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The antimicrobial protein S100A12 identified as a potential autoantigen in a subgroup of atopic dermatitis patients

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Abstract

Background: Atopic dermatitis (AD) is a complex heterogeneous chronic inflammatory skin disease. Specific IgE antibodies against autoantigens have been observed in a subgroup of AD patients, however, little is known about IgG-auto-reactivity in AD. To investigate the presence of autoreactive IgG antibodies, we performed autoantibody profiling of IgG in patients with AD of different severities and in healthy controls (HC).

Methods: First, we performed an untargeted screening in plasma samples from 40 severe AD (sAD) patients and 40 HC towards 1152 protein fragments on planar antigen microarrays. Next, based on the findings and addition of more fragments, a targeted antigen suspension bead array was designed to profile a cohort of 50 sAD patients, 123 patients with moderate AD (mAD), and 84 HC against 148 protein fragments representing 96 unique proteins.

Results: Forty-nine percent of the AD patients showed increased IgG-reactivity to any of the four antigens representing keratin associated protein 17-1 (KRTAP17-1), heat shock protein family A (Hsp70) member 4 (HSPA4), S100 calcium binding proteins A12 (S100A12), and Z (S100Z). The reactivity was more frequent in the sAD patients (66%) than in those with mAD (41%), whereas only present in 25% of the HC. IgG-reactivity to S100A12, a protein including an antimicrobial peptide, was only observed in AD patients (13/173).

Conclusions: Autoantibody profiling of IgG-reactivity using microarray technology revealed an autoantibody-based subgroup in patients with AD. The four identified autoantigens and especially S100A12 could, if characterized further, increase the understanding of different pathogenic mechanisms behind AD and thereby enable better treatment.

Keywords: Affinity proteomics, Antimicrobial protein, Atopic dermatitis/eczema, Autoantibody profiling, Autoantigen, Autoimmunity, Co-morbidity, IgG, Protein microarrays, Suspension bead array

Background

Atopic dermatitis (AD, also designated atopic eczema) is a complex heterogeneous chronic inflammatory skin disease [1] with 15–30% of children and 2–10% of adults being afflicted [2]. Defects in the skin barrier combined with inappropriate immune responses are considered central in the pathogenesis of AD with contribution of both genetic/epigenetic and environmental

factors including microbial agents [3–5]. Today there is an increased awareness that AD not only is associated with other atopic conditions but also has comorbidities with other diseases such as autoimmune and cardiovascular diseases suggesting that AD is a systemic disorder [6]. The different mechanisms behind the development of allergy related symptoms and the presence of comorbidities highlight the need to characterize the biological differences behind the complex clinical heterogeneity of AD to identify the proper endotypes to match novel specific biological treatments [7–9].

AD has been classified into an extrinsic or an intrinsic type according to the presence or absence of allergen-specific IgE antibodies [10]. The extrinsic AD phenotype

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is frequently characterized by coexistence of several IgE-mediated symptoms such as eczema, rhinitis and asthma, and polysensitization indicating that type 2 signaling pathways in the immune system participate in the disease development [7]. The other subgroup, consisting of around 20% of the AD patients, includes those with a clinical picture of AD but who lack detectable IgE antibodies to known food or aeroallergens and they usually have normal total serum IgE levels [10]. At present, one may therefore define this group as suffering from non-IgE-associated forms of AD [11], but it is also likely that the corresponding allergens triggering the skin inflammation in this group are not yet defined [12].

Still another subgroup of patients with AD has, in addition to sensitization against exogenous allergens, an IgE-mediated reactivity against autoantigens like epidermal proteins [13, 14]. These patients usually have a more severe form of AD including high levels of circulating IgE [15]. In addition, to add to the complexity, cross-reactivity between fungal allergens and the human homologues manganese superoxide dismutase [16] and thioredoxin [17] has been observed in AD patients sensitized to the skin commensal yeast *Malassezia sympodialis* [18, 19]. Autoimmunity is usually connected with increased levels of IgG to self-structures. The term autoallergy refers to autoimmunity accompanying an atopic disease with antigen-specific IgE raised against self-proteins [20]. Although IgG-reactivity (and IgE-reactivity) has been detected towards dense fine speckled autoantigen of 70 kD (DFS70) in AD patients [21, 22], little is in general known about IgG-auto-reactivity in AD.

In this study we therefore took the approach to perform a meticulous analysis of the IgG-reactivity profiles towards a broad panel of human protein fragments in AD patients compared with healthy controls (HC) using in-house developed protein microarrays [23–27]. The aim was to obtain information if and how IgG-reactivity may be linked with different clinical phenotypes of AD including co-morbidity with respiratory symptoms. Characterization of autoreactive IgG antibodies and identification of autoantigens could increase the understanding of different pathogenic mechanisms behind AD and thereby enable specific prevention and treatments.

Methods

Study population and IgE serology

Adult AD patients and HC were recruited in Stockholm, Sweden, during September until May to avoid the summer season, as described previously [15]. Inclusion criteria for the AD patients were diagnosis, set by a dermatologist, according to the UK working party criteria

[28]. Exclusion criteria were skin diseases other than AD, autoimmune diseases, immune deficiencies, malignant diseases, pregnancy or lactation, immunosuppressive treatment and an age below 18 or above 65 years. Use of systemic glucocorticoids or systemic antifungal treatment was not allowed for 2 months before the investigation. The severity of the eczema was assessed using SCORing Atopic Dermatitis (SCORAD) [29] where after patients with moderate AD (mAD) or severe AD (sAD) not only restricted to the hands were subjected to the study. The history of patient reported respiratory allergic symptoms was documented as co-morbidity. The HC had no clinical symptoms or history of allergy or skin diseases.

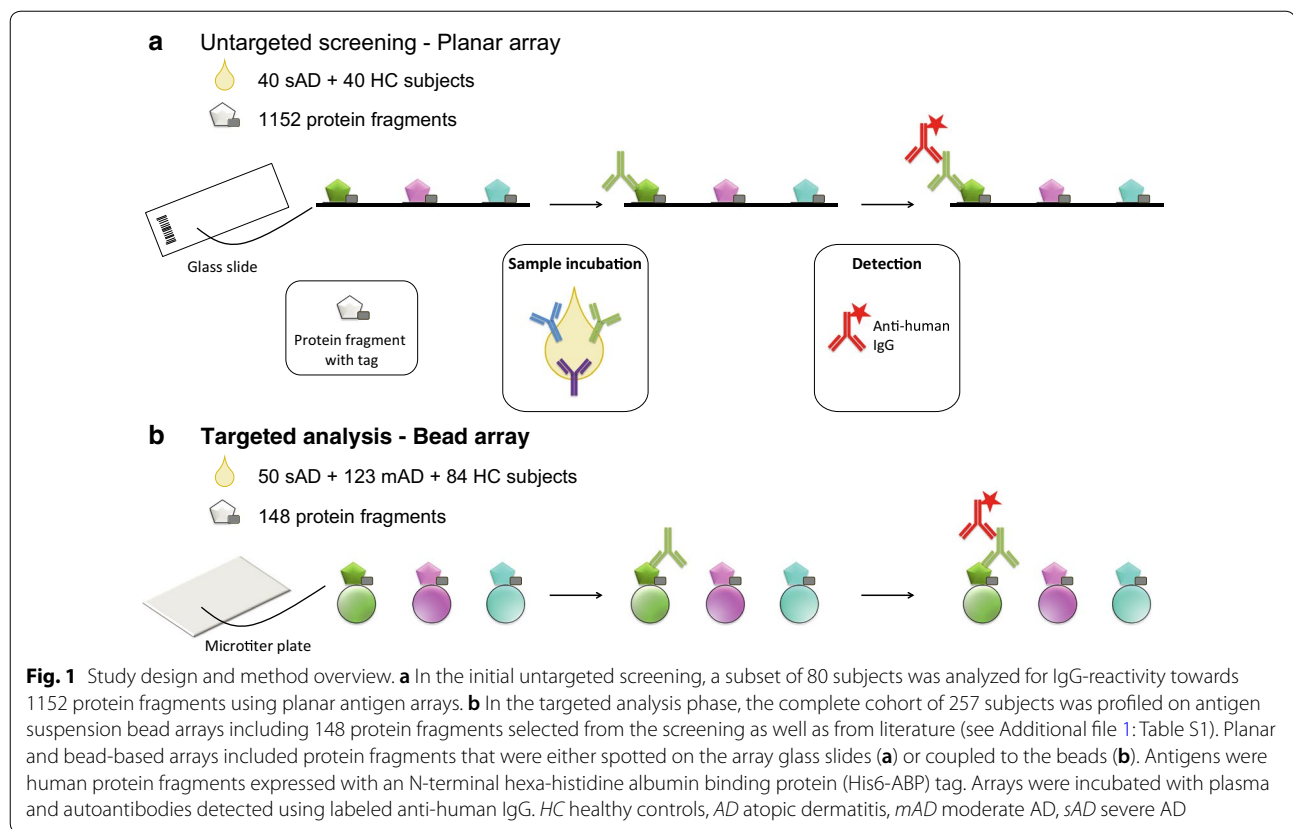
Total IgE and allergen-specific IgE to any of 11 common aeroallergen sources (dog, cat, horse, birch, timothy, mugwort, pellitory, olive tree, *Cladosporium*, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae*) included in the mix Phadiatop, were measured in plasma using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) during 2006–2012 at the Karolinska University Hospital, Stockholm. The reference range for total plasma IgE was 1.6–122 kU/L and for a positive Phadiatop ≥ 0.35 kU/L.

Study design

To profile plasma IgG-reactivity in patients with AD and HC we used two different microarray platforms (see Fig. 1 for an overview of the study design). First, we performed an untargeted screening in 80 subjects using more than 1100 protein fragments on planar antigen microarrays. To verify findings from this initial screening phase and with use of additional fragments (see Additional file 1: Table S1), a targeted antigen suspension bead array was designed to profile the complete cohort of 257 subjects against 148 protein fragments.

Antigens

The antigens used in this study were human protein fragments produced within the Human Protein Atlas project (www.proteinatlas.org) as described previously [30–32]. Briefly, protein fragments with an average length of 80 amino acids and mostly ranging between 50 and 150 amino acid residues were designed in silico with low homology to other human proteins, cloned and expressed in *Escherichia coli* (*E. coli*) as fusion proteins with an N-terminal hexa-histidine albumin binding protein (His6-ABP) tag, and validated with mass spectrometry subsequent to immobilized metal affinity chromatography purification. Protter v 1.0 (<http://wlab.ethz.ch/protter/start/>) [33] was used for visualization of protein sequence and region of fragments.



Planar antigen microarrays

In the screening phase of the study, planar antigen microarrays produced in the routine antibody validation workflow within the Human Protein Atlas project [34] (www.proteinatlas.org) were used to generate autoantibody profiles in plasma for 80 subjects. Each microarray slide consisted of 21 identical subarrays, each subarray containing 384 protein fragments. Three of these array designs comprising in total 1152 protein fragments were selected randomly and used in an untargeted screening. The screening was performed as described previously with minor changes [23], see Supplementary Methods in Additional file 1. Data in the form of median fluorescence intensity (MFI) per array spot were used in further analysis. Cut-off levels for positive IgG-reactivity were set in a sample-specific manner to account for the individual background of each sample. The cut-off was calculated based on the sample median of all 384 protein fragments on each of the subarrays plus 10 or 30 times the median absolute deviation (MAD). For each sample, the signal of individual protein fragments was then compared to the cut-off and positive IgG-reactivity to a particular fragment defined as a fragment signal above the cut-off. The frequency of reactive individuals in sAD and HC were compared and protein fragments selected for further

analysis in the targeted screening based on the following criteria: Fisher's exact test P value <0.05 or $\geq 10\%$ unit difference.

Antigen suspension bead arrays

A targeted antigen suspension bead array was designed to profile the complete cohort including 257 subjects (Fig. 1). This design was based on results from the initial screening, including the top findings as defined from the above mentioned criteria of statistical significance or frequency of reactivity. Additional protein fragments were included to represent targets from the literature [35–38]. Availability of protein fragments within the Human Protein Atlas (www.proteinatlas.org) resulted in a set of 148 fragments representing 96 proteins (see Additional file 1: Table S1 for a list of all fragments, sequences and the source for inclusion). For 62 of the 96 proteins, each protein was represented by two to four fragments that covered different regions of the protein (exemplified in Additional file 1: Fig S1B and D where two fragments were included for these proteins). The protein fragments were coupled to color-coded carboxylated magnetic beads (MagPlex, Luminex corp., Austin, TX, USA) to create an antigen suspension bead array that was incubated with the samples as previously described [23], see

Supplementary Methods for more details. Data in the form of MFI, counted on at least 30 beads per fragment, were compared to sample-specific cut-offs as described for the planar array. For each sample the median MFI across all 148 fragments plus a factor of 10–70 times the MAD were used to create cut-off levels. Again, positive IgG-reactivity was determined by relating the signal of individual protein fragments to the cut-off. Signal intensities were also compared across groups after transformation where the sample median MFI across all fragments was subtracted from the raw MFI and divided by the MAD, thereby creating adjusted MFI values where the signal is expressed in MADs above or below the median. Results are expressed as frequency of reactive individuals (% of subjects with IgG-reactivity above the cut-off level) and as intensity expressed as adjusted MFI.

Statistical analysis

Data analysis and visualization were performed in R [39]. Fisher's exact tests were used per protein fragment to compare reactivity frequencies between groups. Wilcoxon rank-sum tests were used per fragment to compare intensity levels (adjusted MFI) between groups. Unadjusted *P* values < 0.05 were here considered significant. Hierarchical clustering was performed on binary data (IgG-reactivity "yes" or "no").

Results

Study population

In total 173 adult AD patients, 18–65 years (median 28 years), and 84 HC, 18–65 years (median 39 years) were included in the study according to the inclusion and exclusion criteria in the Methods. Clinical and demographic characteristics are presented in Table 1. Among the AD patients, 29% were classified as sAD and 71% as mAD. The majority of patients with AD had current or a

history of rhinoconjunctivitis and/or asthma (80%). Total IgE levels were elevated (≥ 122 kU/L) in 55% of the AD patients, 70% of patients with sAD and in 49% of patients with mAD. A positive Phadiatop, defined as detectable IgE antibodies to a mixture of 11 common aeroallergens, was found in 76% of the AD patients.

Untargeted screening

In the first screening phase (Fig. 1a), antigens representing keratin associated protein 17-1 (KRTAP17-1) and S100 calcium binding protein Z (S100Z) together with 21 additional antigens showed a difference in the frequency of IgG-reactivity between sAD patients (*n* = 40) and HC (*n* = 40) of at least 10% units. Three of these antigens (representing the family with sequence similarity 9 member C, FAM9C; the olfactory receptor family 2 subfamily T member 11, OR2T11; and the S100 calcium binding protein A3, S100A3) had significantly higher reactivity in sAD compared to HC (*P* < 0.05). These in total 23 antigens were selected for further analysis in the targeted analysis phase (listed in Additional file 1: Table S1 as "Screening (*P* < 0.05)" or "Screening ($\geq 10\%$ reactivity difference)"). Additional fragments that had not been represented on the planar antigen arrays and that covered other regions of the represented proteins were also included. This resulted in a final selection of 47 protein fragments based on the screening phase (see Additional file 1: Table S1).

Targeted analysis

The complete cohort of 173 AD patients (123 mAD and 50 sAD) and 84 HC (Table 1) was analyzed using the targeted antigen suspension bead array covering 148 protein fragments representing 96 proteins (see Fig. 1b and Additional file 1: Table S1). Group comparisons were first made on the level of frequency of subjects with positive

Table 1 Characterization of the AD patients and healthy controls

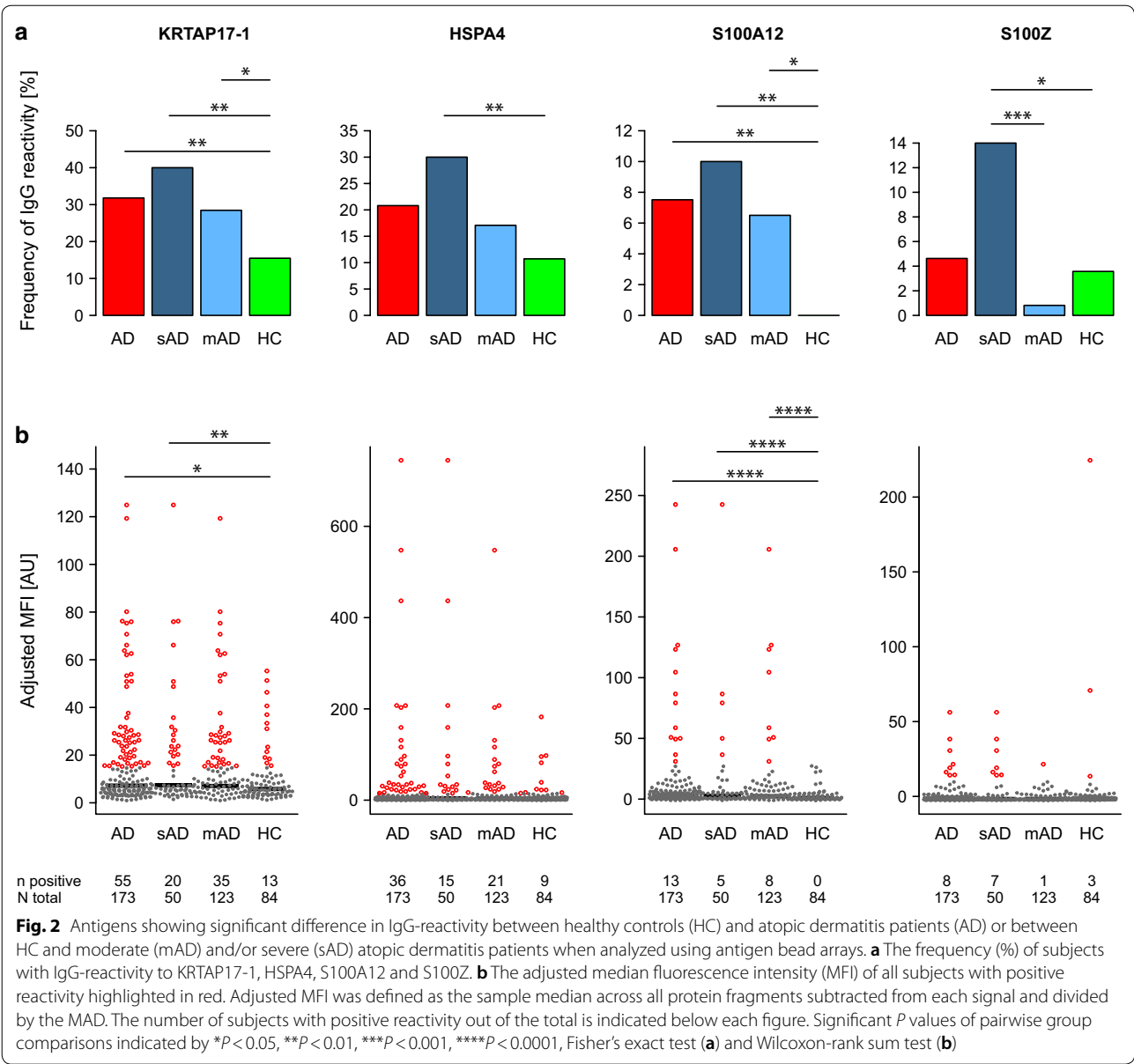
	N	Gender F/M	Age (years)	SCORAD ^a	Rhino-conjunctivitis and/or asthma current or history	Total plasma IgE ^b ≥ 122 kU/L	Total plasma IgE ^b (kU/L)	Phadiatop positive ^c
		n (%)	Median (range)	Median (range)	n (%)	n (%)	Median (range)	n (%)
AD patients	173	99/74 (57)	28 (18–65)	34 (14–70)	139 (80)	95 (55)	160 (2–15,100)	132 (76)
Severe ^a	50	17/33 (34)	29 (18–65)	54 (41–70)	45 (90)	35 (70)	460 (2.5–15,100)	43 (86)
Moderate ^a	123	82/41 (67)	28 (18–63)	31 (14–40)	94 (76)	60 (49)	120 (2–6810)	89 (72)
Healthy controls	84	52/32 (62)	39 (18–65)	NA	0 (0)	0 (0)	19 (2–120)	0 (0)

AD atopic dermatitis, F female, M male, N number of individuals, n number of positive individuals, NA not applicable

^a Objective SCORAD [29], severe AD defined as SCORAD ≥ 41

^b ImmunoCAP (Thermo Fisher Scientific), reference range 1.6–122 kU/L

^c Phadiatop (Thermo Fisher Scientific), plasma IgE-reactivity to any of 11 common aeroallergens, reference range ≥ 0.35 kU/L



IgG-reactivity (Fig. 2a and Table 2). Applying sample- and protein fragment-specific cut-off levels, a significant difference in the frequency of IgG-reactivity was identified for four antigens representing KRTAP17-1; heat shock protein family A (Hsp70) member 4, HSPA4; S100 calcium binding proteins A12, S100A12; and S100Z, differentiating between AD and HC or HC and any of the two subgroups of mAD and sAD (Table 2 and Additional file 1: Table S2). Both KRTAP17-1 and S100Z were included from the untargeted screening where they showed a higher frequency of reactivity in sAD compared to HC. The other two, HSPA4 and S100A12, were not part of the initial screening. The four antigens

showed $\geq 10\%$ reactivity in either AD patients or in any of the two subgroups and were selected for further characterization. Positive IgG-reactivity to KRTAP17-1 was observed in significantly higher frequency in AD patients (32%) as well as in mAD (28%) and sAD (40%) compared to HC (15%) (Fig. 2a and Table 2). KRTAP17-1 was represented by a protein fragment covering amino acid 7–38 (Additional file 1: Fig S1A, in red). HSPA4 was found reactive in 21% of AD patients and showed differential reactivity comparing sAD and HC (Fig. 2a and Table 2). Two protein fragments covering different parts of HSPA4 were included in the analysis and reactivity was identified

Table 2 IgG-reactivity^a and adjusted MFI^b to four antigens detected in the targeted screening of the complete cohort using antigen bead arrays

Gene	KRTAP17-1	HSPA4	S100A12	S100Z
Gene description	Keratin associated protein 17-1	Heat shock 70 kDa protein 4	S100 calcium binding protein A12	S100 calcium binding protein Z
Ensembl ENSG ID ^c	ENSG00000186860	ENSG00000170606	ENSG00000163221	ENSG00000171643
Uniprot	Q9BYP8	P34932	P80511	Q8WVG8
Protein fragment ID	HPRR3460778	HPRR4220528	HPRR400165	HPRR3460547
Number of IgG-reactive individuals	n (%)	n (%)	n (%)	n (%)
AD patients (N = 173)	55 (32)	36 (21)	13 (8)	8 (5)
Severe ^d (N = 50)	20 (40)	15 (30)	5 (10)	7 (14)
Moderate ^d (N = 123)	35 (28)	21 (17)	8 (6)	1 (1)
Healthy controls (N = 84)	13 (15)	9 (11)	0 (0)	3 (4)
P values^e				
Calculated on proportion positive individuals				
AD versus HC	0.006	0.054	0.006	1.00
sAD versus mAD	0.15	0.07	0.53	0.0008
sAD versus HC	0.002	0.009	0.006	0.04
mAD versus HC	0.03	0.23	0.02	0.31
Adjusted MFI of individuals	Median (Q1–Q3)	Median (Q1–Q3)	Median (Q1–Q3)	Median (Q1–Q3)
AD patients (N = 173)	7.2 (4.3–18.2)	3.2 (1.3–9.8)	2.6 (0.8–6.5)	– 1.7 (– 2.1 to – 0.6)
Severe ^d (N = 50)	7.4 (4.6–23.3)	4.0 (1.2–21.3)	2.6 (1.0–6.5)	– 1.8 (– 2.2 to 0.5)
Moderate ^d (N = 123)	7.1 (4.3–15.9)	3.2 (1.3–8.7)	2.3 (0.8–6.3)	– 1.7 (– 2.1 to – 1.0)
Healthy Controls (N = 84)	5.8 (3.4–10.5)	2.8 (1.1–7.2)	0.8 (0.2–2.0)	– 1.6 (– 2.0 to – 0.2)
P values^f				
Calculated on adjusted MFI values				
AD versus HC	0.01	0.36	0.000003	0.16
sAD versus mAD	0.26	0.63	0.50	0.35
sAD versus HC	0.01	0.32	0.00008	0.78
mAD versus HC	0.054	0.48	0.00003	0.09

AD atopic dermatitis, sAD severe AD, mAD moderate AD, HC healthy controls, N number of individuals, n = number of positive individuals

^a IgG-reactivity was defined using sample-specific cut-offs based on the sample median across protein fragments and the median absolute deviation (MAD), see “Methods” section

^b Sample median across protein fragments was subtracted from each signal and divided by the MAD, see “Methods” section

^c Ensembl version 88.38

^d Objective SCORAD [29], severe AD defined as SCORAD $\geq$ 41

^e Fisher's exact test

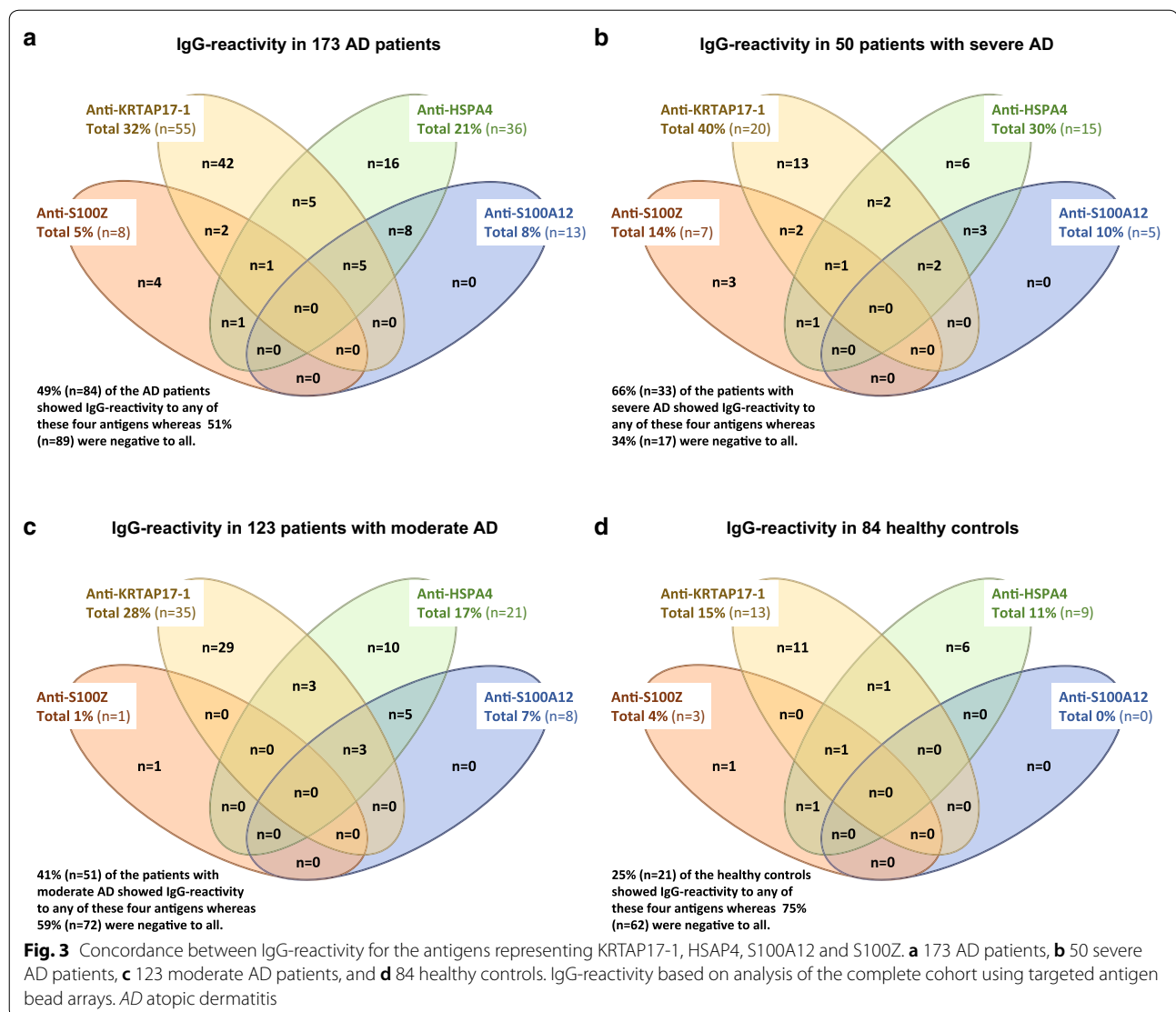
^f Wilcoxon rank-sum test

towards the region of amino acid 526–577 (Additional file 1: Fig S1B, in red).

Interestingly, IgG-reactivity above the cut-off value to S100A12, was only observed in AD patients (8%) with significant differences found between AD or subgroups of AD and HC (Fig. 2a and Table 2). The reactive protein fragment for S100A12 mapped to amino acid 3–92, thereby covering 98% of the full protein sequence (Additional file 1: Fig S1C, in red). Significantly higher reactivity to S100Z was found in sAD patients compared to both

HC and mAD (Fig. 2a and Table 2). S100Z was represented by two protein fragments with the reactive region mapped to amino acid 34–71 (Additional file 1: Fig S1D, in red).

The four antigens with differential reactivity were further explored by group comparisons on the level of adjusted MFI (Fig. 2b and Table 2). For KRTAP17-1 and S100A12, most of the significant differences in IgG-reactivity frequency (Fig. 2a) were also observed on the intensity level (Fig. 2b).

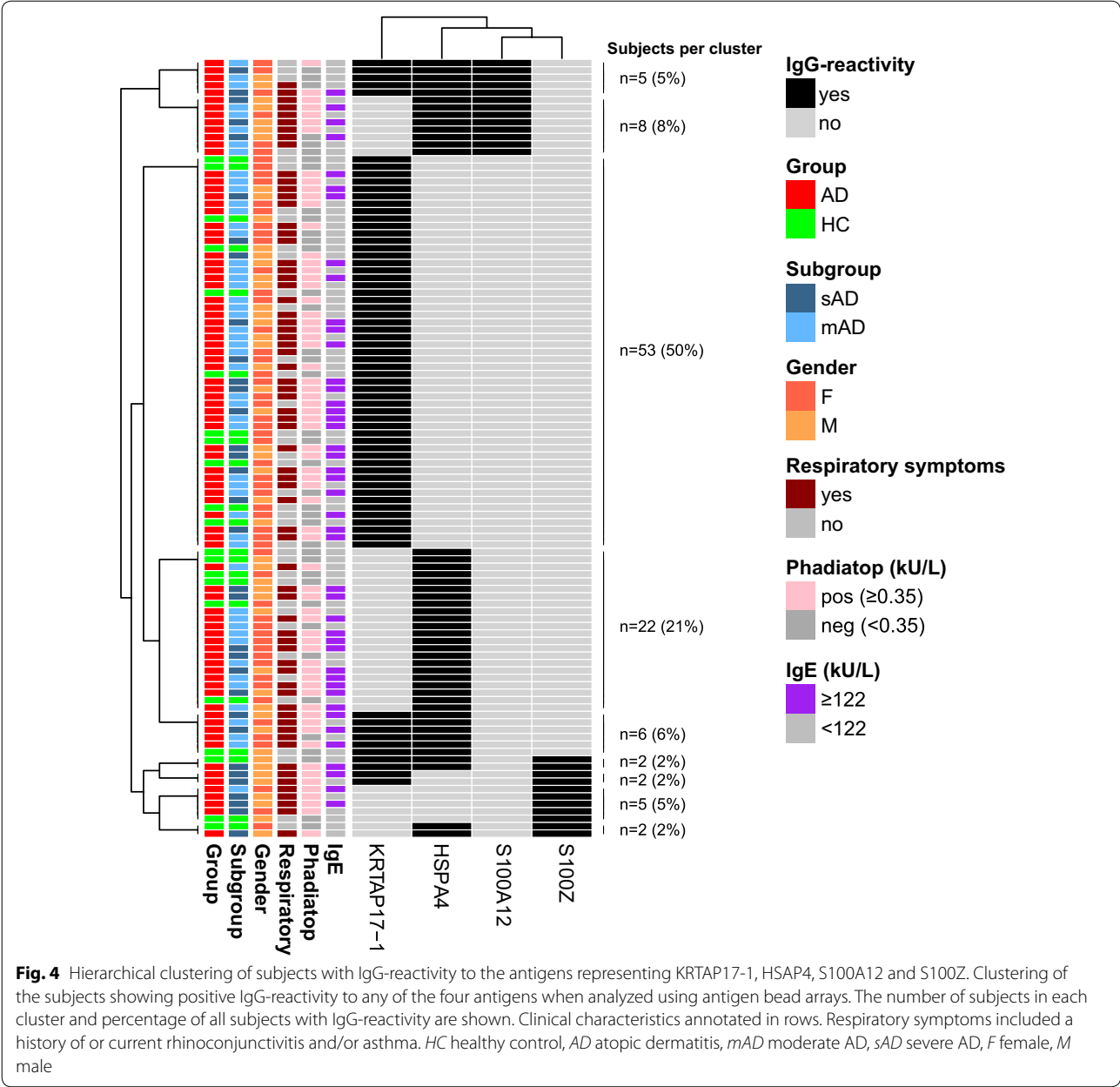


Forty-nine percent of the AD patients showed IgG-reactivity to any of the four antigens representing KRTAP17-1, HSPA4, S100A12 and/or S100Z (Fig. 3a). IgG-reactivity to those antigens was more frequent in the sAD patients (66%) than in those with mAD (41%), whereas reactivity only could be found in 25% of HC (Fig. 3b–d). Subjects reactive to any of the four antigens were subjected to hierarchical clustering to show any overlap in reactivity (Fig. 4). Here it was evident that most subjects were reactive to single antigens only, shown by the three clusters corresponding to 76% of all reactive subjects (single reactivity to KRTAP17-1, HSPA4 or S100Z). Notably, this pattern differed for S100A12 where all 13 AD patients reactive to this antigen also showed reactivity to HSPA4 and five of them also to KRTAP17-1 (Fig. 3a). This pattern was the

same in sAD and mAD, whereas no HC showed IgG-reactivity to S100A12 (Fig. 3b–d). Four clusters with 17% of the IgG-reactive subjects included reactivity to two antigens (Fig. 4). Reactivity to three antigens was observed in two clusters, corresponding to 7% of the IgG-reactive subjects (Fig. 4). There was no significant difference in the frequency of IgG-reactivity between females and males (Fig. 4) and also no association between age and adjusted MFI for any of the four antigens.

Associations to allergen specific IgE and total plasma IgE

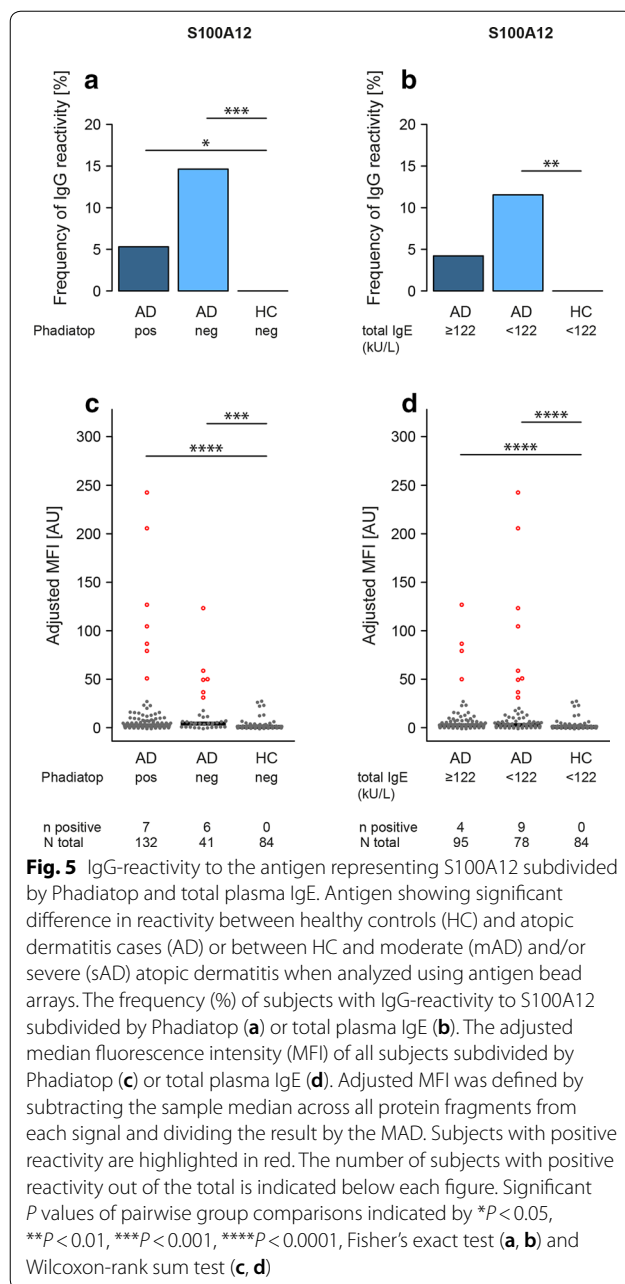
Investigating the relation to allergen specific IgE defined by a positive or negative Phadiatop, revealed significantly higher frequency of IgG-reactivity to KRTAP17-1 in AD patients with positive Phadiatop compared to HC



as well as higher frequency of IgG-reactivity to S100A12 in AD patients irrespective of Phadiatop compared to HC (Fig. 5a and Additional file 1: Table S3). These differences were also observed when comparing adjusted MFI (Fig. 5b and Additional file 1: Table S3). Subdividing AD patients into subgroups of total plasma IgE lower or higher than 122 kU/L, only showed differences between any of the AD subgroups and HC for KRATP17-1 (Additional file 1: Table S3) and S100A12 (Fig. 5b, d and Additional file 1: Table S3). Details for all four antigens can be found in Additional file 1: Table S3.

Co-morbidity with respiratory allergic symptoms

The majority of patients with AD, irrespective of subgroup, had current or a history of rhinoconjunctivitis and/or asthma (Table 1) in combination with elevated total plasma IgE and a positive Phadiatop (Fig. 6a). This pattern was also observed among the 84 AD patients with IgG-reactivity to at least one of the antigens for KRTAP17-1, HSPA4, S100A12 or S100Z (Fig. 6b). Details for the separate antigens are shown in Fig. 6c–f. The proportion of sAD was significantly larger ($P < 0.01$) among the patients with IgG-reactivity to any of the



four antigens compared to the group of patients without any reactivity, whereas the proportion of patients with rhinoconjunctivitis and/or asthma, elevated total

plasma IgE and positive Phadiatop was similar (Fig. 6b compared to Fig. 6g).

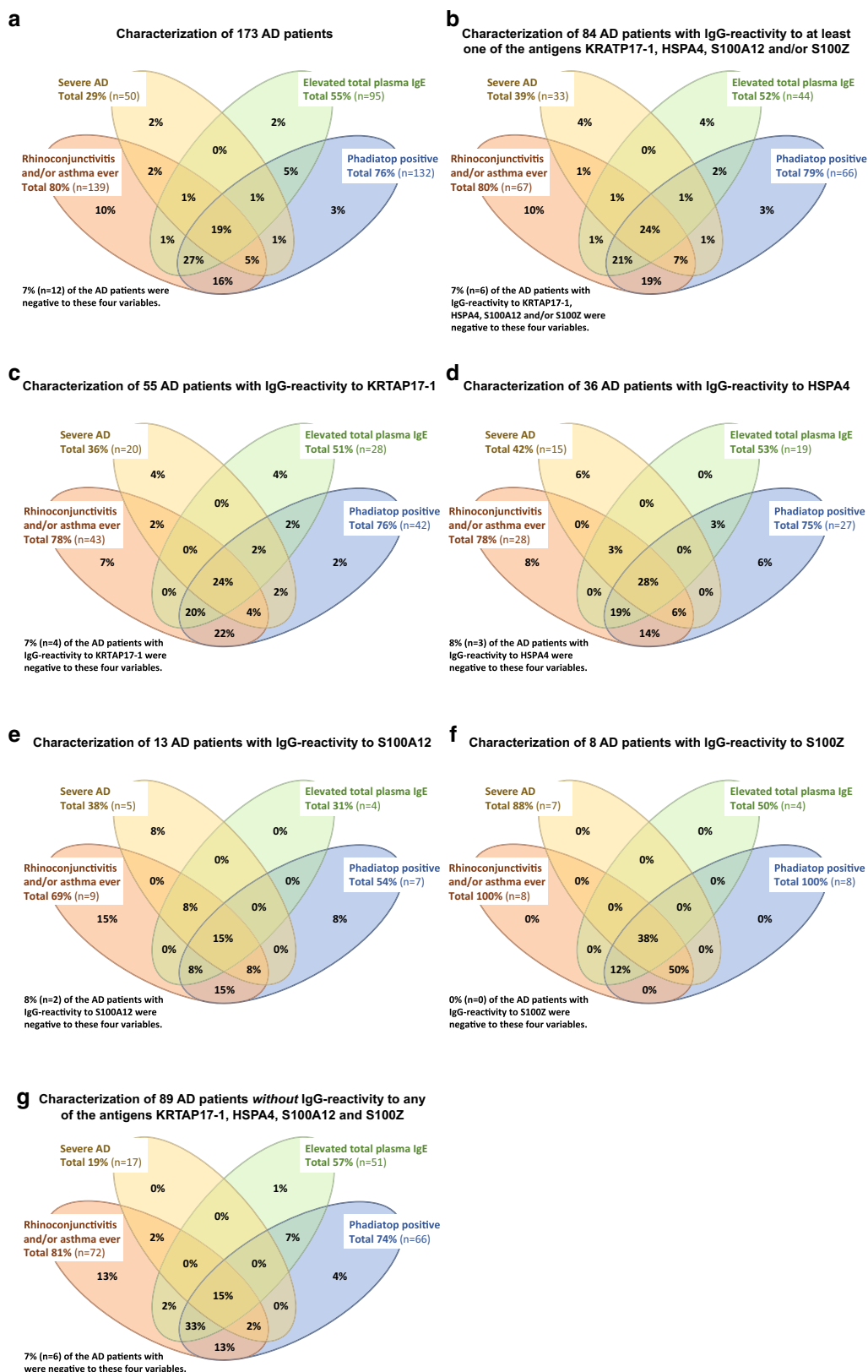
Discussion

With the aim to obtain information if autoantibody-based IgG-reactivity can be associated with AD we discovered that auto-reactivity to S100A12 was only observed in AD patients using antigen microarray technology. Three additional proteins KRTAP17-1, HSPA4, and S100Z had a significant increased IgG binding reactivity in plasma samples from AD patients compared to HC and more frequently in the sAD patients than in those with mAD.

Access to large collections of expressed and purified proteins in combination with microarray technology allows for screening of autoantibody reactivity towards hundreds to thousands of antigens in parallel while consuming only small sample volume enabling detection of potential novel autoimmune targets [24]. Planar array formats offer higher theoretical multiplexing in terms of antigens and bead-based formats provide higher sample throughput, making the use of both formats beneficial [40]. The methods used in this study relied on the use of human protein fragments of various lengths and with low similarity to other proteins. Depending on the coverage of the full protein sequence, different conformations are possible and most likely allow for both linear and conformational epitopes to be recognized. Furthermore, as the fragments are expressed as recombinant products in *E. coli*, post-translational modifications (PTMs) such as glycosylation are not present and reactivity towards epitopes dependent on PTMs will not be detected. Utilizing protein fragments instead of full-length proteins mean that for some proteins only a subset of the possible epitopes are present and could result in that potentially autoreactive sequences are not covered or that the fold makes the epitope inaccessible to binding. However, we included several fragments from different parts of the protein when designing the targeted antigen array, thereby increasing the coverage and allowing for closer localization of the reactive region of a protein. This was the case for both KRTAP17-1 and S100Z, where two fragments were included to represent each protein and reactivity identified to only one of them (Fig. S1). Despite differences between the two used array formats as well as increased heterogeneity of individuals when considering

(See figure on next page.)

Fig. 6 Concordance between rhinoconjunctivitis and/or asthma ever, AD severity (moderate or severe), elevated total plasma IgE and positive Phadiatop. **a** 173 AD patients, **b** 84 AD patients with IgG-reactivity to KRTAP17-1 HSPA4, S100A12 and/or S100Z, **c** 55 AD patients with IgG-reactivity to KRTAP-17, **d** 36 AD patients with IgG-reactivity to HSPA4, **e** 13 AD patients with IgG-reactivity to S100A12, **f** 8 AD patients with IgG-reactivity to S100Z, and **g** 89 AD patients without IgG-reactivity to any of the antigens for KRTAP17-1 HSPA4, S100A12 and/or S100Z. IgG-reactivity based on analysis of the complete cohort using targeted antigen bead arrays, showing frequencies. Elevated total plasma IgE defined as ≥ 122 kU/L. AD atopic dermatitis



the whole cohort, KRTAP17-1 and S100Z were successfully verified in the bead array assay.

S100A12 and S100Z belong to the heterogeneous S100 family of calcium-binding proteins [37]. S100A12, predominantly expressed and secreted by neutrophil granulocytes, is also known as calgranulin C and as extracellular newly identified RAGE-binding protein (EN-RAGE) [41, 42]. S100A12 can trigger pro-inflammatory signaling through TLR4 and exert antimicrobial activity [41]. The reactive protein fragment used for S100A12 in our study mapped to amino acid 3–92, thereby covering 98% of the full protein sequence including the antimicrobial peptide (Fig S1C, in red). Antimicrobial peptides and proteins (AMPs) are effector molecules of the innate defense system and are mainly produced by epithelial cells including keratinocytes [43]. Studies have shown that S100A12, also classified as an epithelial cytokine, is expressed at very low levels in normal epidermis, but it is highly overexpressed in lesions of psoriasis [44, 45], UVB-irradiated skin [46], and tape-stripping of the skin induce fast and long-lasting AMP expression [47]. In addition, S100 proteins are overexpressed in conditions associated with water loss, such as during wound repair why it has been suggested that S100A12 is a potential therapeutic target for dermal scarring [48]. The skin barrier in AD is characterized by dry itchy skin with increased permeability and water loss [1], and with enhanced expression of several AMPs [49, 50] including S100A12 [45]. Regarding serum levels, S100A12 has been found to be the most significant marker for psoriasis disease activity and therapeutic response [45] and as a sensitive and specific diagnostic marker of other inflammatory responses [42], such as asthma where S100A12 provokes mast cell activation and thereby might exacerbate allergic inflammation [51]. Recently, serum levels of S100A12 were found to be differently expressed between four identified AD disease clusters (see Supplemental table E2 in Ref [8]) and were reported significantly upregulated in AD patients versus healthy controls with a correlation between the S100A12 serum levels and SCORAD [52]. In addition, increased serum levels of S100 proteins including S100A12 have been found especially associated with auto-inflammatory diseases, and therefore suggested as promising targets for treatment [53]. In future studies it would thus be interesting to see whether autoantibodies against S100A12 in AD have a mechanistic connection to serum levels of this protein and to increased expression of S100A12 in the skin.

The highest frequency of IgG-reactivity was found against the keratin associated protein 17-1 (KRTAP17-1) among the AD patients (32%) and up to 40% among the sAD patients (Table 2) and with significantly higher frequency compared to HC (15%) for the two subgroups of AD (Fig. 2a). Keratin associated proteins (KRTAPs) are

one of the main structural components of hair, and are expressed in distinct cell populations of the hair follicle involved in hair formation, where KRTAP17-1 shows strong expression in the upper hair cuticle [54]. The hair cuticle structure is severely damaged and distorted in lesional and non-lesional AD [55]. This could result in an increased exposure of altered structural hair components to the immune system inducing autoantibody production.

The majority of the AD patients had also current or a history of rhinoconjunctivitis and/or asthma in combination with elevated total plasma IgE and were positive in Phadiatop with a higher proportion in the sAD compared with the mAD group (Table 1). However, cluster analysis did not reveal any particular subgroup associated with increased IgG-reactivity to any of the antigens for KRTAP17-1, HSPA4, S100A12 and/or S100Z besides more frequent IgG-reactivity in the sAD than in the mAD group. The data would thus, rather suggest that IgG-reactivity to any of these four antigens reflects AD severity rather than co-morbidity with respiratory allergic symptoms.

IgG-reactivity to KRTAP17-1, HSPA4 and S100Z, but not to S100A12 was also observed in 21 of the 84 HC (25%) (Fig. 3d) compared to 49% of the 173 AD patients reactive to any of the four antigens (Fig. 3a). Autoantibodies are not necessarily pathogenic, but often observed in healthy individuals [56]. It has been suggested that “natural” IgG autoantibodies might participate in an adaptive debris-clearance mechanism [57], which could be the case for the three autoantibodies we found both in HC and in AD patients. Pathologic autoantibodies can induce disease through several different pathways [56]. Whether the autoantibodies against S100A12 we detected in a subgroup of AD patients are protective, pathogenic or merely an epiphenomenon remains to be determined. One hypothesis could be that production of these autoantibodies is induced by the chronic inflammation with enhanced expression of S100A12 in the skin and that they could either exhibit anti-inflammatory or pro-inflammatory activities connected to the function of S100A12. A mechanism could be that the autoantibodies possess a pathogenic function by binding to and clearing S100A12, thereby preventing the protein from exerting its antimicrobial function and potentially contributing to increased susceptibility to skin infections. Further studies in additional sample cohorts are needed in addition to the characterization of isotypes and subclasses of autoantibodies in AD and the elucidation of their functional activity in vivo.

Conclusions

We have utilized an untargeted array-based screening on more than 1100 protein fragments followed by the analysis of 257 human AD and HC plasma samples in an antigen suspension bead array representing 96 selected proteins. Introducing array methodologies for detailed phenotype characterization of the autoantibody IgG profile in patients with AD have here provided the possibility for identification of novel candidate autoantigens. We have found a subgroup of AD patients with IgG-reactivity to four autoantigens, among those S100A12 with antimicrobial activity. Further characterization of their implication for AD could be part of the need to develop personalized strategies for prevention and management to break, terminate or reverse the natural course of this chronic disease.

Additional file

Additional file 1. Supplementary Methods, Table S1, Table S2, Table S3 and Fig S1.

Abbreviations

AD: atopic dermatitis; AMPs: antimicrobial peptides and proteins; HC: healthy controls; His6-ABP: N-terminal hexa-histidine albumin binding protein; HSPA4: heat shock protein family A (Hsp70) member 4; KRTAP17-1: keratin associated protein 17-1; mAD: moderate atopic dermatitis; MAD: median absolute deviation; MFI: median fluorescence intensity; PTM: post-translational modifications; S100A12: S100 calcium binding protein A12; S100Z: S100 calcium binding protein Z; sAD: severe atopic dermatitis; SCORAD: scoring atopic dermatitis.

Authors' contributions

PN, AS: Conception of the study; MM, CJ, NA, PN, AS: Study design; CJ, AS: Sample collection; MM, CJ: Acquisition of data; MM, CJ, PN, AS: Data analysis and interpretation; MM, CJ, PN, AS: Drafting the manuscript. Critical revision of the manuscript for intellectual content: all authors. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study as well as the R code used for data analysis are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was conducted in accordance with the Helsinki Declaration ethical principles for medical research, and was approved by the Regional Ethical Review Board in Stockholm (Dnr 04-593/1 with addition Dnr 2008/1122-32). All participants gave their written informed consent.

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Supplementary material to:

The antimicrobial protein S100A12 identified as a potential autoantigen in a subgroup of atopic dermatitis patients

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SUPPLEMENTARY METHODS

Planar antigen microarrays

In the screening phase, 1.5 μ l of plasma from 80 subjects (40 sAD patients and 40 HC patients, Fig 1A) were diluted 1:250 in assay buffer using a liquid handler (SELMA, CyBio, Jena, Germany). The sAD patients were individually age- and gender-matched with the HC (12 females and 28 males in each group, age median (range) were 31 (18-65) years in sAD and 31 (18-64) years in HC). The assay buffer contained 3% (w/v) bovine serum albumin (BSA, Saveen Werner AB, Limhamn, Sweden), 5% (w/v) nonfat milk powder (Semper, Sundbyberg, Sweden) and 0.1% Tween-20 in PBS (Medicago AB, Uppsala, Sweden) (PBS-T), supplemented with 160 μ g/ml His6-ABP. Samples were pre-incubated for 1 h at room temperature on a shake table before applied to the subarrays (60 μ l) in a randomized fashion (equal numbers of AD patients and age- and gender matched HC per subarray) using a liquid handler (EVO150, Tecan Group, Männedorf, Switzerland) and incubated for 2 h. Slides were washed in 0.1% PBS-T and incubated with hen anti-His6-ABP IgY (1:25000, Agrisera, Vännäs, Sweden) for 45 min. After washing, a mixture of Alexa Fluor 647-conjugated goat F(ab')₂ anti-human IgG Fc γ specific (1:25000, Jackson ImmunoResearch, West Grove, PA, USA) and Alexa Fluor 555-conjugated goat anti-hen IgG (1:60000, Invitrogen, Thermo Fisher Scientific) was applied for 60 min. After washing, slides were dried and scanned (Agilent G2565BA array scanner, Agilent Technologies Inc., Santa Clara, CA, USA) followed by image analysis (GenePix Pro 5.1, Molecular Devices, Sunnyvale, CA, USA) , reporting median fluorescence intensity (MFI) for each array spot.

Antigen suspension bead arrays

In the targeted analysis phase, 5×10^5 beads were washed in phosphate buffer (0.1 M NaH_2PO_4) before activated with 0.5 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, Pierce, Thermo Fisher Scientific, Waltham, MA, USA) and 0.5 mg N-hydroxysulfosuccinimide (S-NHS, Pierce) in phosphate buffer for 20 min. Following washing of beads in 2-(N-Morpholino)ethanesulfonic acid (MES, 0.05 M) using a plate washer (EL406, Biotek, Winooski, VT, USA), protein fragments (40 $\mu\text{g}/\text{ml}$ in MES) were added to beads and incubated for 2 h. After a final wash in 0.05% PBS-T, beads were stored overnight in a blocking reagent for ELISA (Roche Diagnostics, Mannheim, Germany) before combined into a multiplex suspension bead array. Successful immobilization of protein fragments was confirmed using hen anti-His6-ABP IgY (Agrisera) and R-phycoerythrin conjugated donkey F(ab')₂ anti-hen IgY (Jackson ImmunoResearch). Before the assay, 50 μl of plasma samples were randomly distributed in 96-well microtiter plates (Thermo Fisher Scientific), controlling for an even distribution of AD patients and HC across plates with regard to numbers, gender and age. In addition, a buffer blank in duplicates and a pool of all samples were aliquoted in triplicates across each plate for determination of technical variation. For the assay, 1 μl of plasma was diluted 1:250 as described above for the planar antigen array using a liquid handler (SELMA, CyBio) and 45 μl diluted samples were incubated with the beads in a 384-well plate (Greiner Bio-One, Kremsmünster, Austria) for 2 h. After washing with 0.05% PBS-T using a plate washer, bound IgG were crosslinked using 0.2% paraformaldehyde and later detected by R-phycoerythrin conjugated goat F(ab')₂ anti-human IgG Fc γ specific (1:750, 30 min, Jackson ImmunoResearch). Read-out was performed in a FlexMAP3D system (Luminex corp.), reporting MFI for each bead identity.

SUPPLEMENTARY TABLES AND FIGURES

Table S1. List of the 148 protein fragments included in the targeted analysis using antigen bead arrays. This selection of protein fragments was based on findings from the untargeted screening process on planar antigen arrays. In addition, protein fragments representing targets from literature [1-3] and mRNA [4] and DNA methylation [4] screenings were also included. The four antigens identified to be reactive in the targeted analysis are highlighted in red.

Gene	Gene description	Uniprot	ENSG	Protein fragment ID	Sequence	Fragment length (aa)	Source
ARRB1	arrestin beta 1	P49407	ENSG00000137486	HPRR3460202	LKGMKDDKEEEEDGTGSPQLNNR	23	Screening (≥10% reactivity difference)
ARRB1	arrestin beta 1	P49407	ENSG00000137486	HPRR3460203	PKPKEEPPHREVPENETPVDTNL	23	Screening (different region)
C2orf61	chromosome 2 open reading frame 61	Q8N801	ENSG00000239605	HPRR3690182	HEGPGPGHYNVKMPPTSSVTSCFQSRVPRFLPSCSKTPGPGAY TTLRQFPKQSPITAKMGQEHSLEFFNNNNWL	73	Screening (≥10% reactivity difference)
C2orf61	chromosome 2 open reading frame 61	Q8N801	ENSG00000239605; ENSG00000273269	HPRR3400242	VGGESFITASKPAQKTSSFEREQWWRIALDTPIPGTYHLKTFIEE SLLNPVIATYNFKNEGRKKPLVQRNNPVLNDLPQYMPPDFLDLL	91	Screening (different region)
CPNE1	copine 1	Q99829	ENSG00000214078	HPRR3560103	KNNLNPTWKRFSVPVQHFCGGNPSTPIQVQCSDYDSGDS	39	Screening (≥10% reactivity difference)
CPNE1	copine 1	Q99829	ENSG00000214078	HPRR3560102	LAQTVLAEVPTQLVSYFRAQGWAPLKPLPPSAKDPAQA	38	Screening (different region)
CPNE1	copine 1	Q99829	ENSG00000214078	HPRR3560104	DLIGTFHTSLAQLQAVPAEFECIHPEKQKQK	30	Screening (different region)
DGKG	diacylglycerol kinase gamma	P49619	ENSG00000058866	HPRR2960061	TEGASNSEANSADTNIQNADNATKADEACAPDTESNMAEKQAP AEDQVAATPLEPPVPRSSSSSESPVYVKDV	73	Screening (≥10% reactivity difference)
DGKG	diacylglycerol kinase gamma	P49619	ENSG00000058866	HPRR2960060	HCVWCRMTFHRKCELSTLCDGGELRDHILLPTSICPITRDRPGEK SDGCVSAKGELVMQYKII	63	Screening (different region)
DGKG	diacylglycerol kinase gamma	P49619	ENSG00000058866	HPRR2960062	MGEERWVSLTPEEFDQLQKYSEYSSKKIKDALTEFNEGGSLKQY DPHEPISYDVFKLFMRAYLEVLDLPQLSTHLFLA	78	Screening (different region)
FAM9C	family with sequence similarity 9 member C	Q8IZT9	ENSG00000187268	HPRR3690171	ETDEHTGVDTKELEDIAADIKHELAAKRKRIEKIACACSEIKNRIK	46	Screening ($P < 0.05$)
FAM9C	family with sequence similarity 9 member C	Q8IZT9	ENSG00000187268	HPRR4050353	MAAKDQLEVQVMAAQEMELAGKDPVSHEHEERKPVETETKEG	41	Screening (different region)
GPR180	G protein-coupled receptor 180	Q86V85	ENSG00000152749	HPRR3140926	KLSKAQLTMTMNQTEHNLTVSQIPSPQTVHVFYADKYTCQDDK ENSQVEDIPFEMVLLNPDAEGNPFDHFSAGES	75	Screening (≥10% reactivity difference)
GPR180	G protein-coupled receptor 180	Q86V85	ENSG00000152749	HPRR3140925	QRIGHFEFHGDHALLCVRINNIAVAVGKEAKLYLFQAQEWLKLQQ SSHGYSCSE	54	Screening (different region)
GPR180	G protein-coupled receptor 180	Q86V85	ENSG00000152749	HPRR3140927	RLFLSHSLYWEVSSLSVTLPITISSGHK	29	Screening (different region)
HHIPL1	HHIP like 1	Q96JK4	ENSG00000182218	HPRR3450164	DVDRKERGLPYGIPPDNPFVGDPAAPQPEVYALGVRNMWRCSFD RGDPSSGT	51	Screening (≥10% reactivity difference)
HHIPL1	HHIP like 1	Q96JK4	ENSG00000182218	HPRR3450163	GRLMSLQENPGTGQWQYSEICMGHGQTCEFPGLINNYPIISF G	45	Screening (different region)

Gene	Gene description	Uniprot	ENSG	Protein fragment ID	Sequence	Fragment length (aa)	Source
KRTAP17-1	keratin associated protein 17-1	Q9BYP8	ENSG00000186860	HPRR3460778	DCFTCCTQEQNCCCECCCPGCCGCCGCCGC	32	Screening (≥10% reactivity difference)
MFSD2B	major facilitator superfamily domain containing 2B	A6NFX1	ENSG00000205639	HPRR3400359	MLPDVVDDFQLQHRHGPGETIFYSSY	27	Screening (≥10% reactivity difference)
MFSD2B	major facilitator superfamily domain containing 2B	A6NFX1	ENSG00000205639	HPRR3400360	VGSTPKTPSRDASSRLSLRRRTSYSL	26	Screening (different region)
MICU1	mitochondrial calcium uptake 1	Q9BPX6	ENSG00000107745	HPRR3020124	IFYTLGECGLISFSDYIFLTTVLSTPQRNFEIAFKMFDLNGDGEVD MEEFEQVQSIIRSQTSMGMRHRDRPTTGNTLKSGLCALTTY	88	Screening (≥10% reactivity difference)
MICU1	mitochondrial calcium uptake 1	Q9BPX6	ENSG00000107745	HPRR3020125	LKGKLTIKNFLEFQRKLQHDVLKLEFERHDPVDGRITERQFGGML LAYSGVQSKKLAMQRQLKKHFKEGKGLTFQEVENFFTL	85	Screening (different region)
MRPL43	mitochondrial ribosomal protein L43	Q8N983	ENSG00000055950	HPRR3590069	CCVPRVVAEYLNAGVREESIHCKSVVEISTLVQKLADQSGLDVIRI RKPFHTDNPSIQGQWHPFTNKPTTF	71	Screening (≥10% reactivity difference)
MRPL43	mitochondrial ribosomal protein L43	Q8N983	ENSG00000055950	HPRR3590068	GTPSRFLASVLHNLGRYVQQLQRLSFSVSRDGASSRGAREFV EREVIDFARRNPGVVIYVNSRPC	66	Screening (different region)
NXT1	nuclear transport factor 2 like export factor 1	Q9UKK6	ENSG00000132661	HPRR3690076	SGQESLSEFFEMLPSEFQISVD	24	Screening (≥10% reactivity difference)
OAF	out at first homolog	Q86UD1	ENSG00000184232	HPRR3050954	RFWLEQGVDSVFEALPKASEQAELPRCRQVDRGKPCVCHY GLSLAWYPCMLKYCHSRDRPTYKCGIRSCQKSYSFDFYVPQ	84	Screening (≥10% reactivity difference)
OAF	out at first homolog	Q86UD1	ENSG00000184232	HPRR4050009	SFTADFKKDVKVFRAILGELEKGQSQFQALCFVTQLQHNEIIPSE AMAKLRQKNPRAVRQAEVVRGLEHLHMDV	75	Screening (different region)
OR2T11	olfactory receptor, family 2, subfamily T, member 11 (gene/pseudogene)	Q8NH01	ENSG00000183130	HPRR3580070	MTNTSSSDFTLLGLLVNSEAA	21	Screening (P < 0.05)
OR2T11	olfactory receptor, family 2, subfamily T, member 11 (gene/pseudogene)	Q8NH01	ENSG00000183130	HPRR4080070	RNKDVIGAFKKVFACCSARKVATSDA	27	Screening (different region)
RAB26	RAB26, member RAS oncogene family	Q9ULW5	ENSG00000167964	HPRR3420131	SGTALSGPDAPPNGPLQGRPSLGGGVDFYDFAKVMVLV	39	Screening (≥10% reactivity difference)
RAB26	RAB26, member RAS oncogene family	Q9ULW5	ENSG00000167964	HPRR3420132	AKELKQRSMKAPSEPRFRLHDYVKREGR	28	Screening (different region)
RUFY2	RUN and FYVE domain containing 2	Q8WXA3	ENSG00000204130	HPRR3020918	EELAIAKNNIIKLQEENHQLRSENKLIIMKTQQHLEVTKVDVETEL QTYKHSRQGLDEMYNEARRQLRDESQLRQDVENELAVQVSMK HEIEL	93	Screening (≥10% reactivity difference)
RUFY2	RUN and FYVE domain containing 2	Q8WXA3	ENSG00000204130	HPRR3020917	EKAQMEAEDDEKYLQECLSKSDSLQKQISQKEKQLVQLETDLKI EKEWRQTLQE	55	Screening (different region)
S100A3	S100 calcium binding	P33764	ENSG00000188015	HPRR3420667	MARPLEQAVAAIVCTFQEYAGRCGDYKLCQAEKELLQK	40	Screening

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	protein A3						(P <0.05)
S100A3	S100 calcium binding protein A3	P33764	ENSG00000188015	HPRR3420666	KELATWTPTEFRECDYNKFMSVLDTNKCCEVDFVEYVRSACLCLYCHEYFKD	53	Screening (different region)
S100Z	S100 calcium binding protein Z	Q8WXG8	ENSG00000171643	HPRR3460547	LKLLQLRELTEFLSCQKETQLVDKIVQDLANKDNEVD	38	Screening (≥10% reactivity difference)
S100Z	S100 calcium binding protein Z	Q8WXG8	ENSG00000171643	HPRR3460548	MPTQLEMAMDTMIRIFHRYSGKARKRFKLS	30	Screening (different region)
SNX11	sorting nexin 11	Q9Y5W9	ENSG00000002919	HPRR3500048	SEVPSLESPTLPPLSSPLCCDFGRPKEGTSTLQSVRRVGGDHA VPLDHGQLETV	55	Screening (≥10% reactivity difference)
SNX11	sorting nexin 11	Q9Y5W9	ENSG00000002919	HPRR3500049	LFLQSQLSVPEIEACVQGRSTMTVSDAILRYAMSNCGWAQEERQ SSSHLAKGDQPKSCCFLPRSGRRSSP	70	Screening (different region)
TEN1	TEN1, CST complex subunit; TEN1-CDK3 readthrough (NMD candidate)	Q86WV5	ENSG00000257949; ENSG00000261408	HPRR3470283	KLVEPFHAQVGSlyIVLGELQHQQDRGSVVKARVLTCEGMNLP LLEQAIREQRLYKQER	60	Screening (≥10% reactivity difference)
TEN1	TEN1, CST complex subunit; TEN1-CDK3 readthrough (NMD candidate)	Q86WV5	ENSG00000257949; ENSG00000261408	HPRR3470282	MMLPKPGTYLPWEVSAGQVPDGSTLRFTGRLCLYDMIQSRVT LMAQHGSQDQHQLVCTK	60	Screening (different region)
TRIM52	tripartite motif containing 52	Q96A61	ENSG00000183718	HPRR3790297	EDEEAVGAMDGWDGSIREVLYRGNADEELFQDQDDDELWLGD SGITNWDNVYMWDEE	58	Screening (≥10% reactivity difference)
TRIM52	tripartite motif containing 52	Q96A61	ENSG00000183718	HPRR3790298	EEVVQEYQEIKLETTLVGILQIEQESIHSKAYNQ	34	Screening (different region)
UTP14A;UTP14C	UTP14A small subunit processome component; UTP14C, small subunit processome component	Q9BVJ6; Q5TAP6	ENSG00000156697; ENSG00000253797	HPRR3360164	ARMMERMSLKHQNSGKWAKSKAIMAKYDLEARQAMQEQLAKN KELTQKLQVASEEEEE	58	Screening (≥10% reactivity difference)
UTP14A;UTP14C	UTP14A small subunit processome component; UTP14C, small subunit processome component	Q9BVJ6; Q5TAP6	ENSG00000156697; ENSG00000253797	HPRR3360161	RVQTLLEELEELGKEDCFQNKELPRPVLEGQQSERTPNNRPDAP KEKKEKEQ	51	Screening (different region)
ZBTB25	zinc finger and BTB domain containing 25	P24278	ENSG00000089775	HPRR3700073	IGLDDGTADQQRACPATQALEEHQKPPVSIKQERCDPESVISQS HPSPSSEVTGPTFTENSVKIHLCHYCGERFDSRSNLR	81	Screening (≥10% reactivity difference)
ZBTB25	zinc finger and BTB domain containing 25	P24278	ENSG00000089775	HPRR3700071	ICGHKFPRKSQLLEHMYTHKGKSYRNYRCQRFGNALAQRFQPY CDSWSDVSLKSSRLSQEHLDLPCALESSELTQENVDTILVE	83	Screening (different region)
CAMP	cathelicidin		ENSG00000164047	HPRR1951441	RSSDANLYRLLDLPRTMDGDPDTPKPVSFVTKETVCPRTTQQ	119	mRNA

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	antimicrobial peptide				SPEDCDFKKDGLVKRCMGTVTLNQARGSFDISCDKDNKRFALLG DFFRKSKKEIGKEFKRIVQRIKDFLRNLVPR		screening[4]
CD58	CD58 molecule	P19256	ENSG00000116815	HPRR1950575	SQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFRA FSSFKNRVYLDTVSGSLTIYNTLSSDEDEYEMESPNITDTMKFFL YVLESLPSPRLTCALTNGSIEVQCMIEHYNSHRGLIMYSWDCPM EQCKRNSTSIYFKMENDLPQKIQCTLSNPLFNTTSSIIITTCIPSSG HSRHR	187	mRNA screening[4]
CD58	CD58 molecule	P19256	ENSG00000116815	HPRR2990063	IMYSWDCPMEQCKRNSTSIYFKMENDLPQKIQCTLSNPLFNTTS SIILTTICIPSSGHSRHR	63	mRNA screening[4]
CD58	CD58 molecule	P19256	ENSG00000116815	HPRR2990064	DTVSGSLTIYNTLSSDEDEYEMESPNITDTMKFFLYVLESLPSPRL TCALTNGSIEVQCMIEHYNSHRGL	71	mRNA screening[4]
CD58	CD58 molecule	P19256	ENSG00000116815	HPRR4420102	CFSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEF RAFSSFKNRVYLD	57	mRNA screening[4]
CFL1	cofilin 1	P23528	ENSG00000172757	HPRR3290106	HELQANCYEEVKDRCTLAELGGSAVISL	29	mRNA screening[4]
CFL1;CFL2; DSTN	cofilin 1; cofilin 2; destrin actin depolymerizing factor	P23528; Q9Y281; P60981	ENSG00000172757; ENSG00000165410; ENSG00000125868	HPRR4290591	FWAPESAPLKSMMIYASSKDAIKKKL	26	mRNA screening[4]
CRNN	cornulin	Q9UBG3	ENSG00000143536	HPRR1420099	YDRQAESQSQERISPPQIQLSGQTEQTQKAGEGKRNQTTEMRPE RQPQTREQDRAHQGTGETVTGSGTQTQAGATQTVEQDSSHQTG RTSKQTQEATNDQNR	100	Literature[2]
DEFA4	defensin alpha 4	P12838	ENSG00000164821	HPRR3760857	SSALQVSGSTRGMVCSCLVFCRRTELVRGNCILGGVSFTYCCT RVD	47	mRNA screening[4]
DEFA4	defensin alpha 4	P12838	ENSG00000164821	HPRR3970309	ARGDEAPGQEQRGPEDQDISISFAWDK	27	mRNA screening[4]
EXT1	exostosin glycosyltransferase 1	Q16394	ENSG00000182197	HPRR1370208	DSSVHISPRQKRDANSSIIYKGGKCRMESCFDFTLCKKNGFKVYV YPQQKGEKIAESYQNILAAIEGSRFYTSQPSQACLFVLSLDTLDR DQLSPQYVHNLRS	102	DNA methylation screening[4]
EXT1	exostosin glycosyltransferase 1	Q16394	ENSG00000182197	HPRR1370209	EDVGFDIGQAMLAASISTENFRPNFDVSIPLFSKDHPRTGGERG FLKFNTIPLRKYMLVFKGKRYLTIGSDTRNALYHVHNGEDVVL LTTCKHGKDWQKHKDSRCDRDNTYEKYDYREMLHNATFCL	131	DNA methylation screening[4]
FKBP4	FK506 binding protein 4	Q02790	ENSG00000004478	HPRR4160063	DFELARADFQKVLQLYPNNKAAKTQLAVCQQRIIRQLAREKKLY ANMFERLAEENKAKAEASSGDHPTDTEMKEEQKSNTAGSQSQ VE	89	mRNA screening[4]
FKBP4	FK506 binding protein 4	Q02790	ENSG00000004478	HPRR520156	IATMKVGEVCHITCKPEYAYGSAGSPPKIPPNATLVFEVLEFEFK GEDLTEEEDGGIIRRIQTRGEGYAKPNEGAIWEVALEGYYKDKLF DQRELFEIGEENLDPYGLERAIQRMEKGE	122	mRNA screening[4]
FLG	filaggrin	P20930	ENSG00000143631	HPRR2551092	SERWSGSASRNHLGSAWEQSRDGSRHPSHHEDRAGHGHSA DSSRQSGTRHTESSSRGQAASSHEQARSSAGERHGSRHQLQS A	84	Literature[2]
FLG	filaggrin	P20930	ENSG00000143631	HPRR2551093	SADSSRQSGTPHAETSSGGQAASSHEQARSSPGERHGSRHQQ SADSSRHSGIPRRQASSAVRDSGHWSGSSGSQASDSEGHSEES DTQSVSGHGQDGP HQSHQESAR	107	Literature[2]
FLG	filaggrin	P20930	ENSG00000143631	HPRR2551094	RKRLSERLEEKEDNEEGVYDYENTGRMTQKWIQSGHIATYYTIQ DEAYDTTDSLLEENKIYERSRSSDGKSSSQVNRSRHENTSQVPL QESR	92	Literature[2]
FLG2	filaggrin family	Q5D862	ENSG00000143520	HPRR2551079	CGYSNSSGCGRPQNASSSCQSHRFGGQGNQFSYIQSGCQSGI	102	Literature[2]

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	member 2				KGGQGHGCVSGGQPSGCGQPESNPCSQSYSQRGYGARENGQ PQNCGGQWRTGSSQSSCCG		
HLA-A;HLA-B;HLA-C;HLA-E;HLA-F;HLA-G	major histocompatibility complex, class I, A; B; C; E; F; G	P04439+P13746+P16188; P01889+P30480+P30486+Q31610+Q31612+Q29836; P10321; NA; P30511; P17693	ENSG00000206503; ENSG00000234745; ENSG00000204525; ENSG00000204592; ENSG00000204642; ENSG00000204632	HPRR3430084	QTQDTELVTETPAGDGTGFKWAAVVPSG	29	mRNA screening[4]
HLA-A;HLA-B;HLA-C;HLA-E;HLA-F;HLA-G	major histocompatibility complex, class I, A; B; C; E; F; G	P04439+P13746+P16188; P01889+P30480+P30486+Q31610+Q31612+Q29836; P10321; NA; P30511; P17693	ENSG00000206503; ENSG00000234745; ENSG00000204525; ENSG00000204592; ENSG00000204642; ENSG00000204632	HPRR3430085	DHEATLRCWALGFYPAEITLTWQRD	25	mRNA screening[4]
HLA-A;HLA-B;HLA-C;HLA-E;HLA-F;HLA-G	major histocompatibility complex, class I, A; B; C; E; F; G	P04439+P13746+P16188; P01889+P30480+P30486+Q31610+Q31612+Q29836; P10321; NA; P30511; P17693	ENSG00000206503; ENSG00000234745; ENSG00000204525; ENSG00000204592; ENSG00000204642; ENSG00000204632	HPRR4470247	YFSAAVSRPGRGEPRIAMGYVDDTQFVRFDSDSA	35	mRNA screening[4]
HMGB1;HMGB2	high mobility group box 1; high mobility group box 2	P09429; P26583	ENSG00000189403; ENSG00000164104	HPRR430077	KSKFEDMAKSDKARYDREMKNYVPPKGDKKGKKKDPNAPKRPP SAFFLFCSEHRPKIKSEHPGLSIGDTAKKLGEMWSEQSAKDKQP YEQKAARKLKEYE	100	mRNA screening[4], Literature[1]
HMGB1;HM	high mobility group	P09429;	ENSG00000189403;	HPRR3430022	KGDPKKPRGKMSSYAFFVQTCREEHKKK	28	mRNA

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GB2;HMGB3	box 1; high mobility group box 2; high mobility group box 3	P26583; O15347	ENSG00000164104; ENSG00000029993				screening[4], Literature[1]
HMGB2	high mobility group box 2	P26583	ENSG00000164104	HPRR3440063	AKGKSEAGKKGPRPTGSKKKNEP	24	mRNA screening[4]
HMG2;HMG4	high mobility group nucleosomal binding domain 2; high mobility group nucleosomal binding domain 4	P05204; O00479	ENSG00000198830; ENSG00000182952	HPRR3360098	PKRKAKGDAKGDKAKVKDEPQ	21	mRNA screening[4]
HMG2;HMG4	high mobility group nucleosomal binding domain 2; high mobility group nucleosomal binding domain 4	P05204; O00479	ENSG00000198830; ENSG00000182952	HPRR4050064	KKGEKLPKGRKGADAGKDGNNPAKNRDA	29	mRNA screening[4]
HRNR	hornerin	Q86YZ3	ENSG00000197915	HPRR2552125	WSAGENDSYSRNVRGSLKPGTESISRRLSFQRDFSGQHNSYSG QSSSYGEQNSDSHQSSGRGQCGSGS	68	Literature[2]
HRNR	hornerin	Q86YZ3	ENSG00000197915	HPRR2552126	RDHNKKVDFTEYLLMIFKLVQARNKIIGKDYCQVSGSKLRDDTHQ HQEEQEETEKEENKRQES	63	Literature[2]
HRNR	hornerin	Q86YZ3	ENSG00000197915	HPRR4050552	LLMIFKLVQAHNKIIGKDYCQVSGSKLRDDTHQHQQEEQEETEKEE NKRQES	51	Literature[2]
HSPA4	heat shock protein family A (Hsp70) member 4	P34932	ENSG00000170606	HPRR1920102	FEELGKQIQQYMKIISFFKNKEDQYDHLDAADMTKVEKSTNEAM EWMNKNLNLQNKQSLTMDPVVKSKEIEAKIKELTSTCSPISKPKP KVEPPKEEQKNAEQNGPVDG	110	mRNA screening[4]
HSPA4	heat shock protein family A (Hsp70) member 4	P34932	ENSG00000170606	HPRR4220528	QEEPHVEEQQQTPAENKAEESEEMETSQAGSKDKKMDQPPQA KKAKVKTSTV	52	mRNA screening[4]
IFITM2;IFITM3	interferon induced transmembrane protein 2; interferon induced transmembrane protein 3	Q01629; Q01628	ENSG00000185201; ENSG00000142089	HPRR370324	MNHTVQTFSPVNSGQPPNYEMLKEEHEVAVLGAPHNPAPPTS TVIHIRSETSVPDH	57	mRNA screening[4]
LCN2	lipocalin 2	P80188	ENSG00000148346	HPRR400092	QDSTSDLPAPPLSKVPLQQNFQDNQFQGWYVVLGAGNAILRE DKDPQKMYATIYELKEDKSYNVTSLVFRKKKCDYWIRTFVPGCQ PGEFTLGNISYPGLTSLVLRVWSTNYNQHAMVFFKKVSNREY FKITL	137	mRNA screening[4]
LGALS1	galectin 1	P09382	ENSG00000100097	HPRR220971	EVAPDAKSFVLNLGKDSNNLCLHFNPRFNAHGDANTIVCNSKDG GAWGTEQREAVFPFQPGSVAEVCITFDQANLTVKLPDGYEFKFP NRLNLEAINYMAADGDFKIKCV	110	mRNA screening[4]
LGALS1	galectin 1	P09382	ENSG00000100097	HPRR252469	LKPGELRVRGVAPDAKSFVLNLGKDSNNLCLHFNPRFNAHG DANTIVCNSKDGAWGTEQREAVFPFQPGSVAEVCITFDQANLT VKLPDGYEFKFPNRLNLEAINYMAADGDF	116	mRNA screening[4]

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LTF	lactotransferrin	P02788	ENSG00000012223	HPRR3720081	PNHAVVSRMDKVERLKQVLLHQQAKFGRNGSDCPDKFCLFQSE TKNLLFNDNTECLARLHGK	62	mRNA screening[4]
LTF	lactotransferrin	P02788	ENSG00000012223	HPRR3720082	RVVWCAVGEQELRKCNQWSGLSEGSVTCSSASTTEDC	37	mRNA screening[4]
LYZ	lysozyme	P61626	ENSG00000090382	HPRR3700032	WCNDGKTPGAVNACHLSCSALLQDNIADAVACAKRVVRDPQGI RAWVAWRNRCQNRDVRQYVQCGG	66	mRNA screening[4]
LYZ	lysozyme	P61626	ENSG00000090382	HPRR3700033	GKVFERCELARTLKRGLMDGYRGISLANWMCLAKWESGYNTRA TNYNAGDRSTDY	55	mRNA screening[4]
MAN1A1	mannosidase alpha class 1A member 1	P33908	ENSG00000111885	HPRR3700097	MKHEFEEAKSWVEENLDFNVNAEISVFEV	29	DNA methylation screening[4]
MAN1A1	mannosidase alpha class 1A member 1	P33908	ENSG00000111885	HPRR3700098	TLQKLPEEIQRDILLEKKKVAQDQLRDKAPFRGLPPVDFVPPIGVE SREPADAAIREK	58	DNA methylation screening[4]
MED1	mediator complex subunit 1	Q15648	ENSG00000125686	HPRR3830164	SHPVDNKWTPSFSSITSANSVDLPACFFLKFPQPIPVSRFVQKL QNCTGIPLFETQPTYAPLYELITQFELSKDPDPIPLNHNMRFYAAL PGQQH	96	DNA methylation screening[4]
MED1	mediator complex subunit 1	Q15648	ENSG00000125686	HPRR3830165	MEKRVVMSSGGHQHLVSCLETQKALKVTSLPAMTDRLESIAHQ NGLGSHLSASGTECYITSDMFYVEVQLDPAGQLCDVKVAHHGE NPVSCP	93	DNA methylation screening[4]
MED13L	mediator complex subunit 13 like	Q71F56	ENSG00000123066	HPRR3070311	PALYGNLELQQLSTLDDRTVLVGQRLPLMAEVSETALYCGIRP SNPESSEKWWHSYRLPPSDDAEFRPPELQGERCDAKMEVNSE STALQRLLAQPNKRFKIWQD	106	DNA methylation screening[4]
MED13L	mediator complex subunit 13 like	Q71F56	ENSG00000123066	HPRR3070312	MMCQSTFLPQVEGTTKPPQEPPIISLLLLQNQHTQPFASLNFLDYI SSNNRQTLPCVSWSYDRVQADNNDYWTECFNALE	79	DNA methylation screening[4]
MNDA	myeloid cell nuclear differentiation antigen	P41218	ENSG00000163563	HPRR4190869	EKSKVAKKIQTQEKAPVKKINQEEVGLAAPAPTARNKLTSEARGR IPVAQKRKTPNKEKTEAKRNKVSQEQSKPPGPSGASTSAAVDHP	89	mRNA screening[4]
MNDA	myeloid cell nuclear differentiation antigen	P41218	ENSG00000163563	HPRR610035	PQTSSSTPSNTSFTPNQETQAQRQVDARRNVQNDPVTVVVLK ATAPFKYESPENGKSTMFHATVASKTYFHVVKVFDINLKEKFVRK KVITISDYSECKGVMEIKEASSVSDFN	115	mRNA screening[4]
NCF4	neutrophil cytosolic factor 4	Q15080	ENSG00000100365	HPRR2800094	MAVAQQLRAESDFEQLPDDVAISANIADIEEKGFTSHFVFIIEVK TKGGSKYLIYRRY	59	mRNA screening[4]
NCF4	neutrophil cytosolic factor 4	Q15080	ENSG00000100365	HPRR2800095	EDIALNYRDAEGDLVRLSDEDVALMVRQARGLPKQKRLFPWKL HITQKDNRYVYNTMP	59	mRNA screening[4]
NCOR2	nuclear receptor corepressor 2	Q9Y618	ENSG00000196498	HPRR430039	EHPVKSECTEEAEEGPAKGKDAEAAEATAEGALKAEEKEGGSG RATTAKSSGAPQSDSSATCSADEVDEAEGGDKNRLLSPRPSLL TPTGDPANASQKPLDLKQLKQRAAIPPIQVTKV	123	DNA methylation screening[4]
PADI1	peptidyl arginine deiminase 1	Q9ULC6	ENSG00000142623	HPRR2550898	PKRVVQLSLKMPTHAVCVVGVEAHVDIHSVDPKGANSFRVSGSS GVEVFMVYNRTVKEVIGKARWPLDT	70	Literature[2]
PADI1	peptidyl arginine deiminase 1	Q9ULC6	ENSG00000142623	HPRR3930191	PSACLKLFQEKKEEGYGEAAQFDGLKHQAKRSINEMLADRHLQR DNLHAQKCIDWN	56	Literature[2]
PADI2	peptidyl arginine deiminase 2	Q9Y2J8	ENSG00000117115	HPRR3700197	PWLPKEDCRDEKVYSKEDLKDMSQMLRTKGPDRLPAGYEIVLYI SMSDDKVGVFYVENPFFGQRYIHILGRRLYHVVKYTGGG	86	Literature[2]
PADI3	peptidyl arginine deiminase 3	Q9ULW8	ENSG00000142619	HPRR3500166	FRMLLASPGACFKLFQEKQKCGHGRALLFQGVVDDEQVKTISIN QVLSNKDLINYNKQVQSCIDWNREV	69	Literature[2]
PADI4	peptidyl arginine deiminase 4	Q9UM07	ENSG00000159339	HPRR1060019	GTLTQLDICSSAPEDCTSF SINASPGVVVDIAHGPPAKKKSTGSS TWPLDPGVEVTLTMKVASGSTGDKVQISYYGPKTPPVKALLYL TGV	92	Literature[2]
PADI4	peptidyl arginine	Q9UM07	ENSG00000159339	HPRR1060020	CEDDEVLDSEDLQDMSMLTSLTKTPKDDFTNHTLVLHVARSEMD	108	Literature[2]

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	deiminase 4				KVRVFQATRGLSSKCSVVLGPKWPSHYLMVPGGKHNMDFYVE ALAFPDTDFFGLITLTISLLD		
PADI6	peptidyl arginine deiminase 6	Q6TGC4	ENSG00000276747	HPRR2552151	SACYKLFREKQKEGYGDALLFDELRADQLLSNGREAKTIDQLLA DESLKKQNEYVEKCIHLNRDILKTELGLVEQDIIIEPQLFCLEKLTNI PSDQQPK	99	Literature[2]
PADI6	peptidyl arginine deiminase 6	Q6TGC4	ENSG00000276747	HPRR2552153	ETFYVEAIAFPSAEFSGSLISYSVSLVEESQDPSIPETVLYKDTVVF RVAPCVFIPCTQVPLEVYLCRELQLQGFVDVTVKLSEK	84	Literature[2]
PRDX1	peroxiredoxin 1	Q06830	ENSG00000117450	HPRR1370091	KGKYVVFYPLDFTFVCPTEIIAFSDRAEEFKKLNQCQVIGASVDS HFCHLAWVNTPKKQGGLGPMNIPLVSDPKRTIAQDYGVLKADG ISFRGLFIIDDKGILRQITVNDLPVGRSVDETLRL	125	mRNA screening[4]
RPTN	repetin	Q6XPR3	ENSG00000215853	HPRR2551740	SSHYIQSQTGEIQGNKYFQGTGTRKASYVEQSGRSGRLSQQ TPGQEGYNQGGQFQSRDSQQNGHQVWEPEEDSQHHQHKL AQIQQERP	92	Literature[2]
RPTN	repetin	Q6XPR3	ENSG00000215853	HPRR2551741	ETVETILNLLDQDRDGHIDFHEYLLLVFQLVQACYHKLDNKSHGG RTSQQERGQEGAQDCKFPGNTGRQHRQRHEEERQNSHHSQ	85	Literature[2]
RPTN	repetin	Q6XPR3	ENSG00000215853	HPRR2551743	KSGSYCGQSERLGQELGCGQTDRQGQSSHYGQTDRQDQSYH YGQTDQRQGQSSHYQTDRQGQSSHYSPDRQGQSSHYGQMD	82	Literature[2]
S100A1	S100 calcium binding protein A1	P23297	ENSG00000160678	HPRR1420141	GSELETAMETLINVFHAHSGKEGDKYKLSKKELKELLQTELSGFL DAQKDVAVDKVMKELDENGDEVDQFEYVVLVAALTVACNNF FWENS	93	Literature[2]
S100A2	S100 calcium binding protein A2	P29034	ENSG00000196754	HPRR2760377	KELLHKELPSFVGEKVDEEGLKKLMGSLDENSDQQVDFQEYAVF LALITVMCNDFQGCCP	60	Literature[2]
S100A2	S100 calcium binding protein A2	P29034	ENSG00000196754	HPRR4090188	EMKELLHKELPSFVGEKVDEEGLKKLMGSLDENSDQQVD	39	Literature[2]
S100A4	S100 calcium binding protein A4	P26447	ENSG00000196154	HPRR1350146	MACPLEKALDVMSTFFHKYSGKEGDKFLNKSELKELLTRELP FLGKRTDEAAAFQKLMSNLDNRDNEVDQFEYCVFLSCIAMMCN EFFEGFPDKQPRKK	101	Literature[2]
S100A5	S100 calcium binding protein A5	P33763	ENSG00000196420	HPRR2552087	GREGSKLTLSRKELKELIKELCLGEMKESSIDLMKSLDKNSDQ EIDFKEYSVFLTMLCMAYNDFLEDNK	72	Literature[2]
S100A5	S100 calcium binding protein A5	P33763	ENSG00000196420	HPRR3790623	KELIKELCLGEMKESSIDLMKSLDKNSDQEIDFKEYSVFLTMLC MAYNDFLEDNK	58	Literature[2]
S100A6	S100 calcium binding protein A6	P06703	ENSG00000197956	HPRR1420196	AIFHKYSGREGDKHTLSKKELKELIQELTIGSKLQDAEIARLMED LDRNKDQEVNFQEYVTFLGALALIYNE	73	mRNA screening[4], Literature[2]
S100A7A;S100A7	S100 calcium binding protein A7A; S100 calcium binding protein A7	Q86SG5; P31151	ENSG00000184330; ENSG00000143556	HPRR1420206	EKPSLLTMMKENFPNLSACDKKGTNYLADVFEKKDKNEDKKID FSEFLSLLGDIATDYHKQSHGAAPCS	70	Literature[2]
S100A7L2	S100 calcium binding protein A7 like 2	Q5SY68	ENSG00000197364	HPRR3420742	SDMDYLSNALEKKDDNKDKKVNYS	25	Literature[2]
S100A9	S100 calcium binding protein A9	P06702	ENSG00000163220	HPRR400189	CKMSQLERNIETINTFHQYSVKLGHPDTLNQGEFKELVRKDLQN FLKKENKNEKVIHIMEDLDTNADKQLSFEEFIMLMARLTWASHE KMHEGDEG	98	mRNA screening[4], Literature[2]
S100A10	S100 calcium binding	P60903	ENSG00000197747	HPRR410031	MPSQMEHAMETMMFTFHKGAGDKGYLTKEDLRVLMEKEFFPGFL	67	Literature[2]

Gene	Gene description	Uniprot	ENSG	Protein fragment ID	Sequence	Fragment length (aa)	Source
	protein A10				ENQKDPLAVDKIMKDLQCRDGKV		
S100A11	S100 calcium binding protein A11	P31949	ENSG00000163191	HPRR3410200	SLIAVFQKYAGKDGYNITLSKTEFLSFMNTELAFTKNQKDPGVL DRMMKKLDTNSDGLDFSEFLNLIGGLAMACHDSF	80	Literature[2]
S100A12	S100 calcium binding protein A12	P80511	ENSG00000163221	HPRR400165	KLEEHLLEGIVNIFHQYSVRKGHFDTLKSGELKQLLTKEANTIKNIK DKAVIDEIFQGLDANQDEQVDFQEFISLVAIALKAAHYHTHKE	90	mRNA screening[4], Literature[2]
S100A13	S100 calcium binding protein A13	Q99584	ENSG00000189171	HPRR2330156	ELEESIETVVTFFTFARQEGRKDSLVSNEFKELVTQQLPHLLKD VGSLDEKMKSLDVNQDSELKFNEYWRLIGELAKEIRKKKDLKI	88	Literature[2]
S100A14	S100 calcium binding protein A14	Q9HCY8	ENSG00000189334	HPRR2552068	GQCRSANAEDAQEFSDVERAIETLIKNFHQYSVEGGKETLTPSEL RDLVTQQLPHLMPSNCGLEEKIANLNGSCNDSKLEFRSFWELIGE A	90	Literature[2]
S100A16	S100 calcium binding protein A16	Q96FQ6	ENSG00000188643	HPRR2552038	VIVLVENFYKYVSKYSLVKNKISKSSFREMLQKELNHMLSDTGNR KAADKLIQNLNANHDGRISFDEYWTLLIGGITGPIAKLIHEQEQ	88	Literature[2]
S100G	S100 calcium binding protein G	P29377	ENSG00000169906	HPRR233077	KSPEELKRIFEKYAAKEGDPDQLSKDELKLLIQAEFSSLKGPNTL DDLQELDKNGDGEVSFE	64	Literature[2]
S100G	S100 calcium binding protein G	P29377	ENSG00000169906	HPRR3920159	KSPEELKRIFEKYAAKEGDPDQLSKDELKLLIQAEFSSLKGPNTL DDLQELDK	55	Literature[2]
S100P	S100 calcium binding protein P	P25815	ENSG00000163993	HPRR2350024	MTELETAMGMIIDVFSRYSGSEGSTQTLTKGELKVLMEKELPGFL QSGKDKDAVDKLLKDLNANGDAQVDFSEF	74	mRNA screening[4], Literature[2]
S100P	S100 calcium binding protein P	P25815	ENSG00000163993	HPRR3970245	FIVFVAITSACHKYFEKAGL	21	mRNA screening[4], Literature[2]
SPRR3	small proline rich protein 3	Q9UBC9	ENSG00000163209	HPRR1420163	EPCHSKVPQPGNTKIPEPGCTKVPEPGCTKVPEPGCTKVPEPG CTKVPEPGCTKVPEPGYTKVPEPGSIKVPDQGFIFPEPGAIVP EQGYTKVPVP	98	Literature[3]
SRGN	serglycin	P10124	ENSG00000122862	HPRR252377	MQKLLKCSRLVLALILVLESSVQGYPTRRARYQWVRCNPDSN SANCLEEKGPMEFLLPGESNKPRLRTDLFPKTRIQLNRIPLSE DYSGSGFGSGSGSGSGSGSGLTEMEQDYQLVDE	124	mRNA screening[4]
TCHH	trichohyalin	Q07283	ENSG00000159450	HPRR2551234	QEKSRREEQELWQEEQKRRQERERKLREEHIRRQQKEEQRH RQVGEIKSQEGKGHGRLLPEGTHQFASVPVRSSPLYEY	80	Literature[2]
TCHHL1	trichohyalin like 1	Q5QJ38	ENSG00000182898	HPRR3410198	KHSNIQEPPLQREDEPSSQHADLPEQAAARSPSQTQKSTDSDK VCRMFDQEPGKDADQTPAKTKNLGEPEDYGRSETQEKECET KDLVPQYGSRN	97	Literature[2]
TCHHL1	trichohyalin like 1	Q5QJ38	ENSG00000182898	HPRR3410199	RDQEPCSVVERGAVYSSPLYQYLQEKILQQTNTVQEEHQKQVQIA QASGPCLCSVSLTSEISDCSVFFNYSQASQPYTRGLPLDESPAG AQETPAPO	96	Literature[2]
TGM1	transglutaminase 1	P22735	ENSG00000092295	HPRR3140167	DLSLTLLGAADVGECEVQIVFKNPLPVTLTNVVFRLEGSGLQRP KILNVGDIGGNETVTLRQSFVPVRPGPRQLIASLD	80	Literature[2]
TGM1	transglutaminase 1	P22735	ENSG00000092295	HPRR3140168	MMDGPRSDVGRWGGNPLQPPTTPSPEPEPEPDGRSRRGGGR SFWARCCGCCSCRNAADDWGPEPDSRGRGSSSGTRRPGS RGSDSRPVSRRGSGVNAA	100	Literature[2]

Gene	Gene description	Uniprot	ENSG	Protein fragment ID	Sequence	Fragment length (aa)	Source
TGM1	transglutaminase 1	P22735	ENSG00000092295	HPRR3140170	NSDKVYWQRQDDGSFKIVYVEEKAIGTLIVTKAISSNMREDITYLY KHPEGSDAERKAVETAAAHGSKPNVYANRGS AEDVAMQV	85	Literature[2]
TGM2	transglutaminase 2	P21980	ENSG00000198959	HPRR2430028	MAEELVLERCDELETNGRDHHTADLCREKLVVRRGQPPFWLTL HFEGRNYESVDSLTFVVTGPAPSQEAGTK	74	Literature[2]
TGM2	transglutaminase 2	P21980	ENSG00000198959	HPRR2430030	VEPVINSYLLAERDLYLENPEIKIRILGEPKQKRKLVAEVS LQNPLP VALEGCTFTVEGAGLTEEQKTVEIPDPVEAGEEVKVRMDLLP	89	Literature[2]
TGM3	transglutaminase 3	Q08188	ENSG00000125780	HPRR4320672	MAALGVQSINWQKAFNRQAHHTDKFSSQELILRRGQNFQVLMIM NKG LGSNERLEFIVSTGPPYSESAMTKAVFPLSNGSSSGWSAVL QASN	92	Literature[2]
TGM4	transglutaminase 4	P49221	ENSG00000163810	HPRR2700176	SVNFTVILKRKTAALQNVNILGSFELQLYTGKKMAKLCDLNKTSQI QGQVSEVTLTLDSKTYINSLAILDDEPVIRGFIIAIVESKEIMA	91	Literature[2]
TGM4	transglutaminase 4	P49221	ENSG00000163810	HPRR2700177	YPEFSIELPNTGRIGQLLVNCNIFKNLAIPLTDVKFSLES LGISSL QTS DHGTVQPGETIQSQIKCTPIKTGPKKFIVKLSSKQVK	87	Literature[2]
TGM5	transglutaminase 5	O43548	ENSG00000104055	HPRR3100089	FLKALQKLKARSFHGSQRGAELQPSRPTSLSQDSPRSLHTPSLR PSDVVQVSLKFKLLDPPNMGMQDICFVLLALNMSSQFKDLKVNLS AQSL LHD	95	Literature[2]
TGM5	transglutaminase 5	O43548	ENSG00000104055	HPRR3100091	VRAIKEGEVDLNYDTPFVFSMVNADCMSWL VQGGKEQKLHQDT SSVGNFISTKSIQSDERDDITENYKY	69	Literature[2]
TGM6	transglutaminase 6	O95932	ENSG00000166948	HPRR3310212	GSRKERQVYSKAVNRLFGVEASGRRIWIRRAGRCLWRDDLLE PATKPSIAGKFKVLEPPMLGHDRLRLALCLANLTSRAQVRVNL SG ATILYTR	95	Literature[2]
TGM7	transglutaminase 7	Q96PF1	ENSG00000159495	HPRR2700160	VTLTNTLMVALSSCTMVLEGSGLINGQIAKDLGTLVAGHTLQIQLD LYPTKAGPRQLQVLISSNEVKEIKGYKDIFVTVAGA	82	Literature[2]
TOX2	TOX high mobility group box family member 2	Q96NM4	ENSG00000124191	HPRR3300261	IAGVFPQKFDGDSAYVGMSDGNPELLSTSQT YNGQSENNEDYEI PPITPPN	51	DNA methylation screening[4]
TOX2	TOX high mobility group box family member 2	Q96NM4	ENSG00000124191	HPRR3300264	HEASYHSLCHGLTPNGLLPAYSYQAMDLP AIMVSNMLAQDSHLL SGQLPTIQEMVHSEVAAYDSGR	66	DNA methylation screening[4]

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³Marenholz I, Rivera VA, Esparza-Gordillo J, Bauerfeind A, Lee-Kirsch MA, Ciechanowicz A, et al. Association screening in the Epidermal Differentiation Complex (EDC) identifies an SPRR3 repeat number variant as a risk factor for eczema. *J Invest Dermatol.* 2011;131:1644-9.

⁴Katayama S, Bruhn S, Scheynius A, Lundeberg L, Kere J, Andersson A, et al. Epigenetic modifications in skin-homing CD4+CLA+ T-cells of atopic dermatitis patients relate to mRNA expression changes in high mobility group proteins. at Conference: 2(nd) Inflammatory Skin Disease Summit, November 16-19, 2016, New York. *Exp Dermatol.* 2016;25 Suppl 4:36-7.

Table S2. Characterization of the AD patients and healthy controls subdivided according to IgG reactivity^{d)}

		Gender F / M	Age (years)	SCORAD ^{a)}	Rhino- conjunctivitis and/or asthma past or present	Total plasma IgE ^{b)} ≥122 kU/L	Total plasma IgE ^{b)} (kU/L)	Phadiatop positive ^{c)}
	N	n (%F)	median (range)	median (range)	n (%)	n (%)	median (range)	n (%)
AD patients with IgG- reactivity to KRTAP17-1	55	30 / 25 (55)	28 (18 – 63)	35 (17 – 66)	43 (78)	28 (51)	130 (4.5 - 12835)	42 (76)
AD patients with IgG- reactivity to HSPA4	36	18 / 18 (50)	25 (18 – 62)	36 (16 – 69)	28 (78)	19 (53)	140 (12 - 5150)	27 (75)
AD patients with IgG- reactivity to S100A12	13	6 / 7 (46)	22 (18 – 57)	36 (12 – 65)	9 (69)	4 (31)	90 (12 - 1100)	7 (54)
AD patients with IgG- reactivity to S100Z	8	2 / 6 (25)	42 (18 – 65)	49 (19 – 67)	8 (100)	4 (50)	330 (13 - 9800)	8 (100)
Severe ^{a)} with IgG- reactivity to KRTAP17-1	20	6 / 14 (30)	28 (18 - 54)	51 (42 - 66)	16 (80)	14 (70)	390 (4.5 - 12835)	17 (85)
Severe ^{a)} with IgG- reactivity to HSPA4	15	4 / 11 (27)	24 (18 - 57)	51 (42 - 69)	13 (87)	11 (73)	490 (45 - 5150)	12 (80)
Severe ^{a)} with IgG- reactivity to S100A12	5	2 / 3 (40)	22 (18 - 27)	51 (44 - 65)	4 (80)	3 (60)	200 (45 - 1100)	3 (60)
Severe ^{a)} with IgG- reactivity to S100Z	7	1 / 6 (14)	42 (18 – 65)	53 (41 - 67)	7 (100)	3 (43)	89 (13 - 9800)	7 (100)
Moderate ^{a)} with IgG- reactivity to KRTAP17-1	35	24 / 11 (69)	29 (18 - 63)	31 (17 – 40)	27 (77)	14 (40)	89 (16 – 6810)	25 (71)
Moderate ^{a)} with IgG- reactivity to HSPA4	21	14 / 7 (67)	25 (18 - 62)	27 (16 – 40)	15 (71)	8 (38)	100 (12 – 2800)	15 (71)
Moderate ^{a)} with IgG- reactivity to S100A12	8	4 / 4 (50)	27 (18 - 57)	30 (19 – 40)	5 (62)	1 (12)	76 (12 – 240)	4 (50)
Moderate ^{a)} with IgG- reactivity to S100Z	1	1 / 0 (100)	30 (30)	19 (19)	1 (100)	1 (100)	570 (570)	1 (100)

		Gender F / M	Age (years)	SCORAD ^{a)}	Rhino- conjunctivitis and/or asthma past or present	Total plasma IgE ^{b)} ≥122 kU/L	Total plasma IgE ^{b)} (kU/L)	Phadiatop positive ^{c)}
	N	n (%F)	median (range)	median (range)	n (%)	n (%)	median (range)	n (%)
Healthy controls with IgG-reactivity to KRTAP17-1	13	8 / 5 (62)	35 (20 – 62)	NA	0 (0)	0 (0)	17 (3.2 – 70)	0 (0)
Healthy controls with IgG-reactivity to HSPA4	9	5 / 4 (56)	38 (22 – 61)	NA	0 (0)	0 (0)	17 (2.4 – 66)	0 (0)
Healthy controls with IgG-reactivity to S100A12	0	NA	NA	NA	NA	NA	NA	NA
Healthy controls with IgG-reactivity to S100Z	3	1 / 2 (33)	24 (23 – 42)	NA	0	0	17 (11 – 35)	0

a) Objective SCORAD^[5], severe AD defined as SCORAD ≥41.

b) ImmunoCAP (Thermo Fisher Scientific), reference range 1.6 – 122 kU/L.

c) Phadiatop (Thermo Fisher Scientific), plasma IgE to any of 11 common aeroallergens, positive ≥ 0.35 kU/L.

d) IgG reactivity were defined using sample-specific cutoffs based on the sample median across protein fragments and a factor times the median absolute deviation (MAD), see Methods section.
AD = atopic dermatitis, F = Female, M = Male, N = number of individuals, n = number of positive individuals, NA = not applicable.

Table S3: IgG-reactivity^{a)} and adjusted MFI^{b)} to four antigens detected in the targeted screening of the complete cohort using antigen bead arrays subdivided by Phadiatop and total plasma IgE

Gene	KRTAP17-1	HSPA4	S100A12	S100Z
Number of IgG-reactive individuals	n (%)	n (%)	n (%)	n (%)
AD patients (N=173)	55 (32)	36 (21)	13 (8)	8 (5)
Phadiatop positive ^{c)} (N=132)	42 (32)	27 (20)	7 (5)	8 (6)
Phadiatop negative ^{c)} (N=41)	13 (32)	9 (22)	6 (15)	0 (0)
Elevated total IgE ^{d)} (N=95)	28 (29)	19 (20)	4 (4)	4 (4)
Normal total IgE ^{d)} (N=78)	27 (35)	17 (22)	9 (12)	4 (5)
Healthy Controls ^{e)} (N=84)	13 (15)	9 (11)	0 (0)	3 (4)
P values^{f)}				
Calculated on proportion positive individuals				
AD Phadiatop ^{positive} vs AD Phadiatop ^{negative}	1.00	0.83	0.08	0.20
AD Phadiatop ^{positive} vs HC	0.01	0.06	0.04	0.53
AD Phadiatop ^{negative} vs HC	0.06	0.11	0.001	0.55
AD Total IgE ^{high} vs AD Total IgE ^{normal}	0.51	0.85	0.09	1.00
AD Total IgE ^{high} vs HC	0.03	0.10	0.12	1.00
AD Total IgE ^{normal} vs HC	0.006	0.09	0.001	0.71
Adjusted MFI of individuals				
	median (Q1 - Q3)	median (Q1 - Q3)	median (Q1 - Q3)	median (Q1 - Q3)
AD Phadiatop ^{positive}	7.2 (4.3 - 18.9)	3.2 (1.3 - 9.8)	2.2 (0.8 - 5.7)	-1.7 (-2.1 - -0.6)
AD Phadiatop ^{negative}	7.2 (4.8 - 16.9)	3.4 (1.3 - 9.8)	4.2 (0.8 - 7.0)	-1.8 (-2.2 - -0.5)
AD Total IgE ^{high}	7.0 (4.3 - 16.5)	3.2 (1.4 - 8.7)	2.4 (1.0 - 5.8)	-1.7 (-2.1 - -0.6)

AD Total IgE ^{normal}	7.8 (4.7 - 21.0)	3.3 (1.2 - 9.9)	2.7 (0.7 - 6.9)	-1.7 (-2.1 - -0.5)
HC	5.8 (3.4 - 10.5)	2.8 (1.1 - 7.2)	0.8 (0.2 - 2.0)	-1.6 (-2.0 - -0.2)
P values^{g)}				
Calculated on adjusted MFI values				
AD Phadiatop ^{positive} vs AD Phadiatop ^{negative}	0.93	0.81	0.32	0.43
AD Phadiatop ^{positive} vs HC	0.02	0.40	0.00002	0.30
AD Phadiatop ^{negative} vs HC	0.06	0.46	0.0002	0.09
AD Total IgE ^{high} vs AD Total IgE ^{normal}	0.47	0.94	0.71	0.96
AD Total IgE ^{high} vs HC	0.06	0.42	0.00002	0.25
AD Total IgE ^{normal} vs HC	0.01	0.43	0.0001	0.20

a) IgG reactivity were defined using sample-specific cutoffs based on the sample median across protein fragments and a factor times the median absolute deviation (MAD), see Methods.

b) Sample median across protein fragments was subtracted from each signal and divided by the MAD, see Methods.

c) Phadiatop (Thermo Fisher Scientific), plasma IgE-reactivity to any of 11 common aeroallergens, reference range ≥ 0.35 kU/L.

d) ImmunoCAP (Thermo Fisher Scientific), reference range 1.6 – 122 kU/L.

e) All HC were negative in Phadiatop and had normal total IgE levels.

f) Fisher's exact test.

g) Wilcoxon rank-sum test.

AD = atopic dermatitis, HC = healthy controls, N= number of individuals, n = number of positive individuals

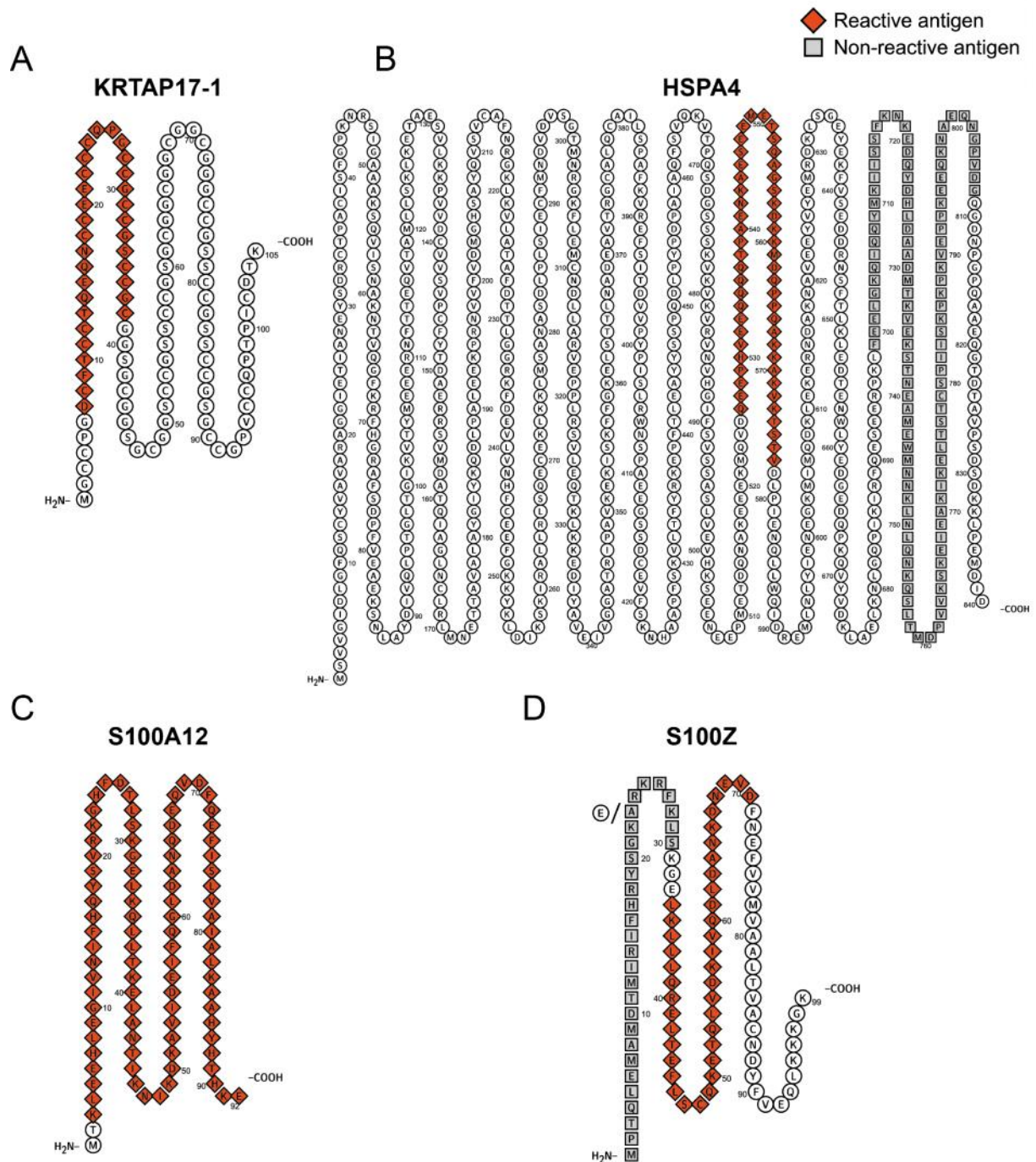


Fig S1. Protein sequence and antigen regions. Illustration of the complete protein sequence of (A) KRTAP17-1, (B) HSPA4, (C) S100A12, and (D) S100Z. The protein sequence is here shown with the one letter abbreviation for each amino acid in white circles, red diamonds or grey squares. The regions of the proteins that were represented as protein fragments and that were identified as either reactive or non-reactive in the targeted analysis are highlighted in red (diamonds, reactive) and grey (squares, non-reactive).

References

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