a substance for treating acne.

In another preferred example, when the level (e.g., content) of *Moraxella osloensis* is decreased or the relative level (e.g., relative content) (M/C) between
20 *Moraxella osloensis* and *Cutibacterium acnes* is decreased, it is indicated that the substance or composition to be screened is a substance for anti-aging of the skin.

In another preferred example, when the level (e.g. content) of *Cutibacterium acnes* is increased or the relative level (e.g. relative content) (C/M) between *Cutibacterium acnes* and *Moraxella osloensis* is increased, it is indicated that the substance or
25 composition to be screened is a substance for anti-aging of the skin.

In another preferred example, when the level (e.g., content) of *Cutibacterium acnes* is decreased or the relative level (e.g., relative content) (C/M) between *Cutibacterium acnes* and *Moraxella osloensis* is decreased, then it is indicated that the substance or composition to be screened is a substance for the treatment of acne.

30 In an eighth aspect of the present invention, it provides a use of a combination of markers as described in the second aspect of the present invention or a combination of

the reagent as described in the fourth aspect of the present invention for the preparation of a kit for (a) skin typing; and/or (b) determining or characterizing skin conditions.

In a ninth aspect of the present invention, it provides a use of the combination of markers described in the second aspect of the present invention or the combination of
5 the reagent described in the fourth aspect of the present invention for screening a substance or composition for improving the skin conditions.

It should be understood that within the scope of the present invention, the above-mentioned technical features of the present invention and the technical
10 features described in detail below (such as embodiments) can be combined with each other to form a new or preferred technical solution. Due to space constraints, I will not repeat them here.

Description of Drawings

15 Figure 1 shows a schematic diagram of the sampled skin sites.

Figure 2 shows the microbiological composition of the facial skin of the Chinese Han population.

Figure 3 shows the optimal cluster analysis for different sites.

Figure 4A, B show the clustering results for the forehead, wherein the box
20 plots indicate the average distance between samples within each of the two groups, and the red lines represent the average distance between samples in different groups. 4A is the JS dispersion (Jensen-Shannon divergence) and 4B is the Bray-Curtis dissimilarity degree (Bray-Curtis dissimilarity).

Figure 4C, D shows the relative levels of *Cutibacterium acnes* or *Moraxella*
25 *osloensis* on the forehead, with each point representing one sample.

Figure 4E,F show the clustering results for the cheeks, wherein the box plots indicate the average distance between samples within each of the two groups, and the red lines represent the average distance between samples in different groups. 4E is the JS dispersion (Jensen-Shannon divergence) and 4F is the Bray-Curtis
30 dissimilarity degree (Bray-Curtis dissimilarity).

Figure 4G, H shows the relative levels of *Cutibacterium acnes* or *Moraxella*

osloensis in the cheeks, with each point representing one sample.

Figure 4I, J shows the clustering results for the wing of nose, wherein the box plots indicate the average distance between samples within each of the two groups, and the red lines represent the average distance between samples in different groups. 4I is the JS dispersion (Jensen-Shannon divergence) and 4J is the Bray-Curtis dissimilarity degree (Bray-Curtis dissimilarity).

Figure 4K, L shows the relative levels of *Cutibacterium acnes* or *Moraxella osloensis* on the wing of nose, with each point representing one sample.

Figure 5 shows the differential microorganisms between the different skin types, the colors represent the relative levels of microorganisms, each column is a sample and each line is a microorganism.

Figure 6 shows the characteristics of the microbial network among different skin types, with the M-cutotype-enriched microbial species on the left and the C-cutotype-enriched microbial species on the right. Each dot represents a species, and bouquet of each color represents a species. From the figure, it can be seen that the enriched microorganisms within one skin type are positively correlated with each other and negatively correlated with the enriched species of another skin type, and different skin types show different microbial network compositions.

Figure 7 shows the differential functional enrichment of skin microbial genes in different skin types.

Figure 8 shows the phenotypic differences between the different skin types.

Figure 9 shows the correlation analysis between *Moraxella osloensis* and age as well as skin phenotype: corrected p-value is less than 0.05.

Figure 10 shows the *aceA/aceB* genes enriched in the M-Cutotype.

Figure 11 shows the beta-Carotene synthesis pathway enriched in the M-Cutotype.

Figure 12 shows the functional enrichment analysis of RNAseq differentially expressed genes in human keratinocytes HaCaT treated with *Moraxella osloensis* supernatant and blank control.

Figure 13 shows the utilization of a variety of water-soluble carbon source compounds by *Moraxella osloensis*. The left panel is measured by the CCK-8 kit

and the right panel is measured by Dye mix A.

Figure 14 shows the results of skin type validation using Singapore Chinese skin metagenome data.

Figure 15 shows the results of skin type validation using Filipino and Italian skin metagenome data.

Figure 16 shows the correlation heat map between species level and host phenotype for the three skin symbiotic strains.

Figure 17 shows a graph of *Moraxella osloensis* - HaCaT -QPCR results.

Figure 18 shows a graph of *Cutibacterium acnes* - HaCaT -QPCR results.

Figure 19 shows a graph of *Staphylococcus epidermidis* - HaCaT -QPCR results.

DETAILED DESCRIPTION

After extensive and intensive research, the present inventors have discovered for the first time that *Moraxella osloensis* can be used to characterize skin conditions or for skin typing, and for the first time, a new combination of markers: *Moraxella osloensis* and *Cutibacterium acnes* have been discovered by the present invention. The combination of markers of the present invention can be used for (a) skin typing; and/or (b) determination of skin conditions, which has the advantage of high sensitivity and specificity and has important application value. On this basis, the inventors have completed the present invention.

Terms

The terms used in the present invention have the meanings commonly understood by those of ordinary skill in the relevant field. However, for a better understanding of the present invention, some definitions and related terms are explained as follows:

According to the present invention, the term "combination of markers" refers to a combination of two and more markers.

According to the present invention, the level of the markers is determined by the ratio of the amount and/or expression of the two microorganisms present.

According to the invention, the term "individual" refers to an animal, especially a mammal, such as a primate, preferably a human.

5 According to the present invention, the terms such as "one", "a" and "this" do not only refer to a singular number of individuals, but include the usual categories that can be used to illustrate a particular embodiment.

As used herein, when used in reference to a specific enumerated value, the term "about" means that the value can vary from the enumerated value by no more
10 than 1%. For example, as used herein, the expression "about 100" includes all values of 99 and 101 and between 99 and 101 (e.g., 99.1, 99.2, 99.3, 99.4, etc.).

As used herein, the terms "contain" or "include (comprise)" may be open-ended, semi-closed, and closed type. In other words, the terms "consisting essentially of", or "consisting of" are also included.

15 It should be noted that the explanation of terms is provided herein only to enable those skilled in the art to better understand the invention and is not a limitation of the invention.

***Moraxella osloensis*, *M. osloensis*, later abbreviated as M bacteria**

20 *Moraxella Osloensis*, genus *Moraxella*, bacillus, Gram-negative, chemoorganotrophic bacteria, which cannot use carbohydrate to produce acid.

***Cutibacterium acnes*, *C. acnes*, later abbreviated as C bacteria**

Cutibacterium acnes, a genus of *Propionibacterium* in the
25 *Propionibacteriaceae* of *Actinomycetales* of the *Actinobacteria*, is a Gram-positive bacillus. It is an important colonized bacteria of human skin, involved in maintaining skin health, and can also be used as the pathogenic bacteria of common acne.

30 ***Staphylococcus epidermidis*, *S. epidermidis***

Staphylococcus epidermidis is a Gram-positive cocci that grows on the

epidermis of living organisms and is found on the skin, vagina, etc. It is named *Staphylococcus epidermidis* because it often accumulates in bunches of grapes.

Skin typing

5 In the present invention, the skin can be further divided into M-Cutotype (referred to as type M), mixed type, and C-Cutotype (referred to as type C).

C-Cutotype

10 As used herein, the terms "C-Cutotype" and "C-type or type C" are used interchangeably and refer to a type of microbial-based skin typing characterized by high-level aggregation of *Cutibacterium acnes* (C.acnes).

M-Cutotype

15 As used herein, the terms "M-Cutotype" and "M-type or type M" are used interchangeably to refer to a type of microbial-based skin typing characterized by a high-level of aggregation of *Moraxella osloensis* (M. osloensis).

Skin elasticity

20 As used herein, skin elasticity depends on, but is not limited to, the adequacy of the skin's water content, the content of collagen, elastin and natural fat.

Skin color

25 As used herein, skin color depends on, but is not limited to, skin gloss, skin color, yellow value, etc.

Degree of Skin Aging

30 As used herein, skin aging depends on, but is not limited to, an increase in skin porphyrins, a decrease in skin water content and gloss, enlarged pores and larger areas, skin oil imbalance, and skin dullness.

Detection method

A microbial sample is obtained by a harvester, such as a sterile cotton swab, dipping into the collection solution and repeatedly rubbing the collection site. Examination of level (e.g., content) of characterizable M or C bacteria by common molecular biology means, such as the following ways to obtain the M/C ratio: 1. 16sRNA sequencing; 2. Metagenome sequencing; 3. design primers for the feature sequences of the two species, and then use qPCR to obtain the M/C ratio; 4. Detection of specific expressed proteins or metabolites of two bacteria for the purpose of quantification, for example, mass spectrometry, western immunoblot, etc.

Kit

In the present invention, the kit of the present invention comprises the combination described in the second aspect of the present invention and/or the combination of the reagent described in the fourth aspect of the present invention.

In another preferred embodiment, each marker in the combination described in the first aspect of the present invention is used as a standard.

The main advantages of the present invention include:

(1) The present invention has the advantages of high sensitivity and specificity with important applications of using *Moraxella osloensis* and *Cutibacterium acnes* as a combination of markers for (a) skin typing; and/or (b) determining skin conditions, such as water content, skin elasticity, and/or degree of aging.

(2) The present invention finds for the first time in an Asian population that *Moraxella osloensis* and *Cutibacterium acnes* can be used as a combination of markers for (a) typing skin into M-Cutotype, mixed type and/or C-Cutotype; and/or (b) determining skin conditions.

(3) The present invention has discovered for the first time a new classification method that differs from the previous classification based on host physiological drive (oily skin, dry skin, and wet skin). The new classification method identifies three skin types with different characteristics using skin microorganisms as the basis for classification. The results of the analysis of these three skin types suggest that the

nutritional differences caused by our host physiology may be the driving factor to produce different skin types. Moreover, the microbiome communities of the different skin types may act back on the host skin by performing specific functions, which has a definite impact on skin health and skin appearance. Therefore, further studies on skin types may contribute to the development of personalized medicine to better maintain skin health.

(4) The present invention is the first to find correlation between M bacteria and skin phenotype, positively correlated with age, and correlated with some skin aging phenotypes, such as decreased skin oil, decreased water content, decreased glossiness, and increased yellow value (dull skin) with increased levels of M bacteria; and correlated with some typical features of acne, such as decreased oil, decreased porphyrins (mostly C bacteria metabolites, which are pro-inflammatory) , and pore area decreases with increased M bacteria.

(5) The present invention is the first to discover that by increasing the relative level (e.g., content) of *Cutibacterium acnes*, M-type skin can be adjusted to C-type skin, which can be used for anti-aging of skin.

(6) The present invention is the first to discover that by increasing the relative level (e.g. content) of *Moraxella osloensis*, C-type skin can be adjusted to M-type skin for the treatment of acne.

(7) This invention is the first to discover that correlation analysis of single-strain with skin phenotypes to investigate whether skin microorganisms may lead to changes in host skin phenotypes, providing new insights and perspectives in the study of microbe-host interactions.

(8) The present invention is the first to discover the use of bacterial supernatant to treat host epidermal cells to explore the interaction between bacteria and host at the molecular level, rather than being limited to correlation studies.

The present invention is further described below in connection with specific embodiments. It should be understood that these embodiments are intended to illustrate the invention only and are not intended to limit the scope of the invention. Experimental methods for which specific conditions are not indicated in the

following embodiments are generally in accordance with conventional conditions, or in accordance with the conditions recommended by the manufacturer. Percentages and servings are by weight unless otherwise noted.

Reagents and materials used in embodiments of the present invention are commercially available products, unless otherwise noted.

Generic methods

1. Correlation analysis:

1) Skin microbial sample collection and metagenome sequencing

The Shanghai resident population was recruited as volunteers for this study. All volunteers participating in this study were examined by a dermatologist at the Shanghai Dermatology Hospital to exclude eczematous dermatitis, acne, psoriasis, infections, and other skin lesions at the subject site, and to ensure that they had not had any skin disease in the past 6 months, volunteers treated with systemic or topical antibiotics in the past 6 months were also excluded. Finally, 294 healthy subjects aged 20- 65 years were included, of which 46 were male (M) and 248 were female (F) (Table 1).

Table 1 Subject demographic data

Age group	male	female	Total
21-35	16	58	74
36-50	19	112	131
51-65	11	78	89
Total	46	248	294

Subjects washed their faces with water only on the day of sampling and avoided using any skin care products or cosmetics the day before sampling. The sampling site was kept at a room temperature of 20°C and 50% humidity. A special sterile swab dipped in 0.15 M NaCl and 0.1% Tween20 solution was used to repeatedly wipe an area of approximately 4 cm² on the subject's forehead (Fh), cheek (Ch) and paranasal (Ns) areas for 20 times back and forth. The swabs were then broken and placed in 1.5 ml sterile EP tubes and frozen at -80°C to be stored for skin microbial genomic DNA extraction, and the sampling section is shown

schematically in Figure 1.

The samples were amplified by whole-genome amplification, followed by metagenome sequencing, and finally sequencing data of 822 facial skin microbial samples were obtained. The relative abundance of each gene was calculated by SOAP2 (version 2.21), and then the sum of the relative abundance of genes from the same strain was calculated based on the comparison results of each gene, and the value represents the relative abundance of the strain.

2) Skin phenotype measurement

All phenotypic measurements were performed on 294 Chinese Han subjects in an indoor environment with a temperature of 20 °C and a humidity of 50%. Prior to performing the tests, the subjects rested for more than 30 minutes to ensure that their blood circulation returned to normal levels after possible physical activity. The area of phenotype measurement was the same as the microbial collection site. Skin sebum content (sebum), Water content of stratum corneum (hydration), transepidermal water loss (TEWL), skin pH, porphyrin, skin color (L*a*b), colored patches (lentigines), pores (pores_area), hemangiectasis (telangiectasia), wrinkles (elasticity) and other phenotypes were measured using the following instruments (Table 2), respectively.

Table 2 Phenotype measuring instruments

The measurement of Phenotype	instruments
sebum	Sebumeter® SM815
pH value	Skin-pH-Meter PH 900
Water content of stratum corneum	MositureMeterD Compact device
transepidermal water loss	Vapometer®
porphyrin, skin color, colored patches, pores, hemangiectasis, wrinkles	VISIA-CR

3) Correlation analysis of bacterial abundance and skin phenotype

Correlation analysis (Spearman Rank method) of species levels and phenotypes of sebum content, water content of stratum corneum, transepidermal water loss, skin pH, colored patches, porphyria, skin color and pores and other phenotypes based on the psych package of R software (version 2.0.12) for the facial distribution of various flora in a population of 294 Chinese Han to assess the

correlation between strain level and phenotype; and the P values of the correlation analysis results were subjected to FDR test, and a corrected P value <0.05 indicated that the differences were statistically significant. The correlation heat map was drawn with the R package pheatmap (version 1.0.12) as a display of the results.

5 Blue, negative correlation; red: positive correlation; significant level of Spearman correlation: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

2. Matrix metalloproteinase (MMP) expression experiments:

1) Preparation of strain supernatant

- 10 i. Pick up the frozen bacterial solution obtained from the skin and scratch the plates into the corresponding Petri dishes for incubation using the three-zone scratch method respectively. When a single flora grows on the plate, pick the single flora to the corresponding medium for liquid culture respectively;
- ii. After 24 h of incubation, the bacterial cultures were extracted for genomic
15 DNA and tested for 16S rDNA to confirm that the three bacterial cultures remained the target strains and no contamination existed;
- iii. the confirmed bacteria were subjected to liquid culture, and pure medium without bacterial inoculation was set as the control group respectively;
- iv. the absorbance value of approximately 0.8 at 600 nm of the incubated
20 bacterial solution was detected by an microplate reader;
- v. The three bacterial solutions were filtered twice through 0.22 μm filters to remove the bacterial organisms, and the bacterial supernatant was retained. The same operation was performed for the pure medium control;
- vi. Store the resulting filtered bacterial supernatant and pure medium
25 supernatant in $-80\text{ }^{\circ}\text{C}$ for use.

2) Incubation of HaCaT cells with bacterial supernatant

- i. Incubate 1×10^6 human keratinocytes (HaCaT cells). Set up a bacterial supernatant treatment group (experimental group) and a pure medium control group with three replicates in each group. The culture plates were gently shaken to
30 distribute the cells evenly and put into $37\text{ }^{\circ}\text{C}$, 5% CO_2 cell culture chamber for incubation. Table 3 shows the three bacteria used in the present invention;

ii. Continue to incubate in the incubator for 24 h. Discard the cell culture medium and add PBS to rinse lightly. And then add Tyrisin to each dish, put it into the cell incubator for 5 min to digest and then centrifuge at 800×g for 5 min, collect the cells.

5

Table 3 List of bacterial culture medium

	Experimental group	Control group
Moraxella osloensis	Bacterial supernatant: 10% DMEM is 1:25	R2A medium: 10% DMEM is 1:25
Cutibacterium acnes	Bacterial supernatant: 10% DMEM is 1:25	RCM medium: 10% DMEM is 1:25
staphylococcus epidermidis	Bacterial supernatant: 10% DMEM is 1:25	LB medium: 10% DMEM is 1:25

3) Extraction of cellular RNA

i. Add collected HaCaT cells or primary fibroblasts to Trizol reagent and leave for 10 min at room temperature;

10

ii. Add trichloromethane to the centrifuge tube, shake and mix, and let stand for 5 min;

iii. Centrifuge at 13,200 rpm, 4 °C for 10 min and aspirate the upper aqueous phase (approximately 200 µL) into another mL centrifuge tube;

15

iv. add an equal volume of isopropanol, mix upside down and stand at room temperature for 5 min;

v. Centrifuge at 13,200 rpm, 4 °C for 10 min, discard the supernatant and a white precipitate can be observed at the bottom of the tube;

20

vi. Add 75% ethanol (prepared using anhydrous ethanol and DEPC water, ready to use and cooled at -20 °C before use) and shake gently to suspend the RNA precipitate at the bottom of the tube;

vii. 13,200 rpm, 4 °C, centrifuge for 10 min and discard the supernatant; repeat the above two steps;

viii. Vacuum centrifugation for 2 min and aspirate as much residual ethanol as possible with a pipette, open the cap of the centrifuge tube and allow to dry at room

temperature for 5 min;

ix. After adding DEPC water to lyse RNA, determine the purity and concentration of RNA using NanoDrop, and the RNA concentration of each sample was leveled at 200 ng/μL.

5 4) cDNA synthesis

i. For this experiment, cDNA was synthesized by reverse transcription PCR using HiScript III RT SuperMix for qPCR (+gDNA wiper) kit from Vazyme.

ii. A total of 1 μg of total RNA was taken for reverse transcription reaction and the remaining RNA was stored at -80 °C;

10 iii. Before reverse transcription, potentially mixed genomic DNA will be removed from the RNA sample and prepare the reaction mix for the removal of genomic DNA (RNA sample: 4 × gDNA wiper Mix: RNase-free ddH₂O=5:4:7) in an RNase-free 8 rows of tube, and mixed by gently blowing with a pipette and then add 0.25 times the volume ratio of 5 × HiScript qRT Super Mix and mix.

15 iv. Place the aforementioned product in a PCR instrument at 37 °C for 15 min; 85 °C for 5 s. After the reverse transcription process is completed, use the product for qPCR reaction immediately or store it at -20 °C.

5) Real-time quantitative PCR (Real-time PCR)

20 i. In this experiment, real-time quantitative PCR (Real-time PCR) was performed using the QuantiFast SYBR Green PCR Kit from QIAGEN to detect the relative expression levels of genes.

ii. The primers in Table 4 below were prepared in DEPC water to 2 μM, mixed, and for use;

Table 4 Human primer sequences

Primer Name	Sequence (5'to 3')
<i>MMP1-F</i>	GGGGCTTTGATGTACCCTAGC (SEQ ID NO.1)
<i>MMP1-R</i>	TGTCACACGCTTTTGGGGTTT (SEQ ID NO.2)
<i>MMP10-F</i>	CTCTGGAGTAATGTCACACCTCT (SEQ ID NO.3)
<i>MMP10-R</i>	TGTTGGTCCACCTTTCATCTTC (SEQ ID NO.4)
<i>MMP12-F</i>	CATGAACCGTGAGGATGTTGA (SEQ ID NO.5)
<i>MMP12-R</i>	GCATGGGCTAGGATTCCACC (SEQ ID NO.6)

<i>MMP13</i> -F	CCAGACTTCACGATGGCATTG (SEQ ID NO.7)
<i>MMP13</i> -R	GGCATCTCCTCCATAATTTGGC (SEQ ID NO.8)

iii. Prepare the Real-time PCR reaction system in a 384-well plate, Primers (2 μ M): cDNA: SYBR Master Mix (2 $\times$) = 1:4:5;

iv. Place the 384-well plates in a centrifuge at 4 °C and 3000 rpm for 3 min. Place the 384-well plates in the QuantStudio™ 7 Flex Real-Time PCR System for RT-PCR reactions, and the reaction procedure is shown in Table 5;

Table 5 Real-time PCR procedures

Step	Temperature (°C)	Time	Cycles
1	95.0	4 min	1
2	95.0	15 s	38
2	60.0	36 s	38
3	95.0	15 s	1
3	60.0	15 s	1
3	95.0	15 s	1

v. The experimental results were statistically processed using QuantStudio™ Real-Time PCR Software, and the relative expression of genes in each sample was further calculated according to the $2^{-\Delta\Delta T}$ method, and the experimental results were finally analyzed and plotted using the T-test testing method in GraphPad Prism 5.0 software. Significance levels: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Example 1 Composition of facial skin microbiome in Chinese Han population

Subjects: This study was approved by the Ethics Committee of the School of Life Sciences, Fudan University, and 294 resident healthy subjects in Shanghai were recruited as volunteers for this study. Among them, 46 were males (M) and 248 were females (F). Skin microorganisms were collected from three areas of the head and face: forehead (Fh), cheek (Ch) and paranasal (Ns) (please refer to Figure 1).

The composition and functional characteristics of the healthy skin microbiome of the Chinese Han population were systematically characterized by means of metagenome sequencing combined with bioinformatic analysis. By comparing with the skin microbial sample data of Americans from the Human Microbiome Project (HMP), we have found significantly higher relative levels of a bacterium, *Moraxella osloensis* (*M. osloensis*), in Chinese populations than in Americans. *Moraxella osloensis* is relatively high in all Singaporean Chinese, and this strain may be one of the characteristic strains of skin flora in East Asian populations (please refer to Figure 2).

Example 2 Population typing analysis based on skin microbiome

The skin microbiome, like the gut microbiome, can be affected by a variety of factors and there is significant individual variability. In the study, the present invention draws on the approach of intestinal typing to type the population based on skin microorganisms and to explore the drivers that produce typing in order to find potential patterns behind the skin microbiome and to provide new classification criteria for clinical diagnosis.

Subjects with no missing skin microbiome data at three sites were screened for this invention, for a total of 247 subjects.

Based on the skin microbiome data, these 247 subjects were identified for skin type. First, the samples from each of the three sites were clustered using the PAM method, and the optimal number of clusters was determined using the CH index. Referring to Figure 3, the results of CH index show that the highest score of CH index is obtained when the number of clusters is 2, so that the best cluster number is class 2 in all three sites.

Based on this result, the present invention performed typing analysis of the samples into 2 classes, and PCoA analysis was performed on the typing results of the samples from the three sites using JSD distance and Bray-Curtis distance, respectively. Please refer to Figs. 4A to L. The results show that the typing results of the samples from all three sites can be effectively classified into 2 classes, and the microorganisms that contributed most to this classification were *Cutibacterium*

acnes and *Moraxella osloensis*, i.e., one class was enriched for *Cutibacterium acnes* and the other for *Moraxella osloensis*. Based on this result, we named the two skin types (cutotype) as C-Cutotype and M-Cutotype, respectively.

The present invention analyzes the differential flora of C-Cutotype and M-Cutotype based on the skin microbiome data of the forehead. Referring to Figure 5, the colors represent the relative levels of microorganisms (e.g., relative abundance), with one sample per column and one microorganism per line. The results of the differential analysis show that certain microorganisms prefer a particular skin type, possibly due to interactions between flora. For example, *propionibacterium avidum*, *Propionibacterium granulosum*, *staphylococcus*, *Propionibacterium acnes* phage and *Staphylococcus* phage were enriched in the C-Cutotype. In contrast, M-Cutotype was enriched with *Moraxella bovoculi* and *Psychrobacter sp.*

The present invention performed a correlation analysis based on the relative levels of the differential flora and presented in the form of a network diagram. Referring to Figure 6, the results show a strong positive correlation between microorganisms enriched in the same skin type and there is a strong negative correlation between microorganisms enriched in different skin types. The results of this analysis suggest that microorganisms enriched in the same skin type may occupy different ecological niches, respectively and form a stable ecological network among each other, building a strong microbial community to resist new colonization by other microorganisms including opportunistic and potentially pathogenic microorganisms.

Based on this 247 individuals with a total of 741 samples, we give the typing of skin types based on the ratio (M/C) of M bacteria to C bacteria levels (e.g., content) as shown in Table 6.

Table 6

	Type C	Mixed type	Type M
Cheek	≤ 0.75	$0.75 < M/C < 1.68$	≥ 1.68
forehead	≤ 1.24	$1.24 < M/C < 2.18$	≥ 2.18
wing of nose	≤ 0.34	$0.34 < M/C < 0.55$	≥ 0.55

Based on the different source sites of microorganisms, the reference values for

specific typing are different. The principle of skin typing sampling for clinical application: the facial skin sites that are in urgent need of improvement, and then typing with reference to the above data.

In addition, the full name of C bacteria is *Cutibacterium acnes*, and its level (e.g., abundance) is closely related to skin acne. Very few reports have been made on M bacteria, and the study of the present invention has found for the first time that M bacteria correlates with skin phenotypes, positively correlates with age, and correlates with some skin aging phenotypes.

The inventors conducted experiments on skin texture and M/C changes in subjects and obtained the results in Table 2, showing that the M/C ratio is related to skin texture. As the level of M bacteria increases, skin oil decreases, water content decreases, glossiness decreases, and yellow value (skin dullness) increases; it correlates with some typical characteristics of acne, such as oil decreases, porphyrins (mostly C bacteria metabolites that are pro-inflammatory) decrease, and pore area decreases as M bacteria increases.

Table 7 lists the correlation between the relevant phenotypes and M/C values, and all phenotypes listed are significantly correlated ($p < 0.05$).

Table 7

site	Variable 1	Variable 2	Correlation coefficient	P-value
Fh forehead	Porphyrin	M/C ratio	-0.4788	2.30E-16
	oils	M/C ratio	-0.2592	2.24E-05
	water content	M/C ratio	-0.1782	3.87E-03
	gloss	M/C ratio	-0.1585	1.03E-02
	Pore area	M/C ratio	-0.1405	2.32E-02
Ck Cheek	Porphyrin	M/C ratio	-0.4103	1.65E-12
	water content	M/C ratio	-0.2876	1.35E-06
	oils	M/C ratio	-0.2217	2.22E-04
Ns paranasal	Porphyrin	M/C ratio	-0.3722	1.35E-10
	Skin yellow value	M/C ratio	0.1993	8.16E-04
	Pore size	M/C ratio	-0.1383	2.09E-02
	Pore area	M/C ratio	-0.1320	2.75E-02

	oils	M/C ratio	Paranasal undetected	
	water content	M/C ratio	Paranasal undetected	

Example 3 Biological significance of microbial skin types

PCoA analysis of the samples based on gene level (e.g., abundance) profiles showing that the two skin types can be effectively separated, indicating that there are significant differences in function between the two skin types. Specifically, genes in the C-Cutotype are enriched in carbohydrate and sterol metabolism and fatty acid synthesis, whereas genes in the microbiome of the M-Cutotype are more associated with the synthesis of amino acids, aromatic compounds and some lipids such as inositol. Previous studies have reported that *Cutibacterium acnes* can use carbohydrates as a carbon source, and the results of gene function enrichment have also revealed that 17 KEGG functional modules in the C-Cutotype are associated with the phosphotransferase system (PTS). In prokaryotes, this system is known to be responsible for carbohydrate transport and phosphorylation, and is associated with the ability to metabolize glucose, maltose, lactose, fructose, and cellobiose, which may reflect the dependence of C-Cutotype on carbohydrates as a source of nutrition. Conversely, previous studies with *Moraxella osloensis* have found that the microorganism is unable to utilize any carbohydrates, relying on fatty acids and alcohols as carbon sources. This further suggests that possibly the two skin types constitute two communities with different nutritional requirements.

Since the skin microenvironment is the environment in which the skin microorganisms grow, it determines the nutrients available to the microorganisms. Therefore, to investigate whether the skin phenotype is a driving factor for the different skin types, we further analyzed the phenotypic differences between the two skin types. Referring to Figure 7, it is found that the two skin types have significant differences in stratum corneum moisture, oil, and skin color. In contrast, the C-Cutotype has higher oil and water content, while the M-Cutotype has drier skin. Since oil is the main source of nutrients for microorganisms, this result further suggests that differences in nutritional requirements are the driving factor for the

different skin types.

In addition, the skin phenotypic characteristics of M-Cutotype were similar to those of older adults, and therefore, we compared whether there were differences in age between the two skin types. Referring to Figure 8, as expected, the M - Cutotype group is significantly older than C - Cutotype, but further analysis reveals that both C - Cutotype and M - Cutotype are present in different age groups, i.e., C - Cutotype in older people and M - Cutotype in younger people. Therefore, we speculate that age is not the real determinant, nutritional requirements are the direct determinant. Instead, the difference in age may be due to changes in host physiology during aging that affect the skin phenotype, resulting in a greater age of the M-Cutotype.

Example 4 Correlation of *Moraxella osloensis* with skin aging phenotype

In this study, skin flora was collected from three sites, forehead (Fh), cheek (Ch) and paranasal (Ns), of 248 resident healthy female subjects in Shanghai and analyzed for correlation analysis between *Moraxella osloensis* and age as well as skin phenotype by calculating Spearman's coefficient and correcting the P-value by FDR method, using a corrected P-value less than 0.05 as the screening criterion. Referring to Figure 9, it is found that the levels of *Moraxella osloensis* at all three sites show a significant positive correlation with age and a significant negative correlation with porphyrins. In addition, there is a positive correlation with cheek spots and a negative correlation with moisture and oil in the cheek stratum corneum and with oil content in the forehead. In addition, there is also a trend for a weak positive correlation between *Moraxella osloensis* and other aging phenotypes.

Example 5 Potential targets of skin aging in M bacteria

In the M-Cutotype, we observed an enrichment of *liaza izocytrynianowa* (*aceA*) and malate synthase (*aceB*) genes in the skin microbiome. Whereas the functions of these genes are related to the glyoxylate cycle, it has been demonstrated that the glyoxylate cycle is involved in the catabolism of ethoxy, and these results provide a basis for the involvement of skin microbes in the degradation of

OctaphenylPolyoxyethylene (OPEs). In contrast, the alkylphenol and short polyoxy metabolites formed by OPE degradation have endocrine disrupting activity. Among them, alkylphenol ethoxylates (APEs) have estrogen-like activity, and these compounds have been shown to mimic the effects of estradiol in vivo and in vitro, and are known as environmental estrogens. Therefore, it is possible that skin microorganisms can interfere with the production of estradiol, which is essential for the prevention and control of skin aging (please refer to Figure 10).

1. Please refer to Figure 11 for the results of gene function difference analysis showing that M-Cutotype is enriched in the pathway of β -carotene synthesis, which suggests that M-Cutotype may synthesize more β -carotene. And β -carotene is associated with skin yellowing.

2. We used culture solutions of M bacteria to stimulate keratinocytes and used a transcriptomic approach to explore the potential aging-causing mechanisms of M bacteria.

Signaling pathway enrichment analysis of RNA-Seq differentially expressed genes suggest that *Moraxella osloensis* can affect skin cells through signaling pathways, mainly including regulation of collagen synthesis and decomposition. Referring to Figure 12, RNAseq results suggest that differential genes are heavily enriched in collagen metabolic processes, extracellular matrix breakdown, and other biological processes strongly associated with the aging phenotype.

Example 6 Method for regulating the level of *Moraxella osloensis*

In the previous analysis, it is known that *Moraxella osloensis* is associated with age and skin aging phenotype. In this study, incubation of *Moraxella osloensis* using a single skin surface compound by examining the number of viable bacteria, thus reflecting the utilization of different skin surface compounds by microorganisms and the toxicity of specific compounds to *Moraxella osloensis*. Ultimately, by regulating the amount of preferred compounds or toxic compounds of *Moraxella osloensis*, the growth of *Moraxella osloensis* can be regulated, thus delaying the aging process.

Referring to Figure 13, in this study, two methods, CCK-8 and Dye mix A,

were used to investigate the effect of 32 skin surface compounds on the growth of *Moraxella osloensis*. The compounds were: L-lysine, L-glutamine, L-histidine, L-arginine, Cholic acid, creatinine, D-glucose, L-lactic acid, Glycerin, 2-carboxybenzaldehyde, urea, SDS, L-threonine, L-tryptophan, glycine, L-methionine, L-serine, L-glutamic acid, L-phenylalanine, L-cystine, L-tyrosine, L-leucine, L-isoleucine, L-ornithine hydrochloride, L-citrulline, L-proline, L-valine, L-alanine, D-aspartic acid, trans-4-hydroxy-L-proline, uric acid, taurine. Among them, L-glutamine, L-histidine, L-serine, and L-proline have significant growth-promoting effects on *Moraxella osloensis* and SDS has significant inhibitory effects.

Example 7 Population testing in other countries

To verify whether the obtained skin types are widespread, we downloaded several publicly available data to validate the presence of skin types based on skin microbiome data from different races, sites, and health conditions.

Firstly, we used skin metagenome data from the cubital fossa site (moist type) of Chinese Singaporeans, which had patients with atopic dermatitis (AD) and healthy population. Please refer to Figure 14, results have found that the samples from this data can be effectively classified into 2 classes and are the same as previous results, one enriched for *Cutibacterium acnes* and the other enriched for *Moraxella osloensis*, this result suggests that the presence of our skin type is not influenced by skin health status and site.

Further, we used publicly available skin metagenome data on psoriasis in Filipino children (scalp and neck) and Italians and have found consistent results, please refer to Figure 15, wherein the typing results show that the population can be effectively classified into two classes, one for C-Cutotype and the other for M-Cutotype.

In summary, the present invention demonstrates that skin type is widely available and is not affected by skin site, race, health status, etc.

Example 8 Skin conditions typing system

Based on the aforementioned embodiment we have developed a skin conditions typing system, containing a feature receiving module, a computational processing module and a result output module, each module is connected by wired or wireless means, and the typing steps are as follows:

5 (a) Skin microbial samples are collected from the subject's face and tested to further generate skin sample characterization data. The skin sample characteristic data are the respective levels of *Moraxella osloensis* and *Cutibacterium acnes*. Wherein the collection site is as described in Example 1 and the detection method is as described in the detection method.

10 (b) The skin sample characteristic data is input into the system from the feature receiving module.

 (c) The processing module receives the skin sample characteristic data from the feature receiving module, calculates the respective ratios of *Moraxella osloensis* and *Cutibacterium acnes* quantified or the relative ratio between each other, and
15 based on the respective ratios obtained or relative ratios between each other, a comparison is made with a standard value for skin typing or feature characterization, resulting in a determination result of skin typing and/or skin conditions;

 (d) a result output module, which may be any terminal, such as a display,
20 printer, tablet computer (PAD), smartphone, etc., for receiving and outputting the determination result.

The system includes a storage, wherein the storage contains the threshold information for the standard value.

The skin typing and/or skin conditions corresponds to a standard value of:

25 Skin type C (or type I): when the ratio (M/C) of the level (e.g. content) (M) of the *Moraxella osloensis* to the level (e.g. content) (C) of the *Cutibacterium acnes* meets with the following conditions:

$M/C \leq 1.3$, preferably, $M/C \leq 0.8$, more preferably $M/C \leq 0.4$, the skin condition of this phenotype: Oil, higher water content and good skin elasticity.

30 Skin type M (type III): when the ratio (M/C) of the level (e.g. content) (M) of the *Moraxella osloensis* to the level (e.g. content) (C) of the *Cutibacterium acnes*

meets with the following conditions:

$M/C \geq 0.5$, preferably, $M/C \geq 1.8$, more preferably, $M/C \geq 2.2$, the skin condition of this phenotype: oil and poor water content, poor skin elasticity and high degree of skin aging.

5 Skin type is mixed type (or type II): when the ratio (M/C) of the level (e.g. content) (M) of the *Moraxella osloensis* to the level (e.g. content) (C) of the *Cutibacterium acnes* meets with the following conditions:

$0.3 \leq M/C \leq 2.5$, preferably, $0.3 \leq M/C \leq 2.3$, more preferably, $0.8 \leq M/C \leq 1.8$, the skin conditions of this phenotype is intermediate between type C and type M.

10

Example 9 Correlation analysis of bacterial and skin phenotypes

Correlation analysis of species levels of *Moraxella osloensis*, *Cutibacterium acnes*, and *Staphylococcus epidermidis* and host skin phenotypes such as sebum content, stratum corneum water content, transepidermal water loss, skin pH,
15 colored patches, porphyrins, skin color, and pores in three areas of the host face (cheeks, forehead, and paranasal area) was performed based on R software to assess the correlation between strain levels and phenotypes.

The results show correlation results between bacterial abundance and phenotype at the three sites are consistent in Figure 16, i.e., *Moraxella osloensis* is
20 positively correlated with age and dark and yellow skin color, and negatively correlated with skin water content, sebum, and porphyrin content. *Cutibacterium acnes* shows the opposite trend to *Moraxella osloensis*. *Staphylococcus epidermidis* shows a negative correlation with colored patches and a positive correlation with epidermal water content. The above suggests that there is a correlation between the
25 three bacteria and the aging of the skin.

Example 10 Molecular level validation of bacterial supernatant treatment of skin cells

The bacterial supernatants of *Moraxella osloensis*, *Cutibacterium acnes* and
30 *Staphylococcus epidermidis* were treated with the most abundant cells in the host epidermis, keratinocytes (HaCaT cell line), respectively, and the altered expression

profile of cells after being incubated probed by QPCR, focusing mainly on those related to collagen degradation and extracellular matrix assembly genes-matrix metalloproteinases (MMPs). In particular, MMPs are responsible for degrading extracellular matrix (ECM) proteins and promoting photoaging. The association
5 between bacteria and host skin phenotype was explored by validation at the molecular level.

QPCR results show that, Figure 17-19, HaCaT cells after treatment with *Moraxella osloensis* supernatant. Their expression of MMP1, MMP10, MMP12 and MMP13 is significantly higher compared to the control group, whereas no
10 significant difference in the expression of MMPs is observed after treatment of HaCaT cells with *Cutibacterium acnes* and *Staphylococcus epidermidis* supernatants. It indicates that *Moraxella osloensis* promotes host skin aging, and also suggests that skin microorganisms promote skin aging, not as a general effect, but as an effect of specific strains.

15

All publications mentioned herein are incorporated by reference as if each individual document was cited as a reference, as in the present application. It should also be understood that, after reading the above teachings of the present invention, those skilled in the art can make various changes or modifications,
20 equivalents of which falls in the scope of claims as defined in the appended claims.

sequence listing

<110> FUDAN UNIVERSITY

<120> MARKER COMBINATION FOR SKIN TYPING AND USE THEREOF

<130> P2021-3154

<150> CN202011444009.3

<151> 2020-12-08

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Claims

1. Use of *Moraxella osloensis* (*M. osloensis*) or a detection reagent thereof
5 for (a) skin typing; and/or (b) determining or characterizing skin conditions or for
the preparation of a reagent or kit for (a) skin typing; and/or (b) determining or
characterizing skin conditions.

2. The use of claim 1, wherein the skin conditions includes: skin age, skin
hydration, skin elasticity, skin color, degree of skin aging.

10 3. A combination of markers, comprising *Moraxella osloensis* (*M. osloensis*)
and *Cutibacterium acnes* (*C. acnes*).

4. The combination of markers of claim 3, wherein the combination of
markers further comprises (a) *Moraxella bovoculi* and/or *Psychrobacter* sp.; and/or
(b) *Propionibacterium avidum*, *Propionibacterium granulosum*, *Staphylococcus*,
15 *Propionibacterium acnes* phage and/or *Staphylococcus* phage.

5. A method for skin typing or determining skin conditions, comprising:

(1) providing a sample derived from the skin of a subject to be tested,
detecting the level of each marker in a combination of sample markers, the
combination comprising the following markers: the *Moraxella osloensis* and the
20 *Cutibacterium acnes*, obtaining a level (M) of *Moraxella osloensis* and a level (C)
(e.g. content) of *Cutibacterium acnes*, respectively;

(2) typing the skin of the subject to be tested, and/or determining the skin
conditions, based on the level (e.g. content) (M) of *Moraxella osloensis*, or
comparing the level (M) of *Moraxella osloensis* with the level (C) of *Cutibacterium*
25 *acnes* in the sample.

6. The method of claim 5, wherein in step (2), the skin is typed or the skin
conditions of a sample is determined based on the relative value (e.g. M/C) of the
level (M) of *Moraxella osloensis* to the level (C) of *Cutibacterium acnes*.

7. A combination of the reagent for skin typing and/or detection of skin conditions,
30 comprising a reagent for the detection of each marker in the combination of claim 3.

8. A kit, comprising the combination of the reagent of claim 7.

9. A system for typing the skin of a subject to be tested and/or determining the skin conditions of a subject to be tested, comprising:

(a) a feature receiving module for receiving a skin sample feature data, which
5 comprises: a quantitative information on each of *Moraxella osloensis* (M) and *Cutibacterium acnes* (C) in the skin sample;

(b) a computational processing module for calculating a feature data from the feature receiving module, thereby obtaining a respective ratio of each feature or the ratio relationship between each feature; and comparing with a standard value for skin typing
10 or feature characterization based on the respective ratio obtained or the ratio relationship between each feature, thereby obtaining a determination result of skin typing and/or skin conditions; and

(c) a result output module for receiving and outputting the determination result.

10. The system of claim 9, wherein the method of obtaining quantitative
15 information comprises: sequencing, PCR, protein quantitative detection.

11. A method for screening a substance or composition for improving skin conditions, comprising:

(a) providing a screening bacteria, which is *Moraxella osloensis* (M), *Cutibacterium acnes* (C), or a screening bacteria (a mixed bacteria) comprising
20 *Moraxella osloensis* and/or *Cutibacterium acnes*;

(b) Co-culturing the substance or composition to be screened with the screening bacteria and detecting the respective level of *Moraxella osloensis* or *Cutibacterium acnes*; or the relative level (M/C) between *Moraxella osloensis* and *Cutibacterium acnes*;

25 (c) based on (b) the respective level of *Moraxella osloensis* or *Cutibacterium acnes* after the culture; or the relative level (M/C) between *Moraxella osloensis* and *Cutibacterium acnes*, thereby determining that the substance or composition to be screened is a skin conditions-improving substance or composition.

12. The method of claim 11, wherein when the level of *Moraxella osloensis* in
30 step (c) is increased, or the relative level (M/C) between *Moraxella osloensis* and *Cutibacterium acnes* is increased, or the level of *Cutibacterium acnes* is decreased,

or the relative level (C/M) between *Cutibacterium acnes* and *Moraxella osloensis* is decreased, the substance or composition to be screened is a substance for treating acne; and/or the level of *Moraxella osloensis* in step (c) decreases, or the relative level (M/C) between *Moraxella osloensis* and *Cutibacterium acnes* decreases, or the level
5 of *Cutibacterium acnes* increases, or the relative level (C/M) between *Cutibacterium acnes* and *Moraxella osloensis* increases, the substance or composition to be screened is a substance for anti-aging of the skin.

13. Use of a combination of markers of claim 3 or a combination of the reagent of claim 7 for (a) the preparation of a kit for skin typing; and/or determining or
10 characterizing skin conditions; and/or (b) screening a substance or composition for improving the skin conditions.