

Protein biomarkers in vernix with potential to predict the development of atopic eczema in early childhood

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Abstract

Background: Atopic eczema (AE) is a chronic inflammatory skin disease, which has increased in prevalence. Evidence points toward lifestyle as a major risk factor. AE is often the first symptom early in life later followed by food allergy, asthma, and allergic rhinitis. Thus, there is a great need to find early, preferentially noninvasive, biomarkers to identify individuals that are predisposed to AE with the goal to prevent disease development.

Objective: To investigate whether the protein abundances in vernix can predict later development of AE.

Methods: Vernix collected at birth from 34 newborns within the Assessment of Lifestyle and Allergic Disease During Infancy (ALADDIN) birth cohort was included in the study. At 2 years of age, 18 children had developed AE. Vernix proteins were identified and quantified with liquid chromatography coupled to tandem mass spectrometry.

Results: We identified and quantified 203 proteins in all vernix samples. An orthogonal projections to latent structures-discriminant analysis (OPLS-DA) model was found with $R^2 = 0.85$, $Q^2 = 0.39$, and discrimination power between the AE and healthy group of 73.5%. Polyubiquitin-C and calmodulin-like protein 5 showed strong negative correlation to the AE group, with a correlation coefficient of 0.73 and 0.68, respectively, and a P -value of 8.2×10^{-7} and 1.8×10^{-5} , respectively. For these two proteins, the OPLS-DA model showed a prediction accuracy of 91.2%.

Conclusion: The protein abundances in vernix, and particularly that of polyubiquitin-C and calmodulin-like protein 5, are promising candidates as biomarkers for the identification of newborns predisposed to develop AE.

The prevalence of AE has rapidly increased and is estimated to 15–30% in children and 2–10% in adults with the highest prevalence found in Northern Europe (1). The pathogenesis

of AE is likely to result from the combination of a disturbed skin barrier and inappropriate immune responses with contributions from both genetic and environmental factors. Atopic eczema is often the first symptom early in life of allergic disorders that many times progress to food allergy, asthma, and allergic rhinitis, a phenomenon known as the atopic march (2, 3). A recent report highlighted the need to identify different early biomarkers in AE with the goal to stop or even reverse the atopic march (4).

Inverse relation has been found between the number of anthroposophic lifestyle characteristics and the risk of atopy in school children of families with an anthroposophic lifestyle

Abbreviations

ACN, acetonitrile; AE, atopic eczema; ALADDIN, Assessment of Lifestyle and Allergic Disease During Infancy; AUC, area under curve; LC-MS/MS, liquid chromatography tandem mass spectrometry; OPLS-DA, orthogonal projections to latent structures-discriminant analysis; PANTHER, Protein ANALysis Through Evolutionary Relationships; ROC, receiver operating characteristic.

(5, 6). Anthroposophic lifestyle includes biodynamic food containing live lactobacilli and restrictive use of antibiotics, antipyretics, and vaccinations (7). To investigate how lifestyle factors influence the development of allergic disease early in life, the birth cohort designated Assessment of Lifestyle and Allergic Disease During Infancy (ALADDIN) was initiated (7). In this birth cohort, the children born into families with an anthroposophic or nonanthroposophic life style have been followed prospectively from birth and many different samples have been collected, among those vernix caseosa (vernix). Vernix is a yellow-white, cheese-like, material consisting of sebum, lanugo hairs, and desquamated squamous cells, which covers the neonate's skin during the last trimester of gestation (8, 9). No other animal species produces vernix, making this material a unique human skin barrier film. It is believed to exhibit waterproofing, antioxidant, temperature-regulating, and anti-infective functions (8). The composition of vernix is mainly water (80.5%), lipids (10.3%), and proteins (9.1%) (9). Vernix is produced by the sebaceous glands, and in addition, lanugo hairs and desquamated squamous cells contribute to the formation of vernix (8). Thus, the origin of proteins found in vernix is to a large part from the fetal epidermis.

In this study, we investigated whether protein biomarkers in vernix, which reflects the composition of the infant's epidermal barrier, can predict later development of AE. As a source of biomarkers, vernix is attractive because it is usually abundant and its collection is noninvasive. We also addressed whether lifestyle can influence the protein composition of vernix by further dividing the AE and healthy control groups into three lifestyle categories: anthroposophic, partly anthroposophic, and nonanthroposophic.

Materials and methods

Study population

The study was designed to compare vernix collected at birth from children who had developed AE at 2 years of age with those who remained healthy. According to the inclusion criteria, based on doctor's diagnosis and the criteria of the UK Working Party's refinement of the Hanifin and Rajka criteria (10), 20 children had developed AE at 2 years of age. Due to limited amount of vernix from two of these children, 18 could be included. The healthy controls were selected among children with no current or history of AE or other allergic diseases and not allergen sensitized at 2 years of age. Furthermore, the healthy controls were selected from children where enough amount of vernix had been collected and to match the three lifestyle groups, gender, and parental characteristics of the AE children (see Table 1). Sixteen children who fulfilled all these requirements were included in the study as healthy controls. The anthroposophic, partly anthroposophic, and nonanthroposophic lifestyle groups were classified based on choice of Maternal-Child Health Centers and parental responses to a questionnaire, described in detail elsewhere (7). The study was approved by the Regional Ethical Review Board in Stockholm, and all parents have given their written informed consent.

Determination of sensitization

Parental allergen sensitization was defined by Phadiatop[®] (Phadia AB, Uppsala, Sweden) containing a mix of 11 inhalant allergens. Blood from the children at the age of 2 years were analyzed by ImmunoCAP[®] (Phadia AB) for IgE to seven common allergens (hen's egg, cow's milk, peanut, cat, dog, birch, and timothy). A study subject was classified as allergen sensitized if the IgE level was ≥ 0.35 kU_A/l for the parents in Phadiatop[®] and for the children in at least one of the seven allergens (7).

Collection of vernix

Vernix from newborns was collected by midwives and stored at -80°C . The estimated amount of vernix present on the whole body and the location from where the vernix was collected is presented in Table 1.

Extraction of proteins

Vernix (15 mg) from each individual was mixed with 50 mM ammonium bicarbonate (BDH Laboratory Supplies, Poole, UK)/1 M urea (Invitrogen, Carlsbad, CA, USA), incubated for 15 min on ice followed by sonication (10 cycles, each cycle 20 s with 40% amplitude) on ice using a probe (Vibra-Cell[™] CV18, Sonics & Materials, Newtown, CT, USA). The lipids were pelleted by centrifugation for 30 min at 13 000 g, and the protein concentration in the supernatant was determined with the Bio-Rad detergent compatible protein assay (Bio-Rad, Hercules, CA, USA). A fraction of every sample was separated on 4–12% NuPAGE Bis-Tris gels (Invitrogen) prior to proteomic analysis, to verify the presence of proteins and confirm that the extraction of proteins was not destructive.

Liquid chromatography tandem mass spectrometry

The procedure for protein digestion is described in Supporting information, Data S1. Digested samples were acidified and cleaned with C18 StageTips according to the manufacturer's description (Thermo Fisher Scientific Inc., Waltham, MA, USA). Eluted peptides were dried and re-suspended in 3% acetonitrile (ACN) and 0.2% formic acid (Sigma-Fluka, Munich, Germany). Detailed description of the procedure of liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses can be found in Data S1.

Data analysis

Tandem mass spectra were extracted using Raw2MGF v.2.1.0 [in-house developed software (11)], and the resulting Mascot generic files were searched against the human Swiss-Prot protein database (v. 2012.06, reversed protein sequences were added to the database for decoy search) using the Mascot 2.3.02 (Matrix Science Ltd., London, UK), with trypsin for generation of peptides and allowing

Table 1 Demographic data including allergic symptoms, sensitization, and environmental factors in families with an anthroposophic, partly anthroposophic, and nonanthroposophic lifestyle. [Categorical variables: *n/N* (%). Continuous variables: mean $\pm$ SD]

	Atopic eczema				Healthy				P**	Nonanthro.	Partly anthro.	P***
	Atopic eczema	Healthy	P*	Anthro.	Partly anthro.	Nonanthro.	Anthro.					
n	18	16		5	7	6	6	6	6	4		
Child												
Sex (female)	7/18 (39)	7/16 (44)	1.0	2/5 (40)	2/7 (29)	3/6 (50)	1/6 (17)	3/6 (50)	3/4 (75)	0.22		
Birth weight (g)	3556 ± 500	3607 ± 481	0.77	3797 ± 578	3390 ± 584	3548 ± 278	3474 ± 510	3870 ± 465	3410 ± 374	0.25		
Birth height (cm)	51.0 ± 1.4	50.9 ± 2.6	0.82	51.2 ± 1.3	50.9 ± 1.6	50.3 ± 1.2	49.7 ± 3.2	52.2 ± 2.2	51.0 ± 1.6	0.27		
Gestational week	39.4 ± 1.2	39.5 ± 0.8	0.91	39.2 ± 1.1	39.4 ± 1.0	39.5 ± 1.6	39.3 ± 0.8	39.7 ± 1.0	39.5 ± 0.6	0.76		
Having older siblings	10/15 (67)	9/16 (56)	0.72	3/5 (60)	5/6 (83)	2/4 (50)	4/6 (67)	1/6 (17)	4/4 (100)	0.05		
24 months SCORAD	19.3 (4.5–41)	–	–	24.5 (10.5–37)	16.2 (4.5–41)	14 (4.5–27.5)	–	–	–	–		
median (range) (10)												
Reactions to food†	8/18 (44)	–	–	2/5 (40)	2/7 (29)	4/6 (67)	–	–	–	–		
Recurrent wheeze‡	2/18 (11)	–	–	1/5 (20)	1/7 (14)	0/6 (0)	–	–	–	–		
Sensitized§	7/18 (39)	–	–	2/5 (40)	2/6 (33)	3/6 (50)	–	–	–	–		
Parents												
Age mother at birth of child, years	30.0 ± 5.4	31.5 ± 4.6	0.41	27 ± 5.4	31 ± 5.8	31 ± 5.1	30.8 ± 5.1	30.8 ± 5.6	33.5 ± 1.3	0.63		
Age father at birth of child, years	33.8 ± 6.5	34.7 ± 5.4	0.67	32 ± 7	36 ± 7.2	33 ± 5.6	36.7 ± 6.9	31.0 ± 3.1	36.5 ± 2.6	0.16		
Mother reported eczema	3/18 (17)	4/16 (25)	0.68	1/5 (20)	1/7 (14)	1/6 (17)	3/6 (50)	0/6 (0)	1/4 (25)	0.13		
Father reported eczema	4/18 (22)	2/15 (13)	0.67	1/5 (20)	1/7 (14)	2/6 (33)	1/6 (17)	0/5 (0)	1/4 (25)	0.71		
Mother sensitized¶	4/18 (22)	6/16 (38)	0.46	2/5 (40)	0/7 (0)	2/6 (33)	3/6 (50)	2/6 (33)	1/4 (25)	0.83		
Father sensitized¶	5/17 (29)	5/14 (36)	1.0	2/5 (40)	2/6 (33)	1/6 (17)	2/6 (33)	2/5 (40)	1/3 (33)	1.0		
Pregnancy												
Mother												
Antibiotics	2/18 (11)	2/16 (13)	1.0	1/5 (20)	0/7 (0)	1/6 (17)	0/6 (0)	1/6 (17)	1/4 (25)	0.70		
Vegetarian diet	3/18 (17)	4/16 (25)	0.68	1/5 (20)	1/7 (14)	1/6 (17)	3/6 (50)	1/6 (17)	0/4 (0)	0.15		
Organic/biodynamic diet	5/18 (28)	1/15 (7)	0.18	3/5 (60)	2/7 (29)	0/6 (0)	1/6 (17)	0/6 (0)	0/4 (0)	1.0		
Smoking	1/18 (6)	0/16 (0)	1.0	0/5 (0)	0/7 (0)	1/6 (17)	0/6 (0)	0/6 (0)	0/4 (0)	–		
Snuffing	0/18 (0)	0/16 (0)	–	0/5 (0)	0/7 (0)	0/6 (0)	0/6 (0)	0/6 (0)	0/4 (0)	–		
Father smoking	4/18 (22)	1/15 (7)	0.35	1/5 (20)	2/7 (29)	1/6 (17)	1/6 (17)	0/5 (0)	0/4 (0)	1.0		
Delivery												
Home delivery	4/18 (22)	2/16 (13)	0.66	2/5 (40)	2/7 (29)	0/6 (0)	2/6 (33)	0/6 (0)	0/4 (0)	0.30		
Vacuum extraction	0/18 (0)	2/16 (13)	0.21	0/5 (0)	0/7 (0)	0/6 (0)	1/6 (17)	1/6 (17)	0/4 (0)	1.0		
Acute cesarean	2/18 (11)	1/16 (6)	1.0	0/5 (0)	0/7 (0)	2/6 (33)	1/6 (17)	0/6 (0)	0/4 (0)	–		
Elective cesarean	1/18 (6)	0/16 (0)	1.0	0/5 (0)	1/7 (14)	0/6 (0)	0/6 (0)	0/6 (0)	0/4 (0)	–		
Anesthesia	7/18 (39)	11/16 (69)	0.1	2/5 (40)	0/7 (0)	5/6 (83)	3/6 (50)	4/6 (67)	4/4 (100)	0.33		
Antibiotics	1/18 (6)	1/16 (6)	1.0	0/5 (0)	0/7 (0)	1/6 (17)	1/6 (17)	0/6 (0)	0/4 (0)	1.0		
Vernix												
Location (n)††	2a, 8b, 3c, 3e, 2f (18)	6b, 2c, 1d, 4e, 3f (16)	0.62	4b, 1e (5)	1a, 2b, 2c, 2e (7)	1a, 2b, 1c, 1e, 1f (6)	2b, 1c, 1e, 2f (6)	1b, 1c, 1d, 2e, 1f (6)	3b, 1e (4)	0.86		
Estimated amount on the body [Median (range)] n‡‡	3 (1–4) (18)	3 (1–4) (16)	0.92	3 (1–4) (5)	3 (1–4) (6)	3 (2–4) (5)	3 (1–4) (4)	3 (3–4) (5)	3.5 (3–4) (4)	0.22		
Protein concentration after extraction (mg/ml) n§§	1.26 ± 0.47 (18)	1.27 ± 0.45 (16)	0.94	0.93 ± 0.17 (5)	1.33 ± 0.41 (7)	1.44 ± 0.60 (6)	1.36 ± 0.61 (6)	1.11 ± 0.20 (6)	1.37 ± 0.49 (4)	0.58		

Categorical variables: *P* for trend (Fisher's exact test), continuous variables: *P* for comparisons of means (one-way ANOVA).Bold indicates *P* < 0.05.**P* for comparison of the atopic eczema (AE) group vs the healthy group.***P* for comparison of the lifestyle groups anthro. vs partly anthro. vs nonanthro within the AE group and the healthy group, respectively.

†Reactions to food were defined as acute onset of symptoms such as skin reactions, wheezing, vomiting, or diarrhea on more than one occasion after ingestion or contact with a particular type of food (24).

‡Recurrent wheeze was defined as three or more episodes of wheeze since the last examination (24)

§Classified as sensitized if IgE level was ≥ 0.35 kU_A/l for at least one of the seven allergens analyzed using ImmunoCap[™] (Phadia AB).¶Classified as sensitized if IgE level was ≥ 0.35 kU_A/l measured using Phadiatop[®] (Phadia AB).

††a: arm pit, b: back, c: groin, d: leg, e: other or mixed, f: not indicated; reported by the midwives.

‡‡1: very small, 2: small, 3: medium, 4: large; reported by the midwives.

§§Measured by the DC protein assay (Bio-Rad).

for one missed cleavage site. Mass tolerance was set to 10 ppm for peptides and 0.02 Da for the fragment ions. Carbamidomethylation of cysteine was specified as a fixed modification, whereas oxidation of methionine and deamidation of asparagine and glutamine were defined as variable modifications. Proteins were grouped into protein classes with the tools in Protein ANalysis THrough Evolutionary Relationships (PANTHER) available at <http://www.pantherdb.org>.

Quantitation

Quantitative information was extracted using in-house developed software Quanti v 2.5.3.1 (11). For quantitative purposes only reliably identified (false discovery rate <0.01), unmodified peptides with unique sequences were considered and only proteins discovered in all samples with at least two such peptides were quantified. For each protein, one database identifier (ID) was selected, covering all the identified peptide sequences for this specific protein. If two protein IDs had partial intersection, then all the peptides belonging to this intersection were excluded from the analysis. The results from Quanti v 2.5.3.1 are reported as a set of relative protein abundances scaled to 1.0 as the geometric mean of the abundance of each protein over all samples. Relative abundance values were log-scaled to reveal down-regulated proteins with the same significance as up-regulated.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 20 software (Chicago, IL, USA). A P -value < 0.05 was regarded as statistically significant. Fischer's exact test (for categorical variables) and ANOVA (for comparisons of means of continuous variables) were used for the comparison of demographic and exposure variables between the groups in Table 1. ANOVA was also used in Fig. 3C and D to compare the relative protein abundances of polyubiquitin-C and calmodulin-like protein 5 in the different groups. In Table 2 and Supplementary Table S1, P -values were calculated using Student's t -test and correlation coefficients were calculated using Pearson's Product-Moment Correlation. The orthogonal projections to latent structures-discriminant analysis (OPLS-DA) method was employed for discrimination between the AE and healthy control groups. The OPLS-DA is a supervised classification technique which convolutes systematic variation and maximizes the difference between observations according to provided classification. This will display which variables are responsible for class discrimination (12). Classification power was determined with receiver operating characteristic (ROC) analysis. Receiver operating characteristic curves show the predictive accuracy of the cross-validated OPLS-DA model depicting the equal probability of the false-positive and false-negative results (13). OPLS-DA modeling was performed using SIMCA 13 (Umetrics, Umeå, Sweden).

Results

Study population

Demographic data and the amount, location of collection, and protein concentration of vernix for the 34 children are presented in Table 1. There were no significant differences between any groups, except for the lifestyle groups within the AE group regarding anesthesia at delivery ($P = 0.01$) (Table 1).

Proteins identified

After LC-MS/MS analyses, 208 proteins were identified, with at least two peptides matching and a false discovery rate of less than 1%, and quantified in all 34 vernix samples. Three protein entries for the hemoglobin subunits (HBA_HUMAN, HBB_HUMAN, and HBD_HUMAN) were considered blood contaminants and were excluded from further analysis. Furthermore, two proteins were removed due to their high correlation with similar proteins: CALM_HUMAN highly correlates with CALL5_HUMAN ($R = 0.862$) and probably represents a different isoform of the same protein, and K2C4_HUMAN is also known to be co-expressed with K1C13_HUMAN ($R = 0.96$). Thus, a final list of 203 proteins was used for statistical analysis, classification modeling using SIMCA (Supplementary Table S1) and categorization of proteins into functional classes utilizing PANTHER (Fig. 1).

Proteins with most significant differences between the AE and healthy group

The 30 proteins with highest impact on the classification of individuals into the AE or healthy group are presented in Table 2. Among the proteins present at higher levels in the AE group, peroxiredoxin-2 and serpin A12 had the highest impact (OPLS-DA model loadings -0.127 and -0.123 , respectively), and among the proteins present at lower levels in the AE group, polyubiquitin-C and calmodulin-like protein 5 contributed most (OPLS-DA model loadings 0.166 and 0.157 , respectively). Possible confounders were investigated using logistic regressions models. All the variables in Table 1 were included one at a time (due to the limited amount of data) together with peroxiredoxin-2, serpin A12, polyubiquitin-C, and calmodulin-like protein 5 in individual models with AE as outcome. The association between the protein composition of vernix and AE was still statistically significant, regardless of which variable that was included; thus, no confounders were present. To investigate the presence of up- or down-regulated proteins in vernix from newborns that had developed AE at 2 years of age compared with vernix from children that remained healthy, the relative protein abundances were calculated. An OPLS-DA model was found for discrimination between AE and healthy control samples with $R^2 = 0.85$ and $Q^2 = 0.39$ (Fig. 2A). Although the OPLS-DA model itself showed clear separation, we present results of sevenfold cross-validation of this model to avoid overestimation of predictive power in the modeling (14). Receiver operating characteristic curve analysis of the OPLS-DA model cross-validation results showed 73.5% predictive

Table 2 The proteins that differ most between the atopic eczema (AE) and the healthy group

Rank*	Protein ID (SwissProt)		Correlation coefficient to health status§			Protein ID (SwissProt)		Correlation coefficient to health status§		
	Protein	P-value†	OPLS-DA model loadings‡	Protein	P-value†	OPLS-DA model loadings‡	Protein	P-value†	OPLS-DA model loadings‡	Correlation coefficient to health status§
Higher relative abundance in AE										
1	PRDX2_HUMAN	Peroxiredoxin-2	6.35E-04	-0.127	-0.563	UBC_HUMAN	Polyubiquitin-C	8.21E-07	0.166	0.729
2	SPA12_HUMAN	Serpin A12	6.22E-03	-0.123	-0.469	CALL5_HUMAN	Calmodulin-like protein 5	1.80E-05	0.157	0.684
3	ASAH1_HUMAN	Acid ceramidase	6.56E-03	-0.116	-0.489	TPM4_HUMAN	Tropomyosin alpha-4 chain	5.16E-04	0.130	0.559
4	BPIA1_HUMAN	BPI fold-containing family A member 1	3.45E-03	-0.111	-0.512	CD59_HUMAN	CD59 glycoprotein	1.80E-03	0.121	0.514
5	DESP_HUMAN	Desmoplakin	7.64E-03	-0.110	-0.460	INVO_HUMAN	Involucrin	2.28E-03	0.119	0.522
6	BPIB1_HUMAN	BPI fold-containing family B member 1	3.95E-03	-0.109	-0.507	S10A7_HUMAN	Protein S100-A7	3.23E-03	0.118	0.524
7	K1C13_HUMAN	Keratin, type I cytoskeletal 13	1.83E-03	-0.109	-0.525	AATC_HUMAN	Aspartate aminotransferase, cytoplasmic	3.07E-03	0.113	0.492
8	GDPD3_HUMAN	Glycerophosphodiester phosphodiesterase domain-containing protein 3	1.83E-02	-0.106	-0.416	MYH9_HUMAN	Myosin-9	1.28E-02	0.107	0.428
9	HUTH_HUMAN	Histidine ammonia-lyase	2.45E-02	-0.105	-0.397	FLNB_HUMAN	Filamin-B	4.38E-03	0.107	0.485
10	SAP3_HUMAN	Ganglioside GM2 activator	2.53E-02	-0.104	-0.412	FILA_HUMAN	Flaggrin	2.94E-03	0.106	0.489
11	HEM2_HUMAN	Delta-aminolevulinic acid dehydratase	5.93E-03	-0.103	-0.479	PSA3_HUMAN	Proteasome subunit alpha type-3	4.69E-03	0.106	0.477
12	APOA4_HUMAN	Apolipoprotein A-IV	5.68E-03	-0.103	-0.464	CATH_HUMAN	Pro-cathepsin H	2.54E-02	0.103	0.390
13	CATD_HUMAN	Cathepsin D	1.91E-02	-0.100	-0.405	PSB5_HUMAN	Proteasome subunit beta type-5	2.76E-03	0.101	0.504
14	PLEC_HUMAN	Plectin	1.74E-02	-0.100	-0.425	S10A8_HUMAN	Protein S100-A8	2.13E-02	0.098	0.399
15	SAPL1_HUMAN	Proactivator polypeptide-like 1	1.41E-02	-0.100	-0.441	LAMP1_HUMAN	Lysosome-associated membrane glycoprotein 1	6.15E-03	0.097	0.477

*The proteins are ranked according to the orthogonal projections to latent structures-discriminant analysis (OPLS-DA) model loadings (12).

†Two-tailed Student's *t*-test.

‡The model loadings are a measure of how much each protein contributes to the classification into AE or healthy group (12).

§Correlation coefficient calculated using Pearson's product-moment correlation.

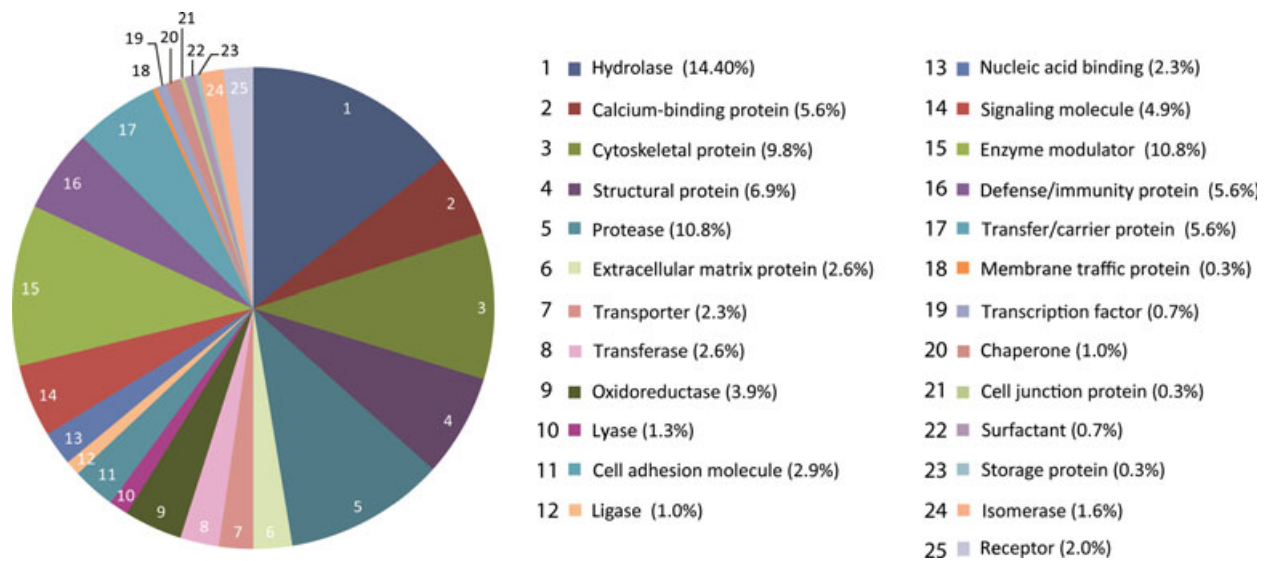


Figure 1 Classification of the identified and quantified 203 vernix proteins according to their function. Proteins were grouped into protein classes with the tools available in Protein ANalysis THrough Evolutionary Relationships (PANTHER).

accuracy (Fig. 2B). No significant difference was found by OPLS-DA in the protein composition of vernix between the lifestyle groups ($R^2 = 0.15$ and $Q^2 = -0.05$).

The potential AE biomarkers polyubiquitin-C and calmodulin-like protein 5

When the intergroup t-test value distributions were plotted, two proteins had much stronger P -values than all the rest, polyubiquitin-C and calmodulin-like protein 5 (Fig. 3A). These two proteins also showed exceptionally high correlation with the healthy state of the children, with a correlation value of 0.73 and 0.68, respectively, and a P -value of 8.2×10^{-7} and 1.8×10^{-5} , respectively (Table 2). The OPLS-DA model providing the optimal linear combination of protein abundances for these two proteins had prediction accuracy of 91.2% (Fig. 3B). The relative abundances of polyubiquitin-C and calmodulin-like protein 5 in all 34 vernix samples were plotted and showed a significant different distribution between the AE and healthy groups ($P = <0.0001$ for both proteins) (Fig. 3C and D). These findings allow considering polyubiquitin-C and calmodulin-like protein 5 as potential biomarkers for AE prediction. No significant differences in the relative abundances of polyubiquitin-C and calmodulin-like protein 5 between the three lifestyle groups or between sensitized and nonsensitized children (Table 1) could be found (Fig. 3C and D).

Discussion

We identified and quantified 203 proteins in vernix collected from 34 newborns. For two proteins, polyubiquitin-C and calmodulin-like protein 5, the abundance variations between the AE and healthy group demonstrated strong significance (Table 2). Together with the ROC curve prediction accuracy

of 91.2%, these two proteins emerge as promising biomarkers in vernix to predict development of AE.

Most vernix proteins identified in this study are hydrolases (14.4%), proteases (10.8%), and enzyme modulators (10.8%) (Fig. 1). These functions are all inter-related (proteases are a kind of hydrolases and both groups are enzymes) and suggest that these processes have important roles in the vernix of newborns. Vernix is produced by the sebaceous glands, and in addition, lanugo hairs and desquamated squamous cells contribute to the formation of vernix (8). Thus, the origin of proteins found in vernix is to a large part from the fetal epidermis. Previously, the vernix proteome has been analyzed on pooled samples from newborns by a 2D-gel electrophoresis-based approach resulting in 41 identified proteins (15). In that study, it was found that almost half of these proteins (39%) are related to immunity, whereas we here demonstrate that only 5.6% of the identified proteins were associated with immunity (Fig. 1). This discrepancy clearly demonstrates that the method used for the proteome analysis affects not only the sensitivity but also the specificity. In the 2D-gel approach, the identification of proteins is restricted to the spots that are selected from the gel, while the method used in our study enables identification of all proteins in a solution. When analyzing the 203 proteins in our study for differences in abundance in vernix from newborns, who had developed AE at 2 years of age and those who remained healthy, an OPLS-DA model was found with $R^2 = 0.85$ and $Q^2 = 0.39$ (Fig. 2A). No significant differences in vernix protein composition could, however, be detected by OPLS-DA between the lifestyle groups.

What is the biological significance of the two potential biomarkers present at lower levels in the AE group found in this study? Polyubiquitin-C is a precursor protein that is cleaved into the active ubiquitin monomer (16). The ubiquitin conjugation system regulates a wide variety of biological processes, including protein degradation and signal transduction

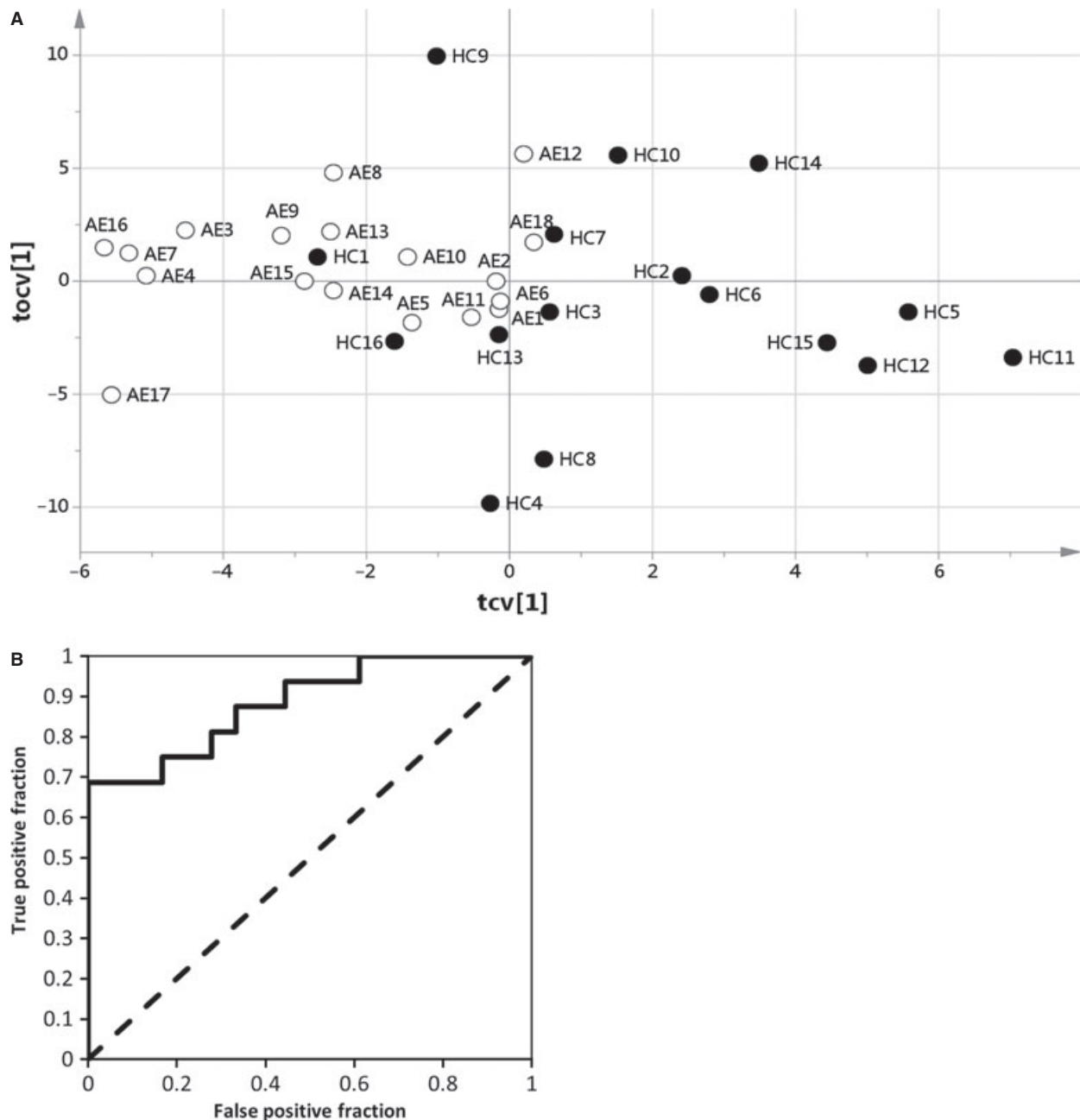


Figure 2 Analysis of the proteome dataset of 203 proteins. (A) Cross-validated score plot from the OPLS-DA model, illustrating the separation of atopic eczema (AE) (○) and healthy control (●) samples. tcv = sample projection to maximum discrimination dimension; tocv = maximum deviation orthogonal to discrimination

dimension in sample set. (B) Receiver operating characteristic (ROC) curve for the predictive score of the OPLS-DA model calculated during sevenfold cross-validation (AUC = 0.885; accuracy = 73.5%).

(17). Furthermore, protein ubiquitylation is of fundamental importance in the regulation of both the innate and the adaptive immune system (18). Decreased ubiquitin level lowers the activation threshold of cells to a variety of chemical and environmental stresses (16), suggesting that the epidermis of the children in the AE group might be more sensitive to external stressors. Calmodulin-like protein 5 (also

called calmodulin-like skin protein) is a calcium-binding protein and a marker for late keratinocyte differentiation (19). Its ability to associate with transglutaminase-3 (19), a key enzyme involved in the formation of the cornified envelope (20), indicates an important role of this protein in the epidermal barrier function. To our knowledge, there are no reports on mutations in the genes coding for polyubiqu-

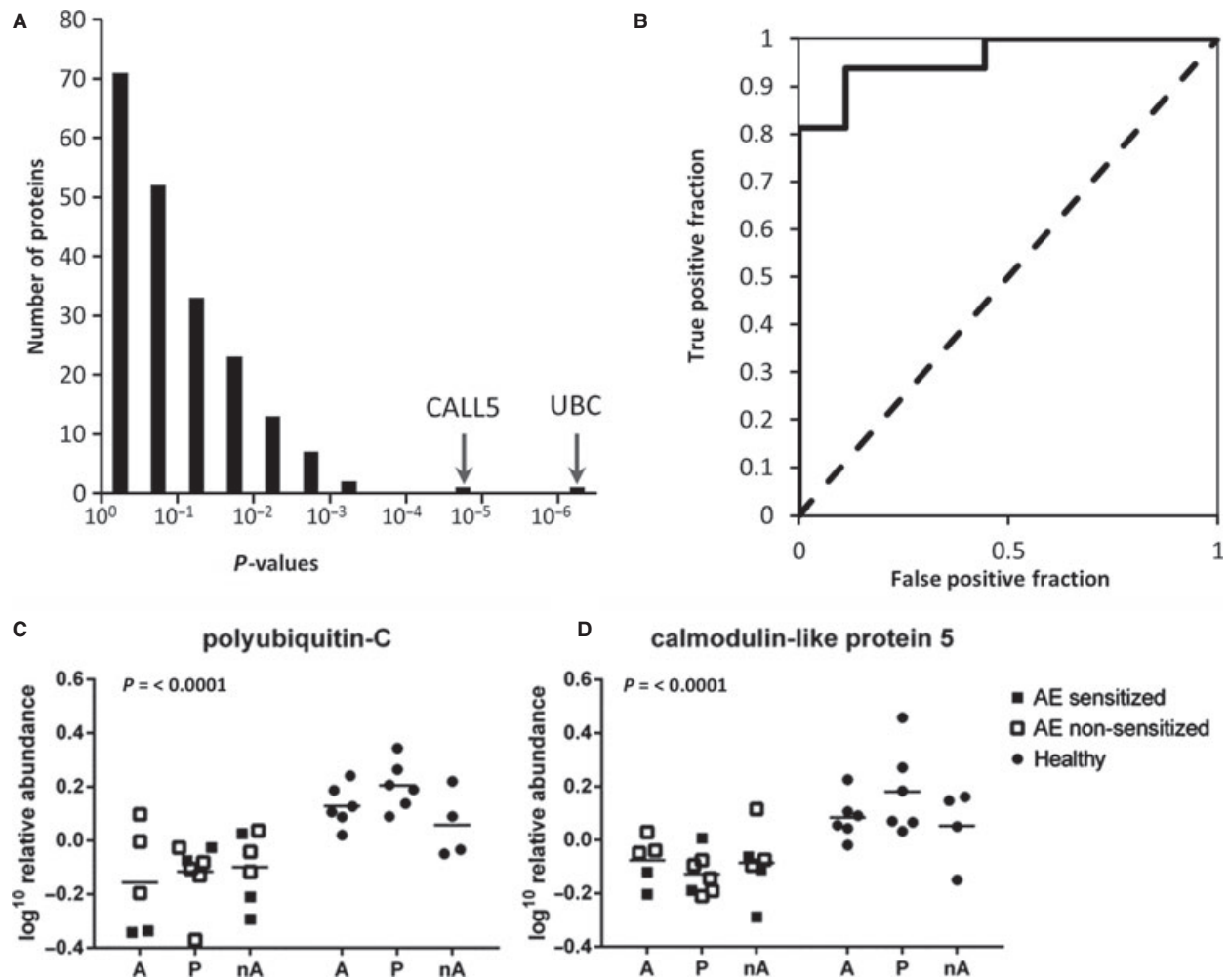


Figure 3 Analysis of polyubiquitin-C and calmodulin-like protein 5. (A) Intergroup t-test value distribution. Polyubiquitin-C (UBC), calmodulin-like protein 5 (CALL5). (B) Receiver operating characteristic (ROC) curve for the predictive score of the OPLS-DA model, based on polyubiquitin-C and calmodulin-like protein 5, calculated during sevenfold cross-validation (AUC = 0.958; accuracy = 91.2%). (C)

Polyubiquitin-C and (D) calmodulin-like protein 5 relative protein abundances within the healthy group (●) and within the atopic eczema (AE) group, divided into sensitized (■) and nonsensitized (□), subdivided into lifestyle groups: anthroposophic (A), partly anthroposophic (P), and nonanthroposophic (nA). Statistical significance was assessed using two-way ANOVA.

itin-C and calmodulin-like protein 5 associated with the pathogenesis of AE, which could be a plausible explanation for their lower levels, as is the case for mutations in the human filaggrin gene (21). Another explanation to the reduced levels of polyubiquitin-C and calmodulin-like protein 5 could be that there are many proteases present in vernix (see Fig. 1).

Polyubiquitin-C and calmodulin-like protein 5 showed strong statistical significance in their negative correlation with the AE group. However, when interpreting the results, it has to be reminded that they are based on two proteins and 34 children (larger populations tend to give less accurate but more realistic models). As nontargeted proteomics analysis is increasingly being used not only in the discovery phase but also for analytical purposes (11), the complete or partial vernix proteome (protein pattern) could also be a potential biomarker. Its 73.5% prediction accuracy was lower than

that of the two proteins, polyubiquitin-C and calmodulin-like protein 5, but full-proteome measurement is more robust as it accumulates signals from hundreds of peptides.

There was also a group of proteins with significantly higher relative abundance in the AE group, although their correlation with AE was not as strong as for polyubiquitin-C or calmodulin-like protein 5 (Table 2). Among those were peroxiredoxin-2 and serpin A12. The level of peroxiredoxin-2 is increased in lymphocytes of patients with rheumatoid arthritis, with a positive correlation with the concentration of the acute-phase protein C-reactive protein (22), indicating its involvement in inflammatory responses. Serpin A12 (also called alpha-1 antitrypsin) is a member of the serpin (serine protease inhibitors) family and is an acute-phase protein, which plasma concentration increases upon inflammatory signals (23). Hence, elevated levels of peroxiredoxin-2 and serpin A12 in

vernix of the AE group indicate that the skin of these neonates might be prone to develop inflammation, which may possibly have a physiological association with AE.

In summary, we have shown that the protein composition of vernix has the potential to function as a predictor of future AE development. Identifying risk groups is of uttermost importance because restoration of the skin barrier function early in life may help prevent the outbreak of the disease.

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Author contributions

TH, FS, JA, and AS involved in conception and design of the study. TH, DR, YKL, YL, RZ, BA, and AS performed acquisition, analysis, and interpretation of data. TH, DR, YKL, and AS contributed to drafting the article. FS, RZ, BA, and JA revised the article. All authors approved the final article.

References

1. Bieber T. Atopic dermatitis. *Ann Dermatol* 2010;**22**:125–137.
2. Ballardini N, Kull I, Lind T, Hallner E, Almqvist C, Ostblom E et al. Development and comorbidity of eczema, asthma and rhinitis to age 12: data from the BAMSE birth cohort. *Allergy* 2012;**67**:537–544.
3. Gustafsson D, Sjöberg O, Foucard T. Development of allergies and asthma in infants and young children with atopic dermatitis—a prospective follow-up to 7 years of age. *Allergy* 2000;**55**:240–245.
4. Bieber T, Cork M, Reitamo S. Atopic dermatitis: a candidate for disease-modifying strategy. *Allergy* 2012;**67**:969–975.
5. Alm JS, Swartz J, Lilja G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. *Lancet* 1999;**353**:1485–1488.
6. Floistrup H, Swartz J, Bergstrom A, Alm JS, Scheynius A, van Hage M et al. Allergic disease and sensitization in Steiner school children. *J Allergy Clin Immunol* 2006;**117**:59–66.
7. Stenius F, Swartz J, Lilja G, Borres M, Bottai M, Pershagen G et al. Lifestyle factors and sensitization in children - the ALADDIN birth cohort. *Allergy* 2011;**66**:1330–1338.
8. Hoath SB, Pickens WL, Visscher MO. The biology of vernix caseosa. *Int J Cosmet Sci* 2006;**28**:319–333.
9. Hoeger PH, Schreiner V, Klaassen IA, Enzmann CC, Friedrichs K, Bleck O. Epidermal barrier lipids in human vernix caseosa: corresponding ceramide pattern in vernix and fetal skin. *Br J Dermatol* 2002;**146**:194–201.
10. Williams HC, Burney PG, Hay RJ, Archer CB, Shipley MJ, Hunter JJ et al. The U.K. working party's diagnostic criteria for atopic dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 1994;**131**:383–396.
11. Lyutvinskiy Y, Yang H, Rutishauser D, Zubarev R. In silico instrumental response correction improves precision of label-free proteomics and accuracy of proteomics-based predictive models. *Mol Cell Proteomics* 2013;**12**:2324–2331.
12. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). *J Chemometric* 2002;**16**:119–128.
13. Fawcett T. An introduction to ROC analysis. *Pattern Recogn Lett* 2006;**27**:861–874.
14. Broadhurst DI, Kella DB. Statistical strategies for avoiding false discoveries in metabolomics and related experiments. *Metabolomics* 2006;**2**:171–196.
15. Tollin M, Jagerbrink T, Haraldsson A, Agerberth B, Jorvall H. Proteome analysis of vernix caseosa. *Pediatr Res* 2006;**60**:430–434.
16. Hanna J, Meides A, Zhang DP, Finley D. A ubiquitin stress response induces altered proteasome composition. *Cell* 2007;**129**:747–759.
17. Iwai K. Linear polyubiquitin chains: a new modifier involved in NFκB activation and chronic inflammation, including dermatitis. *Cell Cycle* 2011;**10**:3095–3104.
18. Liu YC, Penninger J, Karin M. Immunity by ubiquitylation: a reversible process of modification. *Nat Rev Immunol* 2005;**5**:941–952.
19. Mehul B, Bernard D, Simonetti L, Bernard MA, Schmidt R. Identification and cloning of a new calmodulin-like protein from human epidermis. *J Biol Chem* 2000;**275**:12841–12847.
20. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 2005;**6**:328–340.
21. Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med* 2011;**365**:1315–1327.
22. Szabo-Taylor KE, Eggleton P, Turner CA, Faro ML, Tarr JM, Toth S et al. Lymphocytes from rheumatoid arthritis patients have elevated levels of intracellular peroxiredoxin 2, and a greater frequency of cells with exofacial peroxiredoxin 2, compared with healthy human lymphocytes. *Int J Biochem Cell Biol* 2012;**44**:1223–1231.
23. Bergin DA, Hurley K, McElvaney NG, Reeves EP. Alpha-1 antitrypsin: a potent anti-inflammatory and potential novel therapeutic agent. *Arch Immunol Ther Exp (Warsz)* 2012;**60**:81–97.
24. Stenius F, Borres M, Bottai M, Lilja G, Lindblad F, Pershagen G et al. Salivary cortisol levels and allergy in children: the ALADDIN birth cohort. *J Allergy Clin Immunol* 2011;**128**:1335–1339.

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Conflicts of interest

The authors claim no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

Table S1. List of the 203 proteins identified and quantified in vernix.

Protein biomarkers in vernix with potential to predict the development of atopic eczema in early childhood

Supporting information

Data S1 Methods

Protein digestion

Five µg of the protein extract from each vernix sample was dissolved in 80 µl 0.1% ProteaseMax (Promega, Madison, WI, USA), 50 mM ammonium bicarbonate (Sigma-Fluka, Munich, Germany) and 10% acetonitrile (ACN) (Fisher, Loughborough, UK). Resulting protein solutions were incubated for 30 min at 50 °C followed by an additional bath sonication (45hKHz, 120W, VWR, Leuven, Belgium) for 10 min at room temperature. Samples were centrifuged for 10 min at 13 000 g and directly subjected to tryptic digestion carried out by a liquid handling robot (MultiProbe II, Perkin Elmer, Waltham, USA) (24).

Liquid chromatography tandem mass spectrometry

Liquid chromatography tandem mass spectrometry analyses were performed on an Easy-nLC system (Thermo Fisher Scientific, Bremen, Germany) in-line coupled to a QExactive MS (Thermo Fisher Scientific). Coded conditions were used throughout and all samples were processed and analyzed in a randomized manner. From each sample 0.5 µg of the digests were loaded from a cooled auto sampler to the LC column. Separation of the peptides was performed on a 10 cm long fused silica tip column (SilicaTips™ New Objective Inc., Woburn, MA, USA) in-house packed with 3 µm C18-AQ ReproSil-Pur® (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). The chromatographic separation was achieved using an ACN/water solvent system containing 0.2% formic acid. The gradient was set up as follows: 5–35% ACN in 89 min, 48–80% ACN in 5 min, and 80%

ACN for 8 min all at a flow rate of 300 nL/min. The MS acquisition method was comprised of one survey full scan ranging from m/z 300 to m/z 1650 acquired with a resolution of $R=70,000$ at m/z 400, followed by data-dependent higher energy collision induced dissociation scans from maximum ten most intense precursor ions with a charge state ≥ 2 . Fragmentation (MS2) spectra were acquired with a resolution of $R=17,500$, a target value of 2×10^5 , isolation width was set to 4 m/z and normalized collision energy to 30%. For all sequencing events dynamic exclusion was enabled and unassigned charge states were rejected. The instrument was calibrated externally according to the manufacturer's instructions and all samples were acquired using internal lock mass calibration on m/z 429.088735 and 445.120025.

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38 Supplementary Table 1 List of the 203 proteins identified and quantified in vernix

PROTEIN ID (SwissProt)	PROTEIN (SwissProt)	NUMBER OF PEPTIDES	P-value ^a	OPLS-DA model loadings ^b	Correlation coefficient to health status ^c
PRDX2_HUMAN	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5	5	6.35E-04	-0.127	-0.563
SPA12_HUMAN	Serpin A12 OS=Homo sapiens GN=SERPINA12 PE=2 SV=1	11	6.22E-03	-0.123	-0.469
ASAH1_HUMAN	Acid ceramidase OS=Homo sapiens GN=ASAH1 PE=1 SV=5	13	6.56E-03	-0.116	-0.489
BPIA1_HUMAN	BPI fold-containing family A member 1 OS=Homo sapiens GN=BPIFA1 PE=1 SV=1	9	3.45E-03	-0.111	-0.512
DESP_HUMAN	Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3	20	7.64E-03	-0.110	-0.460
BPIB1_HUMAN	BPI fold-containing family B member 1 OS=Homo sapiens GN=BPIFB1 PE=2 SV=1	13	3.95E-03	-0.109	-0.507
K1C13_HUMAN	Keratin, type I cytoskeletal 13 OS=Homo sapiens GN=KRT13 PE=1 SV=4	16	1.83E-03	-0.109	-0.525
GDPD3_HUMAN	Glycerophosphodiester phosphodiesterase domain-containing protein 3 OS=Homo sapiens GN=GDPD3 PE=2 SV=3	5	1.83E-02	-0.106	-0.416
HUTH_HUMAN	Histidine ammonia-lyase OS=Homo sapiens GN=HAL PE=1 SV=1	25	2.45E-02	-0.105	-0.397
SAP3_HUMAN	Ganglioside GM2 activator OS=Homo sapiens GN=GM2A PE=1 SV=4	5	2.53E-02	-0.104	-0.412
HEM2_HUMAN	Delta-aminolevulinic acid dehydratase OS=Homo sapiens GN=ALAD PE=1 SV=1	5	5.93E-03	-0.103	-0.479
APOA4_HUMAN	Apolipoprotein A-IV OS=Homo sapiens GN=APOA4 PE=1 SV=3	9	5.68E-03	-0.103	-0.464
CATD_HUMAN	Cathepsin D OS=Homo sapiens GN=CTSD PE=1 SV=1	6	1.91E-02	-0.100	-0.405
PLEC_HUMAN	Plectin OS=Homo sapiens GN=PLEC PE=1 SV=3	12	1.74E-02	-0.100	-0.425
SAPL1_HUMAN	Proactivator polypeptide-like 1 OS=Homo sapiens GN=PSAPL1 PE=2 SV=2	2	1.41E-02	-0.100	-0.441
K1C19_HUMAN	Keratin, type I cytoskeletal 19 OS=Homo sapiens GN=KRT19 PE=1 SV=4	11	1.64E-03	-0.100	-0.523
A2ML1_HUMAN	Alpha-2-macroglobulin-like protein 1 OS=Homo sapiens GN=A2ML1 PE=1 SV=3	39	4.40E-02	-0.095	-0.361
K2C78_HUMAN	Keratin, type II cytoskeletal 78 OS=Homo sapiens GN=KRT78 PE=2 SV=2	14	2.35E-02	-0.095	-0.414
FIBA_HUMAN	Fibrinogen alpha chain OS=Homo sapiens GN=FGA PE=1 SV=2	8	9.27E-03	-0.095	-0.436
SG3A2_HUMAN	Secretoglobin family 3A member 2 OS=Homo sapiens GN=SCGB3A2 PE=1 SV=1	7	2.01E-02	-0.093	-0.414
ANXA1_HUMAN	Annexin A1 OS=Homo sapiens GN=ANXA1 PE=1 SV=2	21	2.07E-02	-0.093	-0.409
IGHG2_HUMAN	Ig gamma-2 chain C region OS=Homo sapiens GN=IGHG2 PE=1 SV=2	6	2.65E-02	-0.090	-0.379
CRP_HUMAN	C-reactive protein OS=Homo sapiens GN=CRP PE=1 SV=1	5	5.19E-02	-0.089	-0.339
G3P_HUMAN	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3	12	1.21E-01	-0.087	-0.277
K2C79_HUMAN	Keratin, type II cytoskeletal 79 OS=Homo sapiens GN=KRT79 PE=1 SV=2	13	1.22E-02	-0.086	-0.437

K22E_HUMAN	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2	28	8.46E-02	-0.086	-0.308
LUM_HUMAN	Lumican OS=Homo sapiens GN=LUM PE=1 SV=2	8	2.02E-02	-0.084	-0.403
CAN1_HUMAN	Calpain-1 catalytic subunit OS=Homo sapiens GN=CAPN1 PE=1 SV=1	16	8.07E-02	-0.083	-0.320
TGM1_HUMAN	Protein-glutamine gamma-glutamyltransferase K OS=Homo sapiens GN=TGM1 PE=1 SV=4	8	5.10E-02	-0.083	-0.343
K1C10_HUMAN	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6	65	6.92E-02	-0.082	-0.339
ZG16B_HUMAN	Zymogen granule protein 16 homolog B OS=Homo sapiens GN=ZG16B PE=1 SV=3	10	6.18E-02	-0.082	-0.338
K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	64	7.81E-02	-0.080	-0.327
APOA1_HUMAN	Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1	26	4.71E-02	-0.078	-0.359
MA2B1_HUMAN	Lysosomal alpha-mannosidase OS=Homo sapiens GN=MAN2B1 PE=1 SV=3	3	1.37E-01	-0.078	-0.269
ORN_HUMAN	Oligoribonuclease, mitochondrial OS=Homo sapiens GN=REXO2 PE=1 SV=3	3	1.72E-01	-0.076	-0.252
APOD_HUMAN	Apolipoprotein D OS=Homo sapiens GN=APOD PE=1 SV=1	9	9.85E-02	-0.076	-0.300
CAH1_HUMAN	Carbonic anhydrase 1 OS=Homo sapiens GN=CA1 PE=1 SV=2	8	4.61E-02	-0.075	-0.348
SBSN_HUMAN	Suprabasin OS=Homo sapiens GN=SBSN PE=2 SV=1	4	1.47E-01	-0.074	-0.259
K2C5_HUMAN	Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3	24	8.13E-02	-0.071	-0.322
LYSC_HUMAN	Lysozyme C OS=Homo sapiens GN=LYZ PE=1 SV=1	5	1.11E-01	-0.070	-0.278
PEDF_HUMAN	Pigment epithelium-derived factor OS=Homo sapiens GN=SERPINF1 PE=1 SV=4	5	5.69E-02	-0.069	-0.342
PSPB_HUMAN	Pulmonary surfactant-associated protein B OS=Homo sapiens GN=SFTPB PE=1 SV=3	11	1.22E-01	-0.067	-0.276
CPNS2_HUMAN	Calpain small subunit 2 OS=Homo sapiens GN=CAPNS2 PE=2 SV=2	4	6.18E-02	-0.067	-0.337
FINC_HUMAN	Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=4	39	5.99E-02	-0.064	-0.335
CYTB_HUMAN	Cystatin-B OS=Homo sapiens GN=CSTB PE=1 SV=2	2	3.55E-02	-0.064	-0.354
K2C80_HUMAN	Keratin, type II cytoskeletal 80 OS=Homo sapiens GN=KRT80 PE=1 SV=2	10	9.92E-02	-0.064	-0.305
SPB7_HUMAN	Serpin B7 OS=Homo sapiens GN=SERPINB7 PE=2 SV=1	11	4.56E-01	-0.064	-0.133
FIBG_HUMAN	Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3	10	1.08E-01	-0.063	-0.276
APOA2_HUMAN	Apolipoprotein A-II OS=Homo sapiens GN=APOA2 PE=1 SV=1	8	1.15E-01	-0.063	-0.286
HSPB1_HUMAN	Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=2	6	3.42E-02	-0.061	-0.358
K2C1B_HUMAN	Keratin, type II cytoskeletal 1b OS=Homo sapiens GN=KRT77 PE=1 SV=3	36	1.05E-01	-0.061	-0.299
PLAK_HUMAN	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3	7	1.09E-01	-0.060	-0.275
FETUA_HUMAN	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1	5	7.75E-02	-0.059	-0.317
SFTA1_HUMAN	Pulmonary surfactant-associated protein A1 OS=Homo sapiens GN=SFTPA1 PE=1 SV=2	11	1.48E-01	-0.059	-0.250

FILA2_HUMAN	Filaggrin-2 OS=Homo sapiens GN=FLG2 PE=1 SV=1	89	4.55E-01	-0.056	-0.133
K1C17_HUMAN	Keratin. type I cytoskeletal 17 OS=Homo sapiens GN=KRT17 PE=1 SV=2	11	1.32E-01	-0.056	-0.276
IBP1_HUMAN	Insulin-like growth factor-binding protein 1 OS=Homo sapiens GN=IGFBP1 PE=1 SV=1	7	1.30E-01	-0.055	-0.268
K2C6B_HUMAN	Keratin. type II cytoskeletal 6B OS=Homo sapiens GN=KRT6B PE=1 SV=5	3	1.25E-01	-0.054	-0.267
IGLL5_HUMAN	Immunoglobulin lambda-like polypeptide 5 OS=Homo sapiens GN=IGLL5 PE=2 SV=2	2	1.56E-01	-0.054	-0.255
UROM_HUMAN	Uromodulin OS=Homo sapiens GN=UMOD PE=1 SV=1	11	7.68E-02	-0.053	-0.305
PLSL_HUMAN	Plastin-2 OS=Homo sapiens GN=LCP1 PE=1 SV=6	19	1.54E-01	-0.052	-0.265
SBP1_HUMAN	Selenium-binding protein 1 OS=Homo sapiens GN=SELENBP1 PE=1 SV=2	8	4.27E-01	-0.052	-0.143
KPRP_HUMAN	Keratinocyte proline-rich protein OS=Homo sapiens GN=KPRP PE=1 SV=1	10	2.59E-01	-0.052	-0.198
GSDMA_HUMAN	Gasdermin-A OS=Homo sapiens GN=GSDMA PE=2 SV=4	11	1.58E-01	-0.050	-0.257
SAMP_HUMAN	Serum amyloid P-component OS=Homo sapiens GN=APCS PE=1 SV=2	5	1.86E-01	-0.050	-0.236
APOH_HUMAN	Beta-2-glycoprotein 1 OS=Homo sapiens GN=APOH PE=1 SV=3	6	9.80E-02	-0.049	-0.298
PSB6_HUMAN	Proteasome subunit beta type-6 OS=Homo sapiens GN=PSMB6 PE=1 SV=4	4	4.41E-01	-0.049	-0.142
ARGI1_HUMAN	Arginase-1 OS=Homo sapiens GN=ARG1 PE=1 SV=2	19	1.44E-01	-0.048	-0.269
IGHG1_HUMAN	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1	6	1.90E-01	-0.047	-0.237
EF1A3_HUMAN	Putative elongation factor 1-alpha-like 3 OS=Homo sapiens GN=EEF1A1P5 PE=5 SV=1	5	1.15E-01	-0.045	-0.271
KV305_HUMAN	Ig kappa chain V-III region WOL OS=Homo sapiens PE=1 SV=1	3	2.65E-01	-0.044	-0.199
AACT_HUMAN	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	9	2.10E-01	-0.043	-0.222
IGKC_HUMAN	Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1	9	2.66E-01	-0.040	-0.200
PLBL2_HUMAN	Putative phospholipase B-like 2 OS=Homo sapiens GN=PLBD2 PE=1 SV=2	2	8.05E-01	-0.040	-0.045
CRNN_HUMAN	Cornulin OS=Homo sapiens GN=CRNN PE=1 SV=1	11	3.83E-01	-0.040	-0.153
S100P_HUMAN	Protein S100-P OS=Homo sapiens GN=S100P PE=1 SV=2	2	6.48E-01	-0.039	-0.084
IBP4_HUMAN	Insulin-like growth factor-binding protein 4 OS=Homo sapiens GN=IGFBP4 PE=1 SV=2	4	1.93E-01	-0.039	-0.233
PIP_HUMAN	Prolactin-inducible protein OS=Homo sapiens GN=PIP PE=1 SV=1	4	5.34E-01	-0.038	-0.107
IGHA1_HUMAN	Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2	3	3.01E-01	-0.037	-0.184
HPT_HUMAN	Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1	12	2.84E-01	-0.036	-0.191
VTNC_HUMAN	Vitronectin OS=Homo sapiens GN=VTN PE=1 SV=1	8	3.78E-01	-0.036	-0.162
IGHM_HUMAN	Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3	7	6.81E-01	-0.035	-0.073
HV305_HUMAN	Ig heavy chain V-III region BRO OS=Homo sapiens PE=1 SV=1	2	1.88E-01	-0.034	-0.235

MUC5B_HUMAN	Mucin-5B OS=Homo sapiens GN=MUC5B PE=1 SV=3	3	2.79E-01	-0.034	-0.186
ENOA_HUMAN	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	7	1.72E-01	-0.034	-0.242
DMBT1_HUMAN	Deleted in malignant brain tumors 1 protein OS=Homo sapiens GN=DMBT1 PE=1 SV=2	16	1.82E-01	-0.032	-0.239
LDHA_HUMAN	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2	13	5.76E-01	-0.031	-0.100
SPB12_HUMAN	Serpin B12 OS=Homo sapiens GN=SERPINB12 PE=1 SV=1	23	5.79E-01	-0.029	-0.102
VINC_HUMAN	Vinculin OS=Homo sapiens GN=VCL PE=1 SV=4	16	5.54E-01	-0.026	-0.109
ISK5_HUMAN	Serine protease inhibitor Kazal-type 5 OS=Homo sapiens GN=SPINK5 PE=1 SV=2	3	9.71E-01	-0.023	0.006
TIMP1_HUMAN	Metalloproteinase inhibitor 1 OS=Homo sapiens GN=TIMP1 PE=1 SV=1	3	3.96E-01	-0.022	-0.152
CO3_HUMAN	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	57	4.00E-01	-0.022	-0.154
ARSF_HUMAN	Arylsulfatase F OS=Homo sapiens GN=ARSF PE=1 SV=4	3	6.69E-01	-0.021	-0.076
DHB4_HUMAN	Peroxisomal multifunctional enzyme type 2 OS=Homo sapiens GN=HSD17B4 PE=1 SV=3	9	6.16E-01	-0.020	-0.091
K1C9_HUMAN	Keratin. type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3	27	8.90E-01	-0.020	-0.024
ALDOA_HUMAN	Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2	6	2.73E-01	-0.019	-0.192
DCD_HUMAN	Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2	4	9.70E-01	-0.019	-0.007
1433Z_HUMAN	14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1	5	2.26E-01	-0.017	-0.220
SAP_HUMAN	Proactivator polypeptide OS=Homo sapiens GN=PSAP PE=1 SV=2	5	9.63E-01	-0.017	-0.008
PLMN_HUMAN	Plasminogen OS=Homo sapiens GN=PLG PE=1 SV=2	12	7.50E-01	-0.017	-0.059
PARK7_HUMAN	Protein DJ-1 OS=Homo sapiens GN=PARK7 PE=1 SV=2	6	8.79E-01	-0.017	-0.028
PPIA_HUMAN	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2	2	6.36E-01	-0.017	-0.085
H4_HUMAN	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2	5	4.27E-01	-0.017	-0.143
CATL2_HUMAN	Cathepsin L2 OS=Homo sapiens GN=CTSL2 PE=1 SV=2	4	9.77E-01	-0.016	0.005
H32_HUMAN	Histone H3.2 OS=Homo sapiens GN=HIST2H3A PE=1 SV=3	4	4.63E-01	-0.014	-0.132
AMBP_HUMAN	Protein AMBP OS=Homo sapiens GN=AMBP PE=1 SV=1	6	2.94E-01	-0.014	-0.189
MUC7_HUMAN	Mucin-7 OS=Homo sapiens GN=MUC7 PE=1 SV=2	6	5.58E-01	-0.011	-0.105
TRFL_HUMAN	Lactotransferrin OS=Homo sapiens GN=LTF PE=1 SV=6	34	5.83E-01	-0.011	-0.099
PAI2_HUMAN	Plasminogen activator inhibitor 2 OS=Homo sapiens GN=SERPINB2 PE=1 SV=2	6	7.94E-01	-0.010	-0.048
IBP3_HUMAN	Insulin-like growth factor-binding protein 3 OS=Homo sapiens GN=IGFBP3 PE=1 SV=2	6	8.59E-01	-0.010	-0.033
SPB13_HUMAN	Serpin B13 OS=Homo sapiens GN=SERPINB13 PE=2 SV=2	9	9.02E-01	-0.005	-0.022
K1C25_HUMAN	Keratin. type I cytoskeletal 25 OS=Homo sapiens GN=KRT25 PE=1 SV=1	3	5.62E-01	-0.005	0.104

SPB3_HUMAN	Serpin B3 OS=Homo sapiens GN=SERPINB3 PE=1 SV=2	16	8.47E-01	-0.004	0.036
ACTG_HUMAN	Actin. cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1	18	6.60E-01	-0.003	-0.079
PSB4_HUMAN	Proteasome subunit beta type-4 OS=Homo sapiens GN=PSMB4 PE=1 SV=4	6	7.78E-01	-0.003	0.051
TRHY_HUMAN	Trichohyalin OS=Homo sapiens GN=TCHH PE=1 SV=2	40	9.57E-01	-0.002	0.010
TPIS_HUMAN	Triosephosphate isomerase OS=Homo sapiens GN=TPI1 PE=1 SV=3	10	9.91E-01	-0.001	-0.002
K1C16_HUMAN	Keratin. type I cytoskeletal 16 OS=Homo sapiens GN=KRT16 PE=1 SV=4	11	9.67E-01	-0.001	-0.007
GSHB_HUMAN	Glutathione synthetase OS=Homo sapiens GN=GSS PE=1 SV=1	9	8.70E-01	-0.001	0.030
SPB9_HUMAN	Serpin B9 OS=Homo sapiens GN=SERPINB9 PE=1 SV=1	7	9.98E-01	-0.001	0.000
PSB7_HUMAN	Proteasome subunit beta type-7 OS=Homo sapiens GN=PSMB7 PE=1 SV=1	4	6.50E-01	0.001	0.082
RNAS7_HUMAN	Ribonuclease 7 OS=Homo sapiens GN=RNASE7 PE=1 SV=2	3	8.11E-01	0.002	0.042
PLBL1_HUMAN	Phospholipase B-like 1 OS=Homo sapiens GN=PLBD1 PE=1 SV=2	11	6.61E-01	0.003	-0.080
PSA4_HUMAN	Proteasome subunit alpha type-4 OS=Homo sapiens GN=PSMA4 PE=1 SV=1	10	7.05E-01	0.005	0.068
LDHB_HUMAN	L-lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=2	17	5.80E-01	0.006	0.099
GSTP1_HUMAN	Glutathione S-transferase P OS=Homo sapiens GN=GSTP1 PE=1 SV=2	5	7.79E-01	0.008	0.050
DEF3_HUMAN	Neutrophil defensin 3 OS=Homo sapiens GN=DEFA3 PE=1 SV=1	3	8.82E-01	0.009	-0.026
CBPA4_HUMAN	Carboxypeptidase A4 OS=Homo sapiens GN=CPA4 PE=1 SV=2	4	5.75E-01	0.010	0.100
IL36B_HUMAN	Interleukin-36 beta OS=Homo sapiens GN=IL36B PE=2 SV=1	3	8.23E-01	0.010	0.039
GGCT_HUMAN	Gamma-glutamylcyclotransferase OS=Homo sapiens GN=GGCT PE=1 SV=1	17	4.40E-01	0.011	0.140
CALL3_HUMAN	Calmodulin-like protein 3 OS=Homo sapiens GN=CALML3 PE=1 SV=2	4	8.40E-01	0.012	0.036
ANGT_HUMAN	Angiotensinogen OS=Homo sapiens GN=AGT PE=1 SV=1	6	7.69E-01	0.013	-0.054
CUTA_HUMAN	Protein CutA OS=Homo sapiens GN=CUTA PE=1 SV=2	2	5.25E-01	0.013	0.118
CERU_HUMAN	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	18	9.43E-01	0.016	-0.013
SPHM_HUMAN	N-sulphoglucosamine sulphohydrolase OS=Homo sapiens GN=SGSH PE=1 SV=1	4	5.84E-01	0.020	0.100
DLDH_HUMAN	Dihydrolipoyl dehydrogenase. mitochondrial OS=Homo sapiens GN=DLD PE=1 SV=2	6	6.74E-01	0.023	0.077
PNPH_HUMAN	Purine nucleoside phosphorylase OS=Homo sapiens GN=PNP PE=1 SV=2	17	8.55E-01	0.024	0.033
IC1_HUMAN	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2	4	8.45E-01	0.025	0.035
HEXB_HUMAN	Beta-hexosaminidase subunit beta OS=Homo sapiens GN=HEXB PE=1 SV=3	16	4.08E-01	0.028	0.148
ECM1_HUMAN	Extracellular matrix protein 1 OS=Homo sapiens GN=ECM1 PE=1 SV=2	5	2.40E-01	0.033	0.208
HEMO_HUMAN	Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2	9	5.10E-01	0.034	0.122

PSB8_HUMAN	Proteasome subunit beta type-8 OS=Homo sapiens GN=PSMB8 PE=1 SV=3	2	3.40E-01	0.037	0.169
VTDB_HUMAN	Vitamin D-binding protein OS=Homo sapiens GN=GC PE=1 SV=1	13	7.71E-01	0.037	0.054
A1BG_HUMAN	Alpha-1B-glycoprotein OS=Homo sapiens GN=A1BG PE=1 SV=4	11	5.22E-01	0.038	0.119
CATC_HUMAN	Dipeptidyl peptidase 1 OS=Homo sapiens GN=CTSC PE=1 SV=2	9	2.35E-01	0.039	0.219
GGH_HUMAN	Gamma-glutamyl hydrolase OS=Homo sapiens GN=GGH PE=1 SV=2	8	1.88E-01	0.039	0.242
FCGBP_HUMAN	IgGfC-binding protein OS=Homo sapiens GN=FCGBP PE=1 SV=3	12	3.03E-01	0.040	0.182
A1AG2_HUMAN	Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 PE=1 SV=2	2	5.42E-01	0.041	0.112
ANT3_HUMAN	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1	5	6.63E-01	0.042	0.079
ANXA2_HUMAN	Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV=2	20	3.17E-01	0.043	0.180
PSB1_HUMAN	Proteasome subunit beta type-1 OS=Homo sapiens GN=PSMB1 PE=1 SV=2	8	1.28E-01	0.043	0.271
NGAL_HUMAN	Neutrophil gelatinase-associated lipocalin OS=Homo sapiens GN=LCN2 PE=1 SV=2	5	4.05E-01	0.045	0.150
PTGDS_HUMAN	Prostaglandin-H2 D-isomerase OS=Homo sapiens GN=PTGDS PE=1 SV=1	2	7.01E-01	0.046	0.069
ALAT1_HUMAN	Alanine aminotransferase 1 OS=Homo sapiens GN=GPT PE=1 SV=3	5	4.02E-01	0.047	0.152
ALBU_HUMAN	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	62	4.36E-01	0.048	0.143
SPB4_HUMAN	Serpin B4 OS=Homo sapiens GN=SERPINB4 PE=1 SV=2	7	2.60E-01	0.048	0.202
CYC_HUMAN	Cytochrome c OS=Homo sapiens GN=CYCS PE=1 SV=2	2	2.06E-01	0.049	0.226
NNRE_HUMAN	NAD(P)H-hydrate epimerase OS=Homo sapiens GN=APOA1BP PE=1 SV=2	4	2.08E-01	0.049	0.226
PSA1_HUMAN	Proteasome subunit alpha type-1 OS=Homo sapiens GN=PSMA1 PE=1 SV=1	13	1.60E-01	0.051	0.253
TGM3_HUMAN	Protein-glutamine gamma-glutamyltransferase E OS=Homo sapiens GN=TGM3 PE=1 SV=4	46	1.46E-01	0.053	0.259
CATA_HUMAN	Catalase OS=Homo sapiens GN=CAT PE=1 SV=3	26	2.24E-01	0.054	0.212
PSB2_HUMAN	Proteasome subunit beta type-2 OS=Homo sapiens GN=PSMB2 PE=1 SV=1	7	1.11E-01	0.054	0.284
ICAL_HUMAN	Calpastatin OS=Homo sapiens GN=CAST PE=1 SV=4	7	5.70E-02	0.058	0.327
A1AT_HUMAN	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	23	3.08E-01	0.059	0.186
GRP78_HUMAN	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2	8	7.97E-02	0.059	0.308
CASPE_HUMAN	Caspase-14 OS=Homo sapiens GN=CASP14 PE=1 SV=2	31	2.58E-02	0.064	0.386
A1AG1_HUMAN	Alpha-1-acid glycoprotein 1 OS=Homo sapiens GN=ORM1 PE=1 SV=1	3	2.32E-01	0.069	0.213
TRFE_HUMAN	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3	46	1.48E-01	0.069	0.264
ZA2G_HUMAN	Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=AZGP1 PE=1 SV=2	19	4.21E-02	0.071	0.359
TALDO_HUMAN	Transaldolase OS=Homo sapiens GN=TALDO1 PE=1 SV=2	6	4.38E-02	0.072	0.358

PERM_HUMAN	Myeloperoxidase OS=Homo sapiens GN=MPO PE=1 SV=1	24	1.50E-01	0.073	0.257
CYTA_HUMAN	Cystatin-A OS=Homo sapiens GN=CSTA PE=1 SV=1	9	4.90E-02	0.076	0.349
GRN_HUMAN	Granulins OS=Homo sapiens GN=GRN PE=1 SV=2	7	6.05E-02	0.077	0.335
TTHY_HUMAN	Transthyretin OS=Homo sapiens GN=TTR PE=1 SV=1	6	5.51E-02	0.078	0.343
RPIA_HUMAN	Ribose-5-phosphate isomerase OS=Homo sapiens GN=RPIA PE=1 SV=3	4	2.64E-02	0.080	0.377
LMNA_HUMAN	Prelamin-A/C OS=Homo sapiens GN=LMNA PE=1 SV=1	13	1.48E-01	0.081	0.266
PSA7_HUMAN	Proteasome subunit alpha type-7 OS=Homo sapiens GN=PSMA7 PE=1 SV=1	11	2.06E-02	0.081	0.403
PSA2_HUMAN	Proteasome subunit alpha type-2 OS=Homo sapiens GN=PSMA2 PE=1 SV=2	8	3.87E-02	0.082	0.355
S10A9_HUMAN	Protein S100-A9 OS=Homo sapiens GN=S100A9 PE=1 SV=1	6	4.80E-02	0.083	0.345
BLMH_HUMAN	Bleomycin hydrolase OS=Homo sapiens GN=BLMH PE=1 SV=1	34	1.95E-02	0.084	0.404
FLNA_HUMAN	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4	4	3.35E-02	0.084	0.363
PSA5_HUMAN	Proteasome subunit alpha type-5 OS=Homo sapiens GN=PSMA5 PE=1 SV=3	5	1.45E-02	0.084	0.425
PSA6_HUMAN	Proteasome subunit alpha type-6 OS=Homo sapiens GN=PSMA6 PE=1 SV=1	9	1.56E-02	0.084	0.418
PSB3_HUMAN	Proteasome subunit beta type-3 OS=Homo sapiens GN=PSMB3 PE=1 SV=2	5	4.87E-02	0.084	0.349
DPP2_HUMAN	Dipeptidyl peptidase 2 OS=Homo sapiens GN=DPP7 PE=1 SV=3	9	5.62E-03	0.085	0.460
THIO_HUMAN	Thioredoxin OS=Homo sapiens GN=TXN PE=1 SV=3	6	6.34E-02	0.085	0.324
SODC_HUMAN	Superoxide dismutase [Cu-Zn] OS=Homo sapiens GN=SOD1 PE=1 SV=2	6	1.55E-02	0.091	0.418
AATM_HUMAN	Aspartate aminotransferase, mitochondrial OS=Homo sapiens GN=GOT2 PE=1 SV=3	7	1.21E-02	0.096	0.435
LAMP1_HUMAN	Lysosome-associated membrane glycoprotein 1 OS=Homo sapiens GN=LAMP1 PE=1 SV=3	3	6.15E-03	0.097	0.477
S10A8_HUMAN	Protein S100-A8 OS=Homo sapiens GN=S100A8 PE=1 SV=1	7	2.13E-02	0.098	0.399
PSB5_HUMAN	Proteasome subunit beta type-5 OS=Homo sapiens GN=PSMB5 PE=1 SV=3	10	2.76E-03	0.101	0.504
CATH_HUMAN	Pro-cathepsin H OS=Homo sapiens GN=CTSH PE=1 SV=4	7	2.54E-02	0.103	0.390
PSA3_HUMAN	Proteasome subunit alpha type-3 OS=Homo sapiens GN=PSMA3 PE=1 SV=2	7	4.69E-03	0.106	0.477
FILA_HUMAN	Filaggrin OS=Homo sapiens GN=FLG PE=1 SV=3	183	2.94E-03	0.106	0.489
FLNB_HUMAN	Filamin-B OS=Homo sapiens GN=FLNB PE=1 SV=2	8	4.38E-03	0.107	0.485
MYH9_HUMAN	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	9	1.28E-02	0.107	0.428
AATC_HUMAN	Aspartate aminotransferase, cytoplasmic OS=Homo sapiens GN=GOT1 PE=1 SV=3	18	3.07E-03	0.113	0.492
S10A7_HUMAN	Protein S100-A7 OS=Homo sapiens GN=S100A7 PE=1 SV=4	13	3.23E-03	0.118	0.524
INVO_HUMAN	Involucrin OS=Homo sapiens GN=IVL PE=1 SV=2	18	2.28E-03	0.119	0.522

CD59_HUMAN	CD59 glycoprotein OS=Homo sapiens GN=CD59 PE=1 SV=1	2	1.80E-03	0.121	0.514
TPM4_HUMAN	Tropomyosin alpha-4 chain OS=Homo sapiens GN=TPM4 PE=1 SV=3	5	5.16E-04	0.130	0.559
CALL5_HUMAN	Calmodulin-like protein 5 OS=Homo sapiens GN=CALML5 PE=1 SV=2	18	1.80E-05	0.157	0.684
UBC_HUMAN	Polyubiquitin-C OS=Homo sapiens GN=UBC PE=1 SV=2	8	8.21E-07	0.166	0.729

^a Two tailed Student’s t-test.

^b The proteins are ranked according to the OPLS-DA model loadings (12). The model loadings are a measure how much each protein contribute to the classification into AE or healthy group (12).

^c Correlation coefficient calculated using Pearson's Product-Moment Correlation.