Abstract:
The present invention relates to the use, in particular cosmetic and/or therapeutic use, of acid ceramidase, of polypeptides derived from this protein or of analogues thereof, of a nucleic sequence encoding such a polypeptide or of an agent for modulating the expression or the biological or biochemical activity of such a polypeptide, in particular for preventing and/or treating the signs of skin aging. The invention also relates to the use of acid ceramidase, of polypeptides derived from this protein or of analogues thereof, or of a nucleic sequence encoding such a polypeptide, as a marker for evaluating a state of an epithelium.
Cosmetic use of acid ceramidase type proteins

The subject of the present invention is the use, in particular cosmetic and/or therapeutic use, of acid ceramidase, of polypeptides derived from this protein, for example derived from the proteolysis thereof, or of analogues thereof, of a nucleic sequence encoding such a polypeptide or of an agent for modulating the expression or the biological or biochemical activity of such a polypeptide, in particular for preventing and/or treating the signs of skin aging.

The invention also relates to the use of acid ceramidase, of polypeptides derived from this protein or of analogues thereof, or of a nucleic sequence encoding such a polypeptide, as a marker for evaluating a state of an epithelium, and in particular of the epidermis.

Epithelia are tissues of which the cells are joined to and interlinked with one another and lie on a basal membrane. They form either an external covering, for example at the surface of the skin, or the epidermis, or an internal covering, at the surface of a mucosa. They can also form glands.

More specifically, these epithelia are structures of which the homeostasis results from the use of a finely regulated set of intracellular and extracellular signals acting at all the stages of cell proliferation, migration and differentiation, and also of the synthesis of the various extracellular matrix components. These signals can in particular result from the action of factors produced by keratinocytes.

The maintaining of the correct physiological functions of an epithelium involves in particular epithelial terminal differentiation and/or proteoglycan synthesis.

As regards more particularly the epidermis, it is an epithelium, conventionally divided up into a basal layer of keratinocytes containing, in particular, skin stem cells and constituting the germinative layer of the epidermis, a "spiny" layer constituted of several layers of polyhedral cells placed on the basal layer, a "granular" layer comprising one to three layers said to be of flattened cells containing distinct cytoplasmic inclusions, keratohyalin granules, and finally, a set of upper layers, called horny layer (or stratum corneum) constituted of keratinocytes at the terminal stage of their differentiation, called corneocytes.

The stratum corneum, the outermost part of the skin which performs the function of a barrier between the organism and the environment, and the hair shaft, the
emerging part of the hair follicle which constitutes the head of hair, both represent the result of the keratinocyte differentiation process. Epidermal differentiation follows a process of maturation in which keratinocytes from the basal layer differentiate and migrate so as to result in the formation of corneocytes, which are completely keratinized dead cells. This differentiation is the result of perfectly coordinated phenomena which will result in the thickness of the epidermis being kept constant and thus ensure the homeostasis of the epidermis.

Many skin disorders or pathologies can result from a dysfunction of epidermal homeostasis.

For example, in the case of aged skin, this dysfunction is generally manifested through the appearance of wrinkles (micrelief and deep wrinkles), a loss of elasticity, a rough feel and dryness. From the histological point of view, a flattening of the dermo-epidermal junction and a decrease in thickness of the dermis and of the epidermis are observed. The collagen and glycosaminoglycan content decreases. The barrier function of the skin is impaired. All these phenomena are increased by chronic exposure to the sun.

Similarly, this dysfunction may be worsened in women, during the menopause.

The present invention results in particular from the characterization, by the inventors, of the expression of the acid ceramidase protein in the stratum corneum of the human epidermis, in particular in a dry and aged human epidermis.

Acid ceramidase (also known as N-acylsphingosine amidohydrolase) is a heterodimeric protein, the precursor form of which comprises 395 amino acids (SEQ ID NO 2) and has a molecular weight of approximately 55 kDa. The mature form is obtained by cleavage of the precursor form and comprises a nonglycosylated alpha-subunit and a glycosylated beta-subunit. The α-subunit has a molecular weight of approximately 13 kDa and the β-subunit has a molecular weight of approximately 40 kDa.

Post-translational modifications, such as N-acetylglycosylation, are capable of modifying its activity. Acid ceramidase is a lysosomal enzyme of the hydrolase type, responsible for the hydrolysis of ceramide to sphingosine and to fatty acids.

A deficiency in the activity of this enzyme is reflected by an accumulation of ceramides in the lysosomes, and appears to be involved in Farber's disease.

Acid ceramidase is expressed in many tissues, including the heart and the
epidermis. It has recently been observed that acid ceramidase has an activity in keratinocyte differentiation (Houben et al., J Lipid Res, 2006, 47:1063-70).

Jin et al. (Acta Derm Venereol., 1994, 74(5):337-40) have observed an increase in a ceramidase type activity in aged skin.

On the other hand, to the inventors' knowledge, acid ceramidase had not up until now been identified as being a protein of which the expression is decreased in the aged and dry human stratum corneum.

Thus, against all expectations, acid ceramidase is found also to be a potential marker for the physiological state of the skin, in particular in terms of aging. In fact, as emerges from the tests represented hereinafter, the inventors have noted, unexpectedly, a significant decrease in its expression during aging of the epidermis.

Consequently, according to one of its first aspects, a subject of the present invention is a cosmetic or alternatively nontherapeutic use of an effective amount of at least one polypeptide derived from acid ceramidase and, in particular, having an amino acid sequence encoded by a nucleic acid sequence represented entirely or partly by a sequence represented by SEQ ID NO 1, an analogue thereof or a fragment thereof, of at least one nucleic sequence encoding such a polypeptide or of at least one agent for modulating the expression or the biological or biochemical activity of such a polypeptide, as an agent that is of use for preventing and/or treating the signs of skin aging.

According to another of its aspects, a subject of the present invention is also the use of an effective amount of at least one polypeptide derived from acid ceramidase and, in particular, having an amino acid sequence encoded by a nucleic acid sequence represented entirely or partly by a sequence represented by SEQ ID NO 1, an analogue thereof or a fragment thereof, of at least one nucleic sequence encoding such a polypeptide or of at least one agent for modulating the expression or the biological or biochemical activity of such a polypeptide, for the preparation of a composition, in particular a therapeutic composition, for preventing and/or treating the signs of skin aging.

The term "signs of skin aging" is intended to mean all the modifications of the external appearance of the skin due to aging, whether it is chronological and/or photoinduced, for instance wrinkles and fine lines, wizened skin, lack of elasticity and/or of tonicity of the skin, thinning of the dermis and/or degradation of the collagen fibers, thereby leading to the appearance of soft and wrinkled skin.
For the purpose of the present invention, the expression "effective amount" is intended to denote the minimum amount required for the observation of the expected effect, namely a cosmetic effect or a therapeutic effect, it being understood that the effective amounts required for obtaining a cosmetic effect or a therapeutic effect may, as appropriate, be identical or different.

For the purpose of the invention, the term "cosmetic use" is intended to denote a use intended mainly to provide an esthetic effect and/or an effect of comfort.

For the purpose of the invention, the term "therapeutic composition" is intended to denote a composition intended to provide a prophylactic or curative effect with respect to epithelial, and in particular epidermal, disorders recognized as reflecting a pathological state.

For the purpose of the invention, the term "prophylactic" or "preventive" is intended to mean a decreased risk of occurrence of a phenomenon, for example a pathology.

A composition in accordance with the invention may, in particular, be for preventing and/or treating thinning of an epidermis and/or a loss of firmness, of elasticity, of density and/or of tonicity of an epidermis and/or the formation of wrinkles and fine lines.

According to another embodiment, a composition in accordance with the invention may in particular be for preventing and/or treating signs of dryness of the skin, in particular for preventing and/or treating dehydration of an epithelium, especially of an epidermis.

A composition in accordance with the invention may in particular be for preventing and/or treating the effects of chronological aging of an epithelium, in particular of an epidermis or of the lips or of the scalp.

According to another aspect, the present invention also relates to the use of at least one polypeptide in accordance with the invention, as a tool for screening for biological or chemical compounds capable of modulating, and in particular of activating, the expression and/or the biological or biochemical activity of said polypeptide.

In particular, it relates to a method for screening for anti-aging active agents, comprising at least the steps consisting in:

a) bringing at least one cell type capable of expressing a polypeptide in
accordance with the invention, i.e. acid ceramidase or a derivative thereof, into contact
with at least one chemical or biological test compound, under conditions suitable for
manifestation of the expression of said polypeptide, and

b) determining the content of said polypeptide.

According to yet another of its aspects, the present invention also relates to the
use of at least one polypeptide in accordance with the invention, or of at least one nucleic
acid sequence encoding said polypeptide, as a tool for characterizing, *in vitro* or *ex vivo*, a
state of an epithelium, and in particular of an epidermis.

More specifically, according to another of its aspects, the present invention
relates to a noninvasive, in particular cosmetic, method for characterizing the surface state
of an epithelium, in particular of an epidermis, comprising at least the qualitative or
quantitative characterization of the expression and/or of the biological or biochemical
activity of a polypeptide in accordance with the invention, i.e. acid ceramidase, or of a
derivative or fragment thereof.

According to a variant embodiment, the datum or value obtained may be
assessed in comparison to a reference datum or value, obtained for example from at least
one epithelium, in particular one epidermis, which is different from that which is the
subject of the characterization, and the state of which is known.

According to another of its aspects, the present invention is also directed
toward a noninvasive, in particular cosmetic, method for characterizing the effectiveness
of a cosmetic or therapeutic treatment aiming to compensate for the signs of skin aging,
comprising at least the qualitative or quantitative characterization of the expression and/or
of the biological or biochemical activity of a polypeptide in accordance with the invention,
i.e. acid ceramidase, or of a derivative or fragment thereof.

According to a variant embodiment, the datum obtained at the end of the
characterization may also be examined in comparison to a reference value or datum. This
reference value or datum may be a datum obtained from the epithelium, in particular from
the epidermis, that is to be subjected to the treatment, prior to the administration of said
treatment or within a shorter chronological time in relation to the treatment start date.

As it emerges from the description which follows, the methods according to
the invention are particularly advantageous since their implementation does not require an
invasive procedure.
The methods of the invention may be carried out in vitro, ex vivo or in vivo.

Indeed, the localization, by the inventors, of the new biomarker for aging, namely acid ceramidase, in the stratum corneum makes a quantitative or qualitative characterization of the expression of this protein possible by mere topical sampling. The sampling method may, for example, be a stripping technique consisting in applying, to the epithelium under consideration, such as an epidermis, a portion of adhesive tape. On detaching this adhesive tape, a fraction of the epithelium, for example an epidermal fraction, is removed. After protein extraction, said fraction is then analyzed by conventional methods, such as immunoenzymatic assay, or more particularly Western-blot analysis.

**POLYPEPTIDE DEFINITION**

According to one embodiment, a polypeptide suitable for the invention may have an amino acid sequence represented entirely or partly by a sequence represented by SEQ ID NO 2, or an analogue thereof, or a fragment thereof.

For the purpose of the present invention, the term "acid ceramidase" is intended to denote, in general, unless otherwise indicated, the sequence (SEQ ID NO 2) of the protein having or not having undergone post-translational modifications, of the N-acetylglycosylation type, on the asparagine residues at position 173, 195, 259, 286, 342 or 348, capable of modifying its apparent molecular weight or its isoelectric point.

It is, moreover, known that the primary sequence of a polypeptide, i.e. the succession of the amino acids, determines sites specifically recognized by protease-type enzymes, such as trypsin, which, once the recognition of these sites has become effective, will induce cleavage of the polypeptide by proteolysis. This proteolysis results in the generation of various peptides, or proteolytic fragments, of the acid ceramidase.

The inventors have detected the presence of such peptides in the stratum corneum.

Consequently, the invention also extends to the proteolytic fragments of acid ceramidase.

Thus, according to one particular embodiment, a polypeptide suitable for the invention may have an amino acid sequence chosen from SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID...
NO 10, SEQ ID NO 11, SEQ ID NO 12 and SEQ ID NO 13, and mixtures thereof.

The term "analogue of a polypeptide" is intended to denote any polypeptide exhibiting a sequence homology, in particular with respect to one of the characteristic sequences of said polypeptide, and also a biological or biochemical activity of the same nature.

This compound may be a peptidomimetic agent.

The homology may be at least 85%, for example at least 90%, and for example at least 95%. The homology may be determined by visual comparison or by means of any computer tool generally used in the field, such as the BLAST programs available on www.ncbi.nlm.nih.gov and used with the default parameters.

The sequence homology may result from modifications derived from mutation or variation in the sequences of the peptides according to the invention, originating either from the deletion or from the insertion of one or more amino acids, or else from the substitution of one or more amino acids in the characteristic sequences of a polypeptide according to the invention.

For the purpose of the invention, the term "polypeptide fragment" is intended to denote any portion of a polypeptide in accordance with the invention comprising at least 4, at least 6, in particular at least 8, and more particularly at least 12 consecutive amino acids of said polypeptide, and a substantially similar biological or biochemical activity.

The term "characteristic sequence of the polypeptide" is, from the viewpoint of acid ceramidase, intended to be directed in particular toward the sequence represented by SEQ ID NO 2.

In general, the polypeptide analogues may comprise conservative substitutions relative to the amino acid sequence of the natural polypeptide.

Several of these modifications may be combined.

By way of example of mutations that may be considered in the present invention, mention may be made, nonexhaustively, of the replacement of one or more amino acid residues with amino acid residues having a similar hydropathic index, without however substantially affecting the biological properties of the polypeptide, particularly its enzymatic activity.

The hydropathic index is an index assigned to amino acids as a function of their hydrophobicity and of their charge (Kyte et al. (1982), J. Mol. Biol, 157: 105).
A polypeptide or analogue that is also covered by the present invention may be a polypeptide having undergone one or more post-translational modification(s).

The term "post-translational modification(s)" is intended to encompass all the modifications that a peptide or a protein is capable of undergoing at the end of its synthesis in a cell, such as, for example, one or more phosphorylation(s), one or more thiolation(s), one or more acetylation(s), one or more glycosylation(s), one or more lipidation(s), such as a farnesylation or a palmitoylation, a structural rearrangement such as the formation of disulfide bridges and/or cleavage within the peptide sequence.

The analogue has, moreover, substantially the same biological or biochemical activity as the natural polypeptide.

According to one embodiment, a polypeptide suitable for the implementation of the invention may also be a natural or synthetic polypeptide, as appropriate, capable of being obtained after enzymatic or chemical lysis of acid ceramidase, or by chemical or biological synthesis, or by extraction from a biological tissue, for instance the skin, expressing this polypeptide naturally or after transfection thereof, and also the various post-translational forms of said polypeptide, or else any natural or synthetic polypeptide of which the sequence completely or partially (entirely or partly) comprises an amino acid sequence mentioned above, for example the variants and the analogues.


According to another embodiment, a polypeptide suitable for the implementation of the invention may also be a polypeptide as defined above, in which at least one residue has been replaced with an amino acid residue having a similar hydropathic index, as defined above.

According to another embodiment, a polypeptide suitable for the implementation of the invention may also be a polypeptide as defined above, fused with another polypeptide, a hydrophilic or hydrophobic targeting agent, a bioconversion precursor, or a luminescent, radioactive or colorimetric labeling agent.

In a nonlimiting manner, mention may be made, as an example of compounds
that can be coupled with a polypeptide in accordance with the invention, of fluorescent proteins such as Green Fluorescent Protein, fluorescent chemical compounds such as rhodamine, fluorescein or Texas Red®, phosphorescent compounds, radioactive elements, such as $^3\text{H}$, $^{14}\text{C}$, $^{35}\text{S}$, $^{121}\text{I}$ or $^{125}\text{I}$, or colorimetric labeling agents such as chromogenic substrates sensitive to the action of galactosidase, of peroxidase, of chloramphenicol acetyltransferase, of luciferase or of alkaline phosphatase.

Depending on the nature of the compounds that can be coupled with a polypeptide in accordance with the invention, the coupling may be performed by chemical methods, in particular by means of reactive chemical functions, or by molecular biology methods known to those skilled in the art.

**DEFINITION OF NUCLEIC ACID SEQUENCES**

According to one embodiment, the present invention also relates to nucleic acid sequences encoding a polypeptide of the invention and to the employment thereof in the various uses and methods in accordance with the invention.

Thus, the present invention also relates to the use of nucleic acid, in particular deoxyribonucleic acid or ribonucleic acid, sequences encoding a polypeptide in accordance with the invention, in particular the sequences corresponding at least to a nucleic acid sequence represented by SEQ ID NO 1, analogues thereof or a fragment thereof, for the preparation of a composition in accordance with the invention.

For the purpose of the present invention, the term "nucleic acid sequence fragment" is intended to denote a nucleic acid sequence encoding all or part of a polypeptide in accordance with the invention, or an analogue of said polypeptide, and in particular a nucleic acid sequence represented by SEQ ID NO 1 or an analogue thereof.

The expression "analogue of a nucleic acid sequence" is intended to denote any nucleic acid sequence possibly resulting from the degeneracy of the nucleic acid code, and encoding a polypeptide with a sequence identical or analogous to that of the polypeptide encoded by said nucleic acid sequence.

The nucleic acid sequences may be derived from all possible origins, i.e. either of animal, in particular mammalian, and even more particularly human, origin, or of plant origin, or of microbial origin (viruses, phages, bacteria, inter alia) or else of fungal origin, without prejudice regarding whether or not they are naturally present in said organism of
In the case in point, the invention also relates to the use of isolated and purified nucleic acid fragments encoding the polypeptides considered according to the invention.

A nucleic acid sequence in accordance with the invention may comprise a sense, antisense or interfering sequence corresponding to a sequence encoding a polypeptide in accordance with the invention.

Thus, the present invention also relates to the use of nucleic acid, in particular deoxyribonucleic acid or ribonucleic acid, sequences encoding a polypeptide in accordance with the invention.

The nucleic acid sequences according to the invention may in particular be used for preparing the corresponding sense or antisense ribonucleic acid sequences.

A subject of the invention is also the use of any polynucleotide, having a ribonucleic or deoxyribonucleic acid sequence, comprising a sense or antisense sequence, in particular small interfering RNA (siRNA), corresponding at least to the nucleic acid sequence SEQ ID NO 1 or an analogue thereof.

**MODULATING AGENT**

According to another embodiment, the invention relates to the use of an agent for modulating the expression and the activity of a polypeptide in accordance with the invention.

For the purpose of the invention, the term "modulate" is intended to mean, in relation to a given effect, the action of stimulating or inhibiting this effect.

The expression "agent for modulating acid ceramidase" is intended to mean a product capable of interfering with the activity, in particular enzymatic activity, thereof.

The modulator may interfere indirectly by modifying the amount of endogenous enzyme produced, in particular by promoting or, conversely, by decreasing the gene expression thereof and/or the translation thereof. Thus, any agent for modulating the gene or protein expression of acid ceramidase may be suitable for the invention.

The modulating agent may also interfere directly with the enzyme reaction catalyzed by acid ceramidase. It is, for example, an enzyme activator or inhibitor.

The term "biochemical activity" is intended to denote the enzymatic activity of
acid ceramidase, i.e., in particular its ceramide-hydrolyzing activity.

The term "biological activity" is intended to denote any nonenzymatic activity mediated by the acid ceramidase represented by the sequence SEQ ID NO 2, by the mature form, and also by the forms which have or have not undergone glycosylations resulting in molecular weight variants.

This modulating agent may be an agent for activating or inhibiting the gene or protein expression of a polypeptide of the invention.

By way of nonlimiting illustration of the agents for activating the expression of a polypeptide of the invention, mention may in particular be made of compounds for promoting keratinocyte differentiation, such as PPAR-gamma agonists, inhibitors of histone deacetylase, such as butyrate and derivatives thereof, and molecules such as 2-(3,4,5-trimethoxyphenylamino)pyrrolo[2,3-d]pyrimidine and derivatives thereof.

By way of nonlimiting illustration of the agents for inhibiting the expression of a polypeptide of the invention, mention may in particular be made of compounds for decreasing keratinocyte differentiation, such as retinoic acid and isomers thereof, retinol and esters thereof, or vitamin D and derivatives thereof.

More particularly, the modulating agent may be an activator of the gene or protein expression of the polypeptides according to the invention.

A modulating agent suitable for the invention may also be an activator or an inhibitor of the enzymatic activity of acid ceramidase.

By way of nonlimiting illustration of the agents for inhibiting the enzymatic activity, mention may in particular be made of N-oleylethanolamine, certain ceramide analogues such as B13, and D-NMAPPD.

By way of nonlimiting illustration of the agents for activating the enzymatic activity, mention may in particular be made of saposins C and D and certain peptide fragments thereof, or amphiphilic chemical analogues of these natural activators.

More particularly, the modulating agent may be an activator of the enzymatic activity of the polypeptides according to the invention.

The present invention relates, in addition, to a method for screening for biological or chemical compounds or for physicochemical factors capable of modulating a biological or biochemical activity of a polypeptide according to the invention, comprising at least the steps consisting in:
a) bringing at least one polypeptide in accordance with the invention into contact with at least one chemical or biological test compound, and/or subjecting said polypeptide to said physicochemical factor, under conditions suitable for the manifestation of said biological or biochemical activity of said polypeptide, and

b) determining said biological or biochemical activity of said polypeptide.

According to one embodiment, the biological or biochemical activity of the polypeptide may be compared to a reference value.

A reference value may be obtained by measuring the biological or biochemical activity of the polypeptide in the absence of any biological or chemical test compound or physicochemical test factor.

In the event that this reference value measurement is carried out prior to the use of the biological or chemical test compound or of the physicochemical test factor, the method according to the invention may in addition make it possible, where appropriate, to assess the potential effectiveness of said compound.

This biological or biochemical activity may not be affected by the presence of said compound or, on the other hand, may be inhibited or stimulated.

In the event that an inhibitory effect is noted, the compound tested is capable of being used, for example, as an anti-aging active agent.

A method in accordance with the invention may be carried out on an isolated cell sample, obtained either from a skin biopsy or from cells in culture.

Advantageously, by way of a cell sample suitable for the invention, mention may be made of a keratinocyte sample.

Advantageously, a polypeptide used in a method according to the present invention may be acid ceramidase.

The present invention also relates to a method for screening for biological or chemical compounds capable of modulating the expression of a polypeptide in accordance with the invention, comprising at least the steps consisting in:

a) bringing at least one cell type capable of expressing a nucleic acid sequence encoding said polypeptide in accordance with the invention into contact with at least one chemical or biological test compound, under conditions suitable for the manifestation of the expression of said sequence, and

b) determining the expression of said nucleic acid sequence.
The expression of a nucleic acid sequence can be determined, for example, by means of oligonucleotide probes, by any protocol known to those skilled in the art.

By way of example of methods for detecting a nucleic acid sequence, mention may be made of the quantitative (Q-PCR) or nonquantitative polymerase chain reaction (PCR), in the presence or absence of reverse transcriptase (RT-PCR or Q-RT-PCR), of Northern blotting, of the ribonuclease protection assay method, of methods with DNA chips, of methods with transcriptome chips, of methods with oligonucleotide chips, and of in situ hybridization methods.

By way of example of agents suitable for the detection of a nucleic acid sequence, and in particular of mRNA, mention may be made of a labeled nucleic acid probe that can hybridize to said sequence.

Such a nucleic acid probe can be readily obtained by any method known to those skilled in the art.

Thus, the nucleic acid sequences in accordance with the invention may be used to prepare sense and/or antisense oligonucleotide primers, which hybridize, under high stringency conditions, to the sequence SEQ ID NO 1 or an analogue thereof.

The expression of a nucleic acid sequence in accordance with the invention can be compared to a reference value obtained, for example, by carrying out a method in accordance with the invention in the absence of test compound.

The expression of a nucleic acid sequence may also be determined, indirectly, by determining the expression of the polypeptide encoded by said sequence, by means of any technique known in the field, such as Western blotting, ELISA, the Bradford or Lowry method, or as indicated hereinafter.

The present invention also relates to a method for screening for biological or chemical compounds, or even for anti-aging active agents, capable of modulating the expression of a polypeptide in accordance with the invention, comprising at least the steps consisting in:

a) bringing at least one cell type capable of expressing a polypeptide in accordance with the invention into contact with at least one chemical or biological test compound, under conditions suitable for the manifestation of the expression of said polypeptide,

b) determining the polypeptide content, and
c) comparing said content determined in step b) to the content of said polypeptide determined in the absence of chemical or biological test compound.

The comparison carried out in step c) can make it possible to deduce a piece of information regarding the suitability of said tested compound for modulating the expression of a polypeptide in accordance with the invention.

A method in accordance with the invention may be carried out on an isolated cell sample.

The determination of a content of polypeptide in accordance with the invention may be carried out by means of any method known to those skilled in the art.

By way of methods for detecting a polypeptide, mention may be made of Western blotting, slot blotting, dot blotting, ELISA (Enzyme Linked Immunosorbent Assay) methods of the singleplex or multiplex type, proteomics or glycomics methods, staining polypeptides in a polyacrylamide gel with a silver-based stain, with Coomassie blue or with SYPRO, immunofluorescence, UV absorption, immunohistochemical methods in conventional, electron or confocal microscopy, FRET (fluorescence resonance energy transfer), TR-FRET (time resolved FRET) methods, FLIM (fluorescence lifetime imaging microscopy) methods, FSPIM (fluorescence spectral imaging microscopy) methods, FRAP (fluorescence recovery after photobleaching) methods, reporter-gene methods, AFM (atomic force microscopy) methods, surface plasmon resonance methods, microcalorimetry methods, flow cytometry methods, biosensor methods, radioimmunoassay (RIA) methods, isoelectric focusing methods, and enzyme assays, methods using peptide chips, sugar chips, antibody chips, mass spectrometry methods, and SELDI-TOF spectrometry methods (Ciphergen).

The methods in accordance with the invention may be carried out on a sample for example an isolated sample, of epithelium, in particular of epidermis, obtained from a skin biopsy or from an epithelial cell model, for example an epidermal cell model, or more advantageously from a noninvasive surface removal, in particular with adhesive tape (stripping tape), of stratum corneum or by simple washing.

A sample of epidermis can be taken by any method known to those skilled in the art.

These methods may be carried out by "stripping" techniques.

These strippings are sticky surfaces applied to the surface of the epidermis,
such as Blenderm® from 3M, D'squam (commercial adhesive from CuDERM), cyanoacrylate glue or the varnish stripping method. By virtue of these stripplings, the adherent corneocytes and the content of their intercellular spaces can be sampled and subsequently subjected to an extraction which makes it possible to access the protein content.

The taking of a sample suitable for the method may also be carried out more directly by "washing" the skin surface by means, for example, of accessories of the vane turbine type, of the spiral cell type (as described in patent FR 2 667 778) combined with a fluid circuit, or simply by addition/removal of a drop of buffer at the surface of the skin.

By way of indication of other sampling methods suitable for implementing the invention, mention may be made of methods based on scraping the upper part of the stratum corneum by means of a twin blade system. This technique makes it possible to collect squamae which can then be directly analyzed by various techniques in order to determine the mineral, amino acid or lipid contents.

It is understood that all the cosmetic or therapeutic compositions considered according to the invention use a physiologically acceptable medium.

For the purpose of the present invention, the term "physiologically acceptable medium" is intended to denote a medium suitable for the application of a composition to an epithelium or a keratin material, such as the skin, the scalp, the lips, the mucous membranes and keratin fibers such as the hair, the nails and body hairs, or where appropriate, by oral or parenteral administration.

For the purpose of the present invention, the term "therapeutic" is intended to denote a composition that can be used in the context of a prophylactic and/or curative treatment, or of a method for evaluating a state of an epithelium, and in particular of the epidermis.

According to another embodiment, a cosmetic or therapeutic composition in accordance with the invention may also comprise at least one cosmetic and/or therapeutic active agent.

As examples of active agents that can be used in the context of the present invention, mention may be made of cosmetic oils, such as silicone oils, plant oils of the triglyceride type, hydrocarbon-based oils such as Parleam oil and esters of fatty acids and of fatty alcohols.
It may also be possible to use other active agents which make it possible to improve the condition of the skin, such as hydrating or moisturizing active agents or active agents which make it possible to improve the natural lipid barrier, such as ceramides, cholesterol sulfates and/or fatty acids, and mixtures thereof.

It may also be possible to use enzymes which have an activity on the skin, such as proteases, lipases, glucosidases, amidases, cerebrosidases and/or melanases, and mixtures thereof.

As other examples of active agents suitable for implementing the present invention, mention may be made of: analgesic active agents, anti-yeast active agents, antibacterial active agents, antiparasitic active agents, antifungal active agents, antiviral active agents, steroidal anti-inflammatory active agents, anesthetic active agents, antipruritic active agents, keratolytic active agents, free-radical scavenger active agents, antiseborrhoeic active agents, antidendruff active agents, anti-acne active agents, active agents intended for preventing aging of the skin and/or for improving the condition thereof, anti-dermatitis active agents, antiirritant active agents, immunomodulatory active agents, active agents for the treatment of dry skin, antiperspirant active agents, antipsoriatic active agents, active agents for protecting against UV, antihistamine active agents, cicatrizing active agents, self-tanning active agents, antioxidants such as green tea or active fractions thereof, glycerol, laponite, caffeine, aromatic essential oils, colorants, depigmenting active agents, liporegulators, emollient, refreshing, deodorizing, desensitizing, bleaching or nourishing active agents, active agents for reducing skin differentiation and/or proliferation and/or pigmentation, and mixtures thereof.

In general, any composition of the invention may be applied to the skin (on any skin region of the body) or to the mucous membranes (buccal, jugal, gingival, genital, conjunctival, etc.).

In a known manner, a cosmetic composition may also contain adjuvants which are customary in the cosmetics field, such as hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic additives, preservatives, antioxidants, solvents, fragrances, fillers, screens, odor absorbers and dyestuffs.

The amounts of the various constituents of the compositions according to the invention are those conventionally used in the fields under consideration.

The amount of chemical or biological compound or of polypeptide, nucleic
acid sequence or modulating agent in accordance with the invention contained in a composition according to the invention, also referred to as "effective amount", of course depends on the nature of the compound and on the desired effect, and may therefore vary to a large extent.

To give an order of magnitude, a composition may contain a modulating agent in accordance with the invention or a polypeptide in an amount representing from 0.00001% to 50% of the total weight of the composition, in particular in an amount representing from 0.001% to 10% of the total weight of the composition, and more particularly in an amount representing from 0.1% to 1% of the total weight of the composition.

A composition according to the invention may be more particularly intended for reducing and/or treating conditions that may cause deterioration of the state of an epithelium, and in particular of an epidermis.

A state of an epithelium covered by the present invention may be a state linked to a dysfunction of terminal epithelial, in particular epidermal, differentiation, in particular of keratinocytes.

Such a state may be of chronological origin (i.e. linked to the time elapsed, such as skin aging) and/or a sign of a skin disorder, linked for example to photoaging.

Thus, a composition in accordance with the invention, in particular a cosmetic composition, may be for preventing and/or treating signs of epidermal aging.

A composition in accordance with the invention may in particular be for preventing and/or treating thinning of an epidermis and/or a loss of firmness, of elasticity, of density and/or of tonicity of an epidermis and/or the formation of wrinkles and fine lines.

According to another embodiment, a composition in accordance with the invention, in particular a cosmetic composition, may in particular be for preventing and/or treating cutaneous signs of dryness, in particular for preventing and/or treating dehydration of an epidermis.

A composition in accordance with the invention, in particular a therapeutic composition, may be more particularly for use in the treatment of a skin disorder such as a skin hydration disorder, for instance xerosis, parakeratosis, hyperkeratosis, ichthyosis, psoriasis, atopic dermatitis, eczema, rosacea, lichen, pruritus, a skin pathology having an
inflammatory component or resulting from an impairment of the immune response, desquamation, disruption of melanogenesis or of sebogenesis, alopecia, hirsutism, a cicatization disorder, or a skin disorder involving secretion phenomena and cell invasion processes, in particular in the context of malignant or benign neoplasias.

According to another aspect, the present invention also relates to the use of at least one polypeptide in accordance with the invention or of at least one nucleic acid sequence encoding said polypeptide, as a tool for characterizing, *in vitro* or *ex vivo*, a state of an epithelium, and in particular of an epidermis.

By way of example, it is possible to characterize, according to the invention, a state of an epithelium chosen from dryness, chronological aging and photoaging of an epidermis.

Thus, as is specified above, according to another of its aspects, the present invention relates to noninvasive methods for characterizing the surface state of a nonpathological epidermis or else the effectiveness of a cosmetic or therapeutic treatment, directed toward qualitatively or quantitatively characterizing the expression of acid ceramidase, or of a derivative or fragment thereof.

These methods are particularly advantageous since their implementation does not require obligatory recourse to a surgical technique for carrying out such a characterization. An extract of the epidermis can thus be obtained by simple stripping and directly analyzed by a conventional analytical technique, in particular as described above.

According to one embodiment, a method for characterizing a state of an epithelium, for example an epidermis, comprises at least the steps consisting in:

a) determining, in a sample of said epithelium, the content of polypeptide in accordance with the invention, or of a nucleic acid sequence encoding said polypeptide, and

b) comparing said content determined in step a) to a reference value.

Advantageously, a method of the invention is noninvasive.

A method of the invention is advantageously carried out on an isolated sample.

According to one embodiment, a method according to the invention may be carried out on a sample of epithelium, and in particular of an epidermis, taken from an individual.

A method according to the invention may also be carried out on a sample of
epithelium, and in particular of epidermis, taken from an epithelial cell model, in particular an epidermal cell model, or from a reconstructed isolated skin in order to qualify the state thereof.

A sample of epithelium may be taken by any method known to those skilled in the art.

A method according to the invention may be carried out in vivo, in vitro or ex vivo.

A reference value may, for example, be a content of polypeptide or of nucleic acid sequence determined on a sample of epidermis taken from an epithelium, and in particular from normal skin, i.e. skin that is satisfactory from a physiological point of view, like, for example, young skin.

A reference value may be measured in parallel with or following the determination of said content of a polypeptide or of a nucleic acid sequence.

A comparison of a determined content with a reference value may make it possible to evaluate a deviation relative to this value.

The analysis of the intensity and/or of the nature of this deviation (negative or positive) may be informative with regard to the state of the epidermis.

The characterization of a state of an epidermis may be indicative of a possible skin disorder which may be corrected by the use of compounds capable of modulating the expression of a polypeptide of the invention.

According to one embodiment, a method according to the invention may be implemented in a method for the in vivo, in vitro or ex vivo diagnosis of aging of an epithelium, and in particular of the epidermis, in an individual.

A polypeptide suitable for carrying out the method according to the invention may advantageously be acid ceramidase.

The determination of the content of polypeptide in accordance with the invention or of nucleic acids in accordance with the invention in a sample of epidermis may be carried out by any protocol known to those skilled in the art.

By way of methods of detecting a polypeptide, mention may be made of those mentioned above.

Thus, it is possible to envision detecting the presence of a polypeptide in accordance with the invention by means of an antibody, where appropriate in a labeled
An antibody that can be used as a tool for evaluating a state of an epidermis can be obtained by any method known to those skilled in the art, as described in "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990). Advantageously, the antibodies used may be recombinant antibodies such as those developed by the company Antibodies-by-design.

A nucleic acid sequence suitable for implementing a method according to the invention may advantageously be a nucleic acid sequence encoding acid ceramidase, for example of the mRNA type.

By way of example of methods for detecting nucleic acids in accordance with the invention, mention may be made of the methods mentioned above.

The present invention also relates to a nontherapeutic method for demonstrating an effect of a treatment capable of causing regression of an epithelial disorder, in particular a skin or scalp disorder, in an individual, comprising at least the steps consisting in:

a) carrying out, before the treatment, at least a first determination, in a first sample of an epithelium taken from said individual, of a biological or biochemical activity and/or of the expression of a polypeptide in accordance with the invention, or of the expression of a nucleic acid sequence encoding said polypeptide,

b) carrying out, after the treatment, at least a second determination, in a second sample of an epithelium taken from said individual, of said biological or biochemical activity and/or of said expression of said polypeptide or of said expression of said nucleic acid sequence, determined in step a), and

c) comparing the first and second determinations, in particular in order to deduce therefrom information relating to at least one effect of the treatment.

Such a treatment may in particular be a cosmetic treatment.

In particular, the treatment of which the effect is to be evaluated may be a treatment for relieving or reducing the signs of skin aging or a scalp disorder.

The biological or biochemical activity and the expression of a polypeptide may be determined as indicated above.

According to another aspect, the present invention relates to a method for the cosmetic treatment of the signs of skin aging, comprising at least one step consisting in
applying at least one cosmetic composition in accordance with the invention to at least one part of the skin, mucous membranes and/or keratin fibers.

According to another aspect, the present invention relates to the use of an effective amount of at least one polypeptide in accordance with the invention or of at least one agent for modulating the expression of said polypeptide, for the preparation of and/or for improving a pluristratified cell model, in particular of epidermal or mucosal type, and in particular a reconstructed skin model.

For the purpose of the invention, the term "reconstructed skin model" is intended to denote a model in which various cell types are combined, such as in particular the natural constituents of the skin, like for example keratinocytes, fibroblasts, Langerhans cells and melanocytes.

The cells of the fibroblast type may or may not be irradiated.

Such models and the preparation thereof are known to those skilled in the art.

For the purpose of the present invention, "a" should be understood, unless otherwise indicated, in the sense of "at least one".

The examples presented below are given by way of nonlimiting illustration of the invention.

**EXAMPLE 1**

*Analysis of samples taken by varnish stripping on various skin regions of an individual*

The analyses are carried out using varnish stripplings performed on the legs.

The individuals suitable for the study are put into 4 groups.

The AS group corresponds to group 1: dry menopausal individuals, n = 15.

The AN group corresponds to group 2: normal menopausal individuals, n = 13.

The JS group corresponds to group 3: dry young individuals, n = 16.

The JN group corresponds to group 4: normal young individuals, n = 14.

1: Preparation of acetone powders

Two varnish stripplings (B. Mehul et al., J Biol Chem 2000, Apr 28; 275(17): 12841-7) of 10 cm² are placed into 20 ml of acetone. The corneocytes become detached. The mixture is filtered through a 40 µm nylon membrane. Three successive rinses are
carried out with the same volume of acetone. The suspension is finally filtered on a vacuum pump. Acetone powders of *stratum corneum* are obtained in dry form.

2: Sample extraction

An extraction is carried out under denaturing conditions. To do this, a prewash is carried out with a volume (100 µl) of PBS buffer (phosphate buffered saline) +0.1% Triton X100, which is added per mg of acetone powder. The mixture is ground in a Potter and centrifuged. The corneocyte pellet is recovered. It is extracted with the same volume (100 µl/mg) of Laemmli buffer containing 0.0625 mM Tris, pH 6.8, 200 mM DTT, 2% SDS and 10% glycerol. The mixture is heated at boiling temperature for 10 minutes, and is then ground and centrifuged for 10 minutes at 10,000 g. The supernatant is recovered. A protein assay is carried out according to the Bradford technique with the Bradford reagent (Bio-Rad protein assay). The samples are adjusted to 1 mg/ml.

3: Protein analysis by Western blotting

The proteins are separated by SDS-PAGE electrophoresis. After semi-dry blotting onto a PVDF membrane (Immobilon-P, Millipore) according to a standard protocol, the proteins are incubated with the anti-acid ceramidase primary antibody (BD612303) overnight at 4°C. The second incubation is then carried out with the secondary antibody (mouse IgG HRP conjugate) (Bio-Rad) directed against the primary antibody for 1h 30 at ambient temperature. The presence of acid ceramidase on the membrane is revealed by immunodetection using the ECL Plus kit (Amersham). The membrane is then stained with amido black in order to detect the total proteins present on the membrane. The image is acquired with FluorSmax (Biorad) and the bands are quantified using the Quantity-one software (Biorad).

4: Results

The results are expressed as delta cnt*mm² of the protein of interest/delta cnt*mm² of total proteins.

Methodology:

- 2-way (age and type) analysis of variance of skin taking into account the interaction of these two factors + 1-way (group) analysis of variance and Tukey's multiple
comparison test. Since the normality and homoscedasticity conditions were not verified, the analysis was carried out after logarithmic transformation. The statistical analysis was carried out with the SAS version 8.2 and SPSS version 12 software packages.

All the tests were carried out at the 5% two-sided threshold.

The table below gives the mean results and also their standard errors of the mean (SEM).

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A significant decrease is noted in the expression of acid ceramidase during skin aging (p = 0.047).
**Sequence listing**

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SEQ ID NO 11
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SEQ ID NO 12
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SEQ ID NO 13
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CLAIMS

1. The cosmetic use of an effective amount of at least one polypeptide having an amino acid sequence encoded by a nucleic acid sequence represented entirely or partly by a sequence represented by SEQ ID NO 1, an analogue thereof or a fragment thereof, of at least one nucleic sequence encoding such a polypeptide or of at least one agent for modulating the expression or the biological or biochemical activity of such a polypeptide, as an agent that is of use for preventing and/or treating the signs of skin aging.

2. The use of an effective amount of at least one polypeptide having an amino acid sequence encoded by a nucleic acid sequence represented entirely or partly by a sequence represented by SEQ ID NO 1, an analogue thereof or a fragment thereof, of at least one nucleic acid sequence encoding such a polypeptide or of at least one agent for modulating the expression or the biological or biochemical activity of such a polypeptide, for the preparation of a therapeutic composition for preventing and/or treating the signs of skin aging.

3. The use as claimed in claim 1 or 2, in which said polypeptide has an amino acid sequence represented entirely or partly by a sequence represented by SEQ ID NO 2, an analogue thereof or a fragment thereof.

4. The use as claimed in one of claims 1 to 3, in which said polypeptide has an amino acid sequence chosen from SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12 and SEQ ID NO 13.

5. The use as claimed in any one of the preceding claims, in which the modulating agent is an activator of the gene or protein expression of said polypeptide.

6. The use as claimed in the preceding claim, in which said activating agent is chosen from PPAR-gamma agonists, inhibitors of histone deacetylase, such as butyrate and derivatives thereof, and molecules such as 2-(3,4,5-trimethoxyphenylamino)pyrrolo-[2,3-d]pyrimidine and derivatives thereof.

7. The use as claimed in one of claims 1 to 4, in which the modulating agent is an activator of the enzymatic activity of said polypeptide.

8. The use as claimed in any one of the preceding claims, in which said agent
that is of use or said composition is for preventing and/or treating thinning of an epidermis and/or a loss of firmness, of elasticity, of density and/or of tonicity of an epidermis and/or the formation of wrinkles and fine lines.

9. The use as claimed in any one of claims 1 to 7, in which said agent that is of use or said composition is for preventing and/or treating cutaneous signs of dryness, in particular for preventing and/or treating dehydration of an epidermis.

10. The use of at least one polypeptide as defined in any one of claims 1 to 4, or of at least one nucleic acid sequence encoding said polypeptide, as a tool for characterizing, in vitro or ex vivo, a state of an epithelium.

11. The use as claimed in the preceding claim, in which the state of the epithelium is chosen from dryness, chronological aging and photoaging of an epidermis.

12. A method for characterizing a state of an epithelium, comprising at least the steps consisting in:
   a) determining, in a sample of said epithelium, the content of polypeptide as defined according to one of claims 1 to 4 or a nucleic acid sequence encoding said polypeptide, and
   b) comparing said content determined in step a) to a reference value.

13. The method as claimed in the preceding claim, characterized in that it is noninvasive.

14. A method for screening for anti-aging active agents, comprising at least the steps consisting in:
   a) bringing at least one cell type capable of expressing a polypeptide as defined according to any one of claims 1 to 4 into contact with at least one chemical or biological test compound, under conditions suitable for manifestation of the expression of said polypeptide,
   b) determining the content of said polypeptide.

15. A cosmetic method for characterizing the effectiveness of a cosmetic or therapeutic treatment aiming to compensate for the signs of skin aging, comprising at least the qualitative or quantitative characterization of the expression and/or of the biological or biochemical activity of a polypeptide as defined in claims 1 to 4.

16. The use of an effective amount of at least one polypeptide as defined according to one of claims 1 to 4, or of at least one agent for modulating the expression of
said polypeptide, for the preparation of and/or for improving a pluristratified cell model, in particular a reconstructed skin model.
## INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K8/66  A61K8/64  A61K8/72  A61K38/17  A61K38/46
A61P17/00  A61Q19/00  A61Q19/08  C12Q1/02  C12Q1/68
G01N33/68  A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>WO 01/55408 A (MEMOREC MEDICAL MOLECULAR RES [DE]; HOFBRANN KAY [DE]; CONRADT MARCUS) 2 August 2001 (2001-08-02) page 1, paragraph 1 - page 4, paragraph 3; figures 1,2</td>
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<td>EP 1 287 815 A (COSMOFERM B V [NL]) 5 March 2003 (2003-03-05) abstract page 2, paragraph 1-5 page 2, paragraph 7 - page 3, paragraph 16 page 4, paragraphs 26,27; compound 1 page 6, paragraph 36 - page 7, paragraph 56; examples 1-9</td>
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Further documents are listed in the continuation of Box C

See patent family annex

**Date of the actual completion of the international search**

9 April 2009

**Date of mailing of the international search report**

21/04/2009

Name and mailing address of the ISA/
European Patent Office, P B 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040,
Fax (+31-70) 340-3016

Authorized officer
Mateo Rosel I. A
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<td>EP 1 508 328 A (OREAL [FR]) 23 February 2005 (2005-02-23) abstract page 4, paragraphs 23,24 page 8, paragraph 54 - page 9, paragraph 62; cl aims 5,6,10</td>
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<td>JENSEN JENS-MICHAEL ET AL: &quot;Acid and neutral sphingomyelinase, ceramide synthase, and acid ceramidase activities in cutaneous aging.&quot; EXPERIMENTAL DERMATOLOGY AUG 2005, vol. 14, no. 8, August 2005 (2005-08), pages 609-618, XP002489513 ISSN: 0906-6705 abstract page 610, left-hand column, last paragraph - right-hand column, paragraph 2; table 1 page 612, left-hand column, paragraph 2 - page 616, right-hand column, paragraph 3; figure 4</td>
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<td>HOUVEN EVI ET AL: &quot;Differentiation-associated expression of serine palmitoyltransferase and ceramidase isoforms in cultured keratinocytes and epidermis&quot; JOURNAL OF LIPID RESEARCH, vol. 47, no. 5, May 2006 (2006-05), pages 1063-1070, XP002489516 ISSN: 0022-2275 cited in the application abstract page 1063, left-hand column, last paragraph - page 1064, left-hand column, paragraph 2; figure 1; tables 1-4 page 1068, right-hand column, paragraph 2 - page 1069, right-hand column, paragraph 2</td>
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