ORIGINAL ARTICLE
ALLERGY

Nasal upregulation of *CST1* in dog-sensitised children with severe allergic airway disease

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ABSTRACT

Background: The clinical presentation of children sensitised to dog dander varies from asymptomatic to severe allergic airway disease, but the genetic mechanisms underlying these differences are not clear. The objective of the present study was to investigate nasal transcriptomic profiles associated with dog dander sensitisation in school children and to reveal clinical symptoms related with these profiles.

Methods: RNA was extracted from nasal epithelial cell brushings of children sensitised to dog dander and healthy controls. Blood sample analyses included IgE against dog dander, dog allergen molecules, other airborne and food allergens, basophil activation and white blood cell counts. Clinical history of asthma and rhinitis was recorded, and lung function was assessed (spirometry, methacholine provocation and exhaled nitric oxide fraction).

Results: The most overexpressed gene in children sensitised to dog dander compared to healthy controls was *CST1*, coding for Cystatin 1. A cluster of these children with enhanced *CST1* expression showed lower forced expiratory volume in 1 s, increased bronchial hyperreactivity, pronounced eosinophilia and higher basophil allergen threshold sensitivity compared with other children sensitised to dog dander. In addition, multi-sensitisation to lipocalins was more common in this group.

Conclusions: Overexpression of *CST1* is associated with more severe allergic airway disease in children sensitised to dog dander. *CST1* is thus a possible biomarker of the severity of allergic airway disease and a possible therapeutic target for the future treatment of airborne allergy.

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Introduction

Dog allergy, mainly characterised by asthma and rhinoconjunctivitis, affects up to 10% of adolescents in the Western world. A prevalence of IgE reactivity to dog dander of up to ~20% is reported among teenagers in Nordic countries and the clinical presentation varies from asymptomatic to severe allergic airway disease [1–3]. There is still a gap in the understanding of factors determining why some individuals develop allergic disease and others only asymptomatic sensitisation.

The first physical barrier for entry of allergens is the epithelial barrier, predominantly formed by tight junctions at the most apical part of neighbouring epithelial cells [4, 5]. Impaired epithelial structure and function have been recognised as significantly contributing factors in the pathogenesis of asthma [6, 7], and variations in expression and DNA methylation of a number of genes in the airway epithelium have previously been associated with asthma and rhinitis [8–10].

Sampling from the nasal epithelium is an attractive alternative to investigate mechanisms of airborne allergic disease including the underlying transcriptomic profiles, as nasal epithelium gene expression reflects gene expression in the lower airways [11].

Molecular allergy diagnostics provides new opportunities to explore associations between sensitisation to furry animals and clinical disease [12, 13]. Previously, we provided a detailed characterisation of sensitisation profiles in a cohort of 60 dog dander-sensitised children, revealing multi-sensitisation to dog allergen molecules and sensitisation to lipocalins as important risk factors for dog allergy [3].

In this study, we further investigated the same cohort regarding associations between nasal epithelial gene expression, sensitisation and clinical manifestations of allergic disease in detail. Our aim was to identify differences in gene expression between children sensitised to dog dander and healthy controls, as well as to reveal transcriptomic profiles associated with more severe allergic airway disease.

Material and methods

Patients

58 dog dander-sensitised patients aged 10–18 years were recruited from paediatric outpatient clinics. Inclusion criteria consisted of confirmed sensitisation to dog dander through a positive skin prick test (wheal size ⩾3 mm) and/or serum IgE to dog dander ⩾0.10 $kU_A \cdot L^{-1}$ [3]. Patients were included regardless of history of clinical symptoms upon dog exposure. In addition, 20 age-matched healthy controls, without allergic symptoms of rhinitis or asthma were included through advertising at Sach's Children and Youth Hospital (Stockholm, Sweden). The study protocol was approved by the regional ethics committee of Karolinska Institutet (Stockholm; Dnr 2014/1453-31/4). Written informed consent was obtained from parents and/or legal guardians.

Interviews and standardised questionnaires

All children and their guardians were interviewed using a standardised questionnaire which included questions regarding demographic data, such as family history of allergy and asthma, exposure to dogs and other furry animals, history of asthma, rhinitis and other allergic manifestations, symptom triggers, medication and healthcare use [14]. Asthma control was assessed using the Asthma Control Test [15].

Blood sample analyses

Samples of venous blood were collected and white blood cell counts were measured. IgE against airborne allergens (dog, cat and horse dander, timothy, birch, mugwort, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Cladosporium herbarum*) and the food mix fx5 (egg white, peanut, cow's milk, wheat, soy bean and codfish) were analysed. Sera that scored positive (IgE ⩾0.10 $kU_A \cdot L^{-1}$) for furry animal extracts were further analysed for IgE against allergen molecules from dog (Can f 1-Can f 6), cat (Fel d 1, Fel d 2, Fel d 4) and horse (Equ c 1). Furthermore, sera showing an IgE ⩾0.35 $kU_A \cdot L^{-1}$ for fx5 were analysed for the single allergens included in the mix. All IgE determinations were performed using the ImmunoCAP System (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. The results are presented as $kU_A \cdot L^{-1}$ and the cut-off level for single allergens was ⩾0.10 $kU_A \cdot L^{-1}$.

The basophil activation test towards dog dander was performed and the basophil allergen threshold sensitivity (CD-sens) was calculated as described previously [16, 17].

Pulmonary function and bronchial hyper-reactivity

Spirometry and reversibility testing was performed using a Vitalograph™2120 (Vitalograph, Ennis, Ireland), in accordance with recommendations from the European Respiratory Society using the reference values reported by Polgar and Weng [18]. The exhaled nitric oxide fraction (F_{eNO}) was measured prior to

spirometry (Niox Vero). Bronchial hyperreactivity was assessed by methacholine challenge utilising a Spira nebuliser (Spira Respiratory Care Center, Hämeenlinna, Finland) and dose causing a 20% reduction in forced expiratory volume in 1 s (FEV_1) (PD_{20}) was calculated [19].

Nasal provocation testing

Nasal provocation testing was performed as described previously with a commercially available dog dander extract, Aquagen 100 000 SQ-U·mL^{-1} (ALK-Abello, Copenhagen, Denmark) according to a modified Lebel protocol [3, 20]. One spray-dose, 0.1 mL, of diluted dog dander extract (10 000 SQ-U) was deposited in each nostril. Symptoms were recorded at 5, 15 and 30 min after administration and the sum of the score from the three occasions was calculated.

Nasal epithelial brushing

Nasal epithelial brushing was performed in cases and controls. Among cases, nasal provocation tests and nasal epithelial brushings were performed at different occasions, ⩾5 days apart. Nasal epithelial cells were collected using a cervical cytology brush (Bastos Viegas, Penafiel, Portugal) from behind the inferior turbinate. Cells were immediately stored in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA), initially at 4°C overnight, followed by long term storage at −80°C until RNA extraction.

RNA extraction

Total RNA was extracted from nasal epithelial brushings using Qiagen RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA quality and quantity were assessed using NanoDrop 8000, Qubit Fluorometric Quantitation (Thermo Fisher Scientific) and Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA), and an RNA integrity number ⩾8 was used as cut-off for inclusion.

Transcriptome library preparation and sequencing

In total, 74 samples were included (54 cases and 20 healthy controls) and subdivided into two libraries. A modified version of the single-cell tagged reverse transcription (STRT) method [21], described in detail in [22] was used to prepare two 48-plex Illumina-compatible sequencing libraries from 20 ng of each epithelial RNA. The libraries were sequenced on four Illumina HiSeq2000 (Illumina, San Diego, CA, USA) lanes each, using the Illumina TruSeq v3 60-bp single-read protocol. Sequencing was performed at the Bioinformatics and Expression Analysis core facility at Karolinska Institutet.

Sequence data were converted to fastq files using Casava 1.8.2 (Illumina), and quality control performed using the STRTprep pipeline available at https://github.com/shka/STRTprep [22].

Statistical analyses

Differential expression and the statistical significance were tested by SAMstrt [21]. When comparing sample groups q<0.05 was considered as significantly variable expression, and genes with a q-value <0.05 and fold change >1.2 or <0.5 were considered to be significantly differentially expressed. ClustVis (available at https://biit.cs.ut.ee/clustvis/) was used to generate heatmaps and principal component analysis plots of the differentially expressed genes.

Analysis of clinical data was performed with Stata statistical software (release 14.1; Stata Corp, College Station, TX, USA). Categorical data were presented as percentages and two-group comparisons performed using the Chi-squared test, or Fisher's exact test when appropriate. t-test was used for group comparisons of log-transformed IgE values. The Mann–Whitney test was used for other continuous variables. Correlations between *CST1* levels and blood and lung function parameters were investigated using Spearman rank order correlation. A p-value <0.05 was considered significant.

Results

Clinical characteristics

We investigated transcriptomic profiles in nasal epithelial brushings from a cohort of school-aged children. Participants sensitised to dog dander, including children with and without symptoms upon dog exposure, were compared to healthy nonsensitised controls. Figure 1 shows the flow chart leading to the final inclusion of 49 dog dander sensitised cases and 17 healthy controls (figure 1, supplementary table E1). The children sensitised to dog dander had a lower FEV_1 (102% *versus* 110%, p=0.02), a higher median F_{eNO} (32 *versus* 15 ppb, p<0.001), a lower median score on the Asthma Control Test (22 *versus* 25 points, p<0.001) and a higher blood eosinophil count ($0.3\times10^9\cdot L^{-1}$ *versus* $0.2\times10^9\cdot L^{-1}$, p=0.001) compared to nonsensitised healthy controls. Interestingly, dog ownership was more common among the dog dander sensitised children than among the nonsensitised controls (table 1).

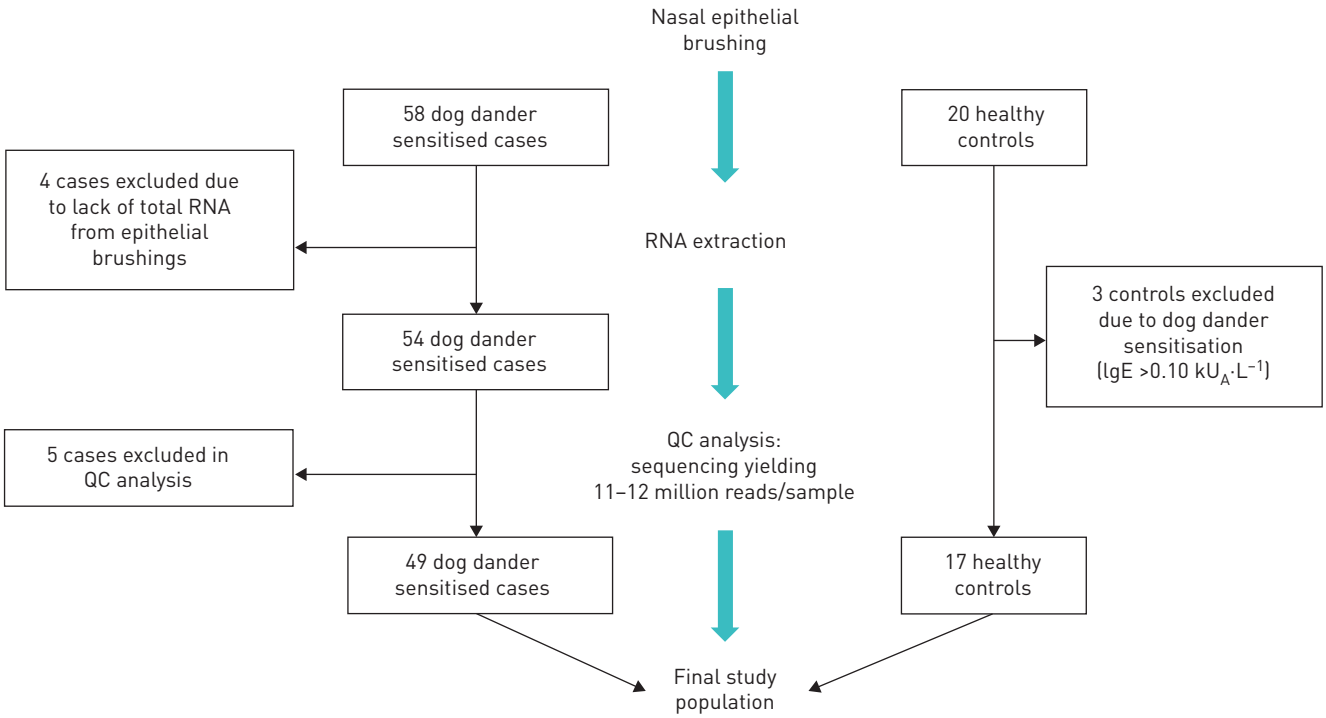

FIGURE 1 Flow chart from patient inclusion to final study population. Total RNA was obtained from 54 children sensitised to dog dander recruited from outpatient paediatric clinics in the Stockholm area and 20 age-matched healthy controls from the same area recruited through advertising. The sequencing yielded ~11–12 million reads per sample (supplementary table E1). Five case samples were removed as part of the quality control (QC) analysis (three had too few spike-ins for high-quality normalisation; two showed degraded RNA). Three of the healthy controls showed to be sensitised to dog dander (IgE >0.10 kU$_A$·L^{-1}) and were therefore excluded from further analysis. For each of the technical duplicates passing QC, the sample with the highest number of raw reads was retained. This resulted in a final set of 49 independent cases and 17 healthy controls.

Transcriptomic analysis

We found that 321 genes were significantly differently expressed among cases sensitised to dog dander compared to nonsensitised controls; 108 genes were upregulated in the cases while 213 genes were

TABLE 1 Demographic and clinical characteristics of dog dander sensitised children and healthy controls

	Dog dander-sensitised cases	Healthy controls	p-value
Subjects	49	17	
Female	17 (35)	9 (53)	0.19
Age years mean (range)	13.2 (10–17)	13.3 (10–17)	0.88
Exposure to dogs			
Dog at home	12 (24)	0 (0)	**0.03**
Dog in the catchment area	39 (80)	11 (65)	0.32
Lung function			
FEV_1 % pred	102 (93–109)	110 (99–119)	**0.02**
Reversibility %	5.7 (1.8–11.5)	3.7 (1.6–5.9)	0.12
F_{eNO} ppb	32 (20–59)	15 (11–18)	**<0.001**
Asthma control test#	22 (19–24)	25 (25–27)	**<0.001**
White blood cell count#			
Leukocytes ×10^9 cells·L^{-1}	5.9 (5.1–7.5)	6.8 (6.0–7.6)	0.28
Neutrophils ×10^9 cells·L^{-1}	2.9 (2.3–4.1)	3.2 (2.9–4.8)	0.13
Eosinophils ×10^9 cells·L^{-1}	0.3 (0.25–0.6)	0.2 (0.1–0.2)	**0.001**

Data are presented as n, n (%) or median (interquartile range), unless otherwise stated. Bold type represents statistical significance. FEV_1: forced expiratory volume in 1 s; F_{eNO}: exhaled nitric oxide fraction. #: dog dander-sensitised cases n=48.

TABLE 2 The top 10 most significantly upregulated or downregulated genes and their fold change in dog sensitised children (cases) *versus* healthy controls

	Gene name	Fold change
Upregulated in cases		
CST1	Cystatin-1	21.33
CCL26	Chemokine (C-C motif) ligand 26	4.58
FOXQ1	Forkhead box Q1	4.51
ZG16	Zymogen granule protein 16	2.75
MTRNR2L6	MT-RNR2-like 6	2.02
ZNF398	Zinc finger protein 398	1.99
XKRX	XK related X-linked	1.94
IFT122	Intraflagellar transport 122	1.82
DEFB1	Defensin β1	1.70
KCNK7	Potassium two-pore domain channel subfamily K member 7	1.68
Downregulated in cases		
CXCL13	C-X-C motif chemokine ligand 13	0.17
SIAH3	Siah E3 ubiquitin protein ligase family member 3	0.20
ATPAF1	ATP synthase mitochondrial F1 complex assembly factor 1	0.21
LRRC45	Leucine rich repeat containing 45	0.22
TMIGD2	Transmembrane and immunoglobulin domain containing 2	0.23
FAM64A	Family with sequence similarity 64, member A	0.23
AGAP3	ArfGAP with GTPase domain, ankyrin repeat and PH domain 3	0.24
KIAA0226L	Rubicon like autophagy enhancer (RUBCNL)	0.24
PZP	α-2-macroglobulin like	0.24
ULBP3	UL16 binding protein 3	0.25

downregulated (q-value <0.05 and fold change >1.2 or <0.5; supplementary table E2). The most upregulated gene among the cases was *CST1*, coding for Cystatin 1, with a median fold change 21 times higher than in controls (table 2). The second most upregulated gene among the cases was *CCL26*, with a median fold change 4.5 times higher than in controls (figure 2). The most downregulated gene in the cases was *CXCL-13*, coding for the C-X-C motif chemokine ligand 13 (fold change 0.17 cases *versus* controls; table 2).

Upregulation of CST1 *and* CCL26 *in a cluster of cases*

Unsupervised clustering of the samples according to the expression of the 10 most upregulated and 10 most downregulated genes identified a distinct cluster of 10 individuals among the 49 dog dander-sensitised cases expressing higher levels of *CST1* and *CCL26* (figure 3, supplementary figure E1). The median fold change for *CST1* in this subgroup was >500 times higher compared with the healthy controls and 47 times higher compared with other cases. Median fold change for *CCL26* was >40 times higher than among controls (figure 2). Hereafter, this subgroup of 10 children is referred to as "*CST1*-high".

Clinical characteristics of CST1*-high children*

CST1-high children showed a lower FEV_1 (% predicted) compared to other children sensitised to dog (94% *versus* 105%, p=0.01), more pronounced bronchial airway responsiveness (PD_{20} methacholine 0.65 *versus* 2.34, p=0.04) and a tendency towards higher reversibility and higher F_{eNO} levels (figure 4a). As shown in figure 4b, they also had a higher median *in vitro* basophil allergen threshold sensitivity (CD-sens) towards dog dander than the other children sensitised to dog (1.8 *versus* 0.20, p=0.01). Furthermore, blood eosinophil counts were significantly higher among *CST1*-high children ($0.65\times10^9\cdot L^{-1}$ *versus* $0.3\times10^9\cdot L^{-1}$, p=0.02) (figure 4b). In addition, the *CST1*-high group displayed an earlier onset of rhinoconjunctivitis, but frequencies of reported rhinoconjunctivitis and asthma were similar between the two groups, as were the results of nasal provocation tests with dog dander extract. We did not see differences in cat or dog ownership between these two groups (supplementary table E3).

Molecular allergy diagnostics in CST1*-high children*

The *CST1*-high children were to a greater extent multi-sensitised towards dog allergen molecules than the other children sensitised to dog (median 5 *versus* 2 allergen molecules, p=0.04). Furthermore, multi-sensitisation to lipocalins from dog, cat and horse was more common in this group (median 6 *versus*

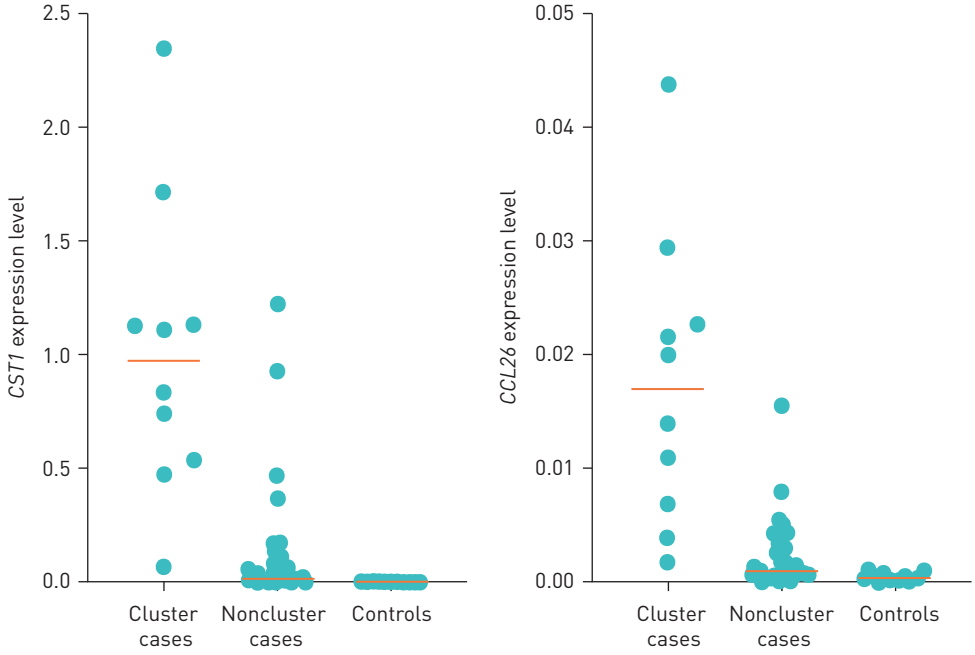

FIGURE 2 Expression of *CST1* (coding for Cystatin-1) and *CCL26* (coding for the C-C motif chemokine ligand 26) among healthy control children (n=17) and dog dander sensitised children. Dog dander sensitised children are separated into *CST1* high "cluster cases" (n=10) and low "noncluster cases" (n=39) subgroups.

3 lipocalins, p=0.03). Regarding single allergen molecules, the frequencies of children sensitised to the dog lipocalins Can f 2 and Can f 6 were higher in the *CST1*-high group than among the other children sensitised to dog (80% *versus* 42% and 80% *versus* 36%, respectively) (figure 5, supplementary table E4).

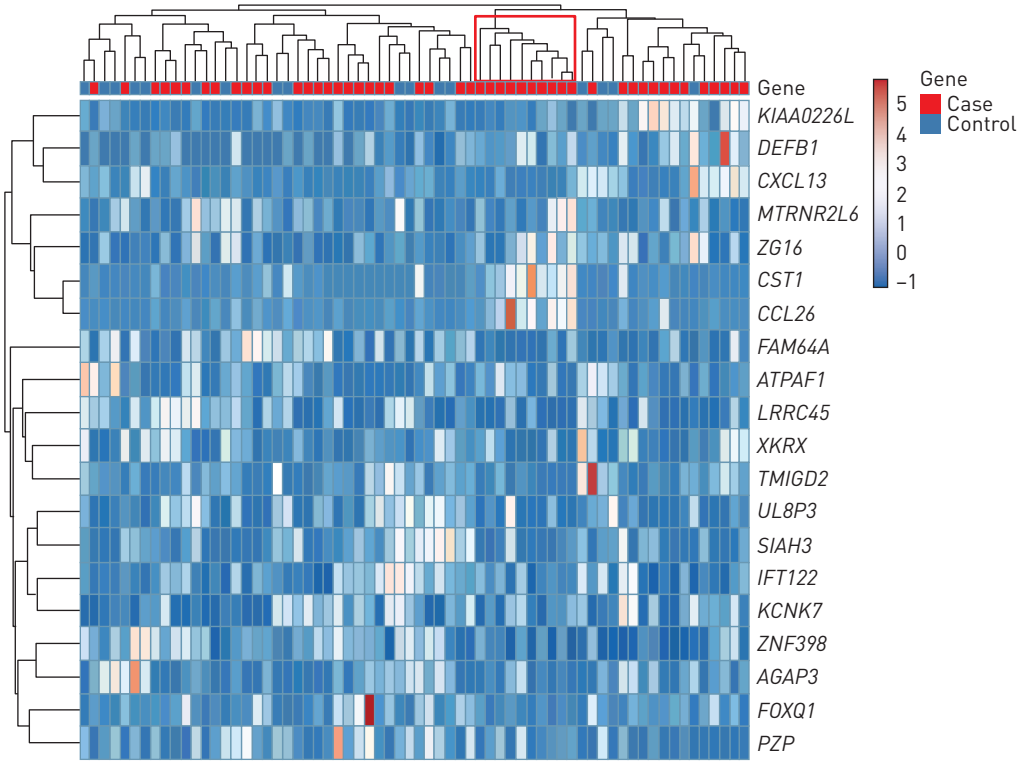

FIGURE 3 Heatmap based on the top 10 most upregulated or downregulated genes in cases *versus* controls. Hierarchical clustering shows the unsupervised clustering of samples.

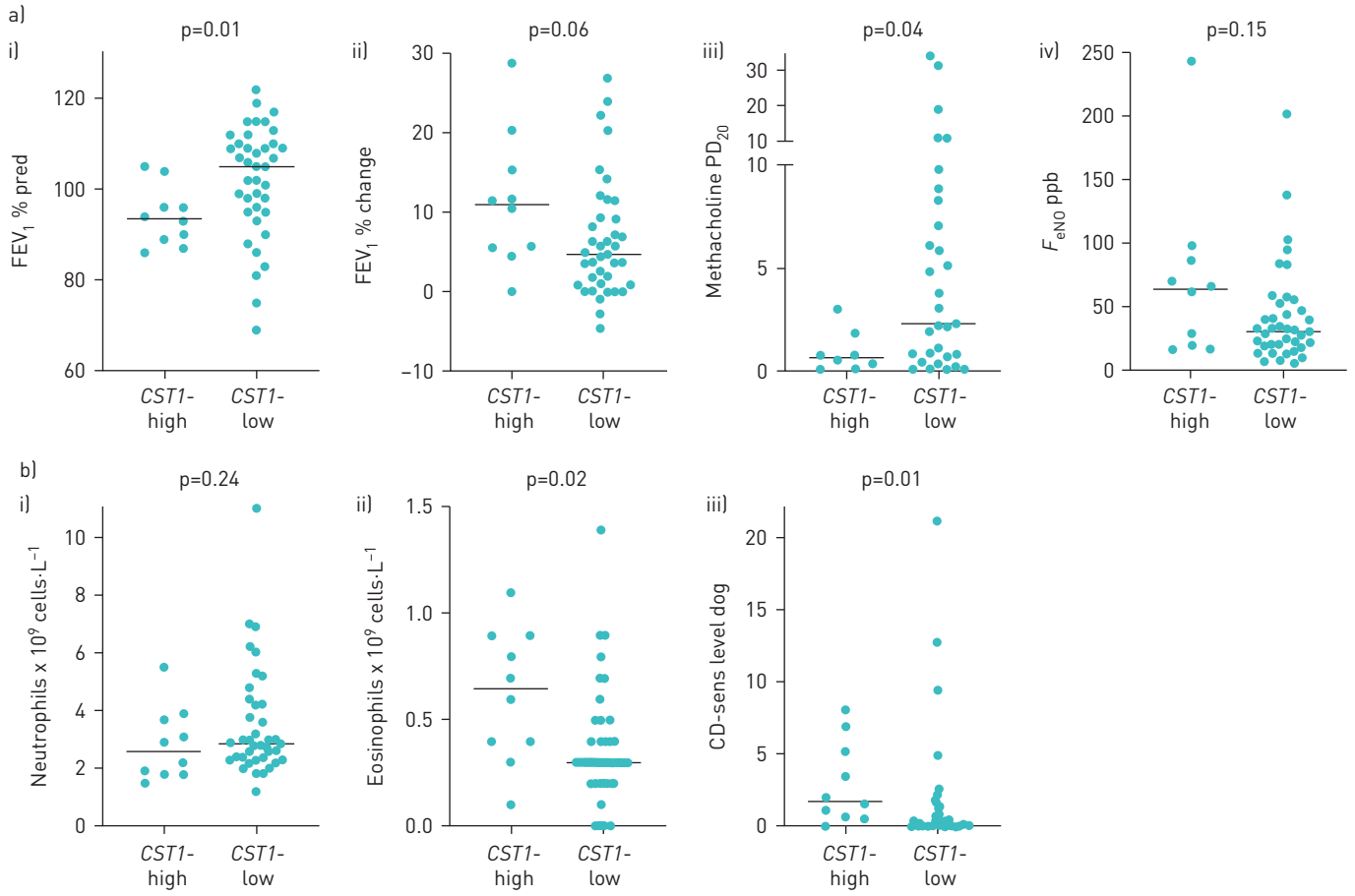

FIGURE 4 a) Lung function parameters in *CST1*-high children compared to other dog dander sensitised children. i) Forced expiratory volume in 1 s (FEV_1) (% predicted) before reversibility test with short-acting β_2-agonist; ii) reversibility: % change in FEV_1 after administration of short-acting β_2-agonist; iii) bronchial hyperreactivity: dose of methacholine leading to a 20% drop in FEV_1 (methacholine PD_{20}); iv) exhaled nitric oxide fraction (F_{eNO}). Two children were excluded due to having FEV_1 ⩽75% of expected at baseline; two children were excluded due to technical issues; and six children did not reach a drop in FEV_1 >20% during the bronchial provocation. b) Blood cell counts among *CST1*-high children (n=10) compared to other children sensitised to dog dander (n=38): i) neutrophils; ii) eosinophils; iii) dog dander CD-sens levels.

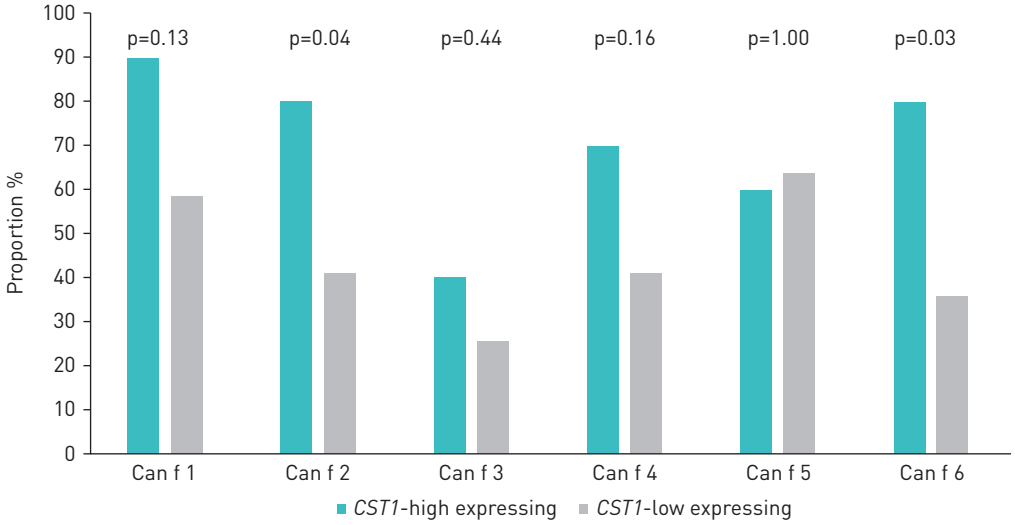

FIGURE 5 Proportions of children (%) sensitised to dog allergen molecules among *CST1*-high (n=10) and other children sensitised to dog dander (n=39).

Despite the higher basophil allergen sensitivity among *CST1*-high children, IgE levels against dog dander did not differ significantly between the groups (46 *versus* 8.9 $kU_A \cdot L^{-1}$, p=0.12) (supplementary table E5). Furthermore, no significant difference in IgE levels towards any furry animal allergen molecule was noted between *CST1*-high children compared to other children sensitised to dog. Sensitisation rates for specific food or airborne allergens did not differ between the groups, and there were only minor differences in IgE levels (supplementary tables E4 and E5).

Correlations between CST1*, inflammatory markers and lung function*

Since the *CST1*-high cluster was not uniquely identified by higher *CST1* levels, but according to the 10 most upregulated and downregulated genes, we further analysed correlations between expression levels of the most upregulated gene *CST1* and lung function parameters and blood cell count. We found a significant correlation between *CST1* levels and F_{eNO} (r_s=0.55, p<0.001) and a negative correlation between *CST1* levels and PD20 methacholine (r_s=−0.56, p<0.001). In addition, we found weaker, but significant correlations between *CST1* levels and impaired FEV_1 % (r_s=−0.37, p<0.002), spirometry reversibility (r_s=0.32, p=0.009) and blood eosinophil count (r_s=0.47, p<0.001).

Discussion

To our knowledge this is the first study to investigate associations between nasal gene expression and multiple clinical characteristics of children sensitised to dog. We found several genes that were upregulated in the nasal airways of children sensitised to dog compared to healthy controls, the highest of which was *CST1*, with more than a 20-fold upregulation. Among the children sensitised to dog dander, there was a cluster of children demonstrating a marked nasal upregulation of *CST1* and *CCL26*, and these children had impaired lung function, pronounced eosinophilia, higher basophil allergen threshold sensitivity and more frequent multi-sensitisation towards dog allergen molecules.

Our data underline the heterogeneity in clinical presentation among children sensitised to dog dander, from being asymptomatic to having persistent rhinitis and asthma. By analysing differences in nasal transcriptomic profiles, we could identify a distinct cluster of children among those sensitised to dog dander with more severe allergic disease. These transcriptomic profiles can provide information on possible underlying mechanisms and may serve as useful biomarkers.

CST1 codes for the protein Cystatin 1 (also called Cystatin SN), belonging to a group of type 2 cysteine protease inhibitors. In humans *CST1* is specifically highly expressed in salivary glands and there are hundreds of minor salivary glands in the nasal cavity. Cystatin 1 has recently been suggested to play a crucial role in the activation and recruitment of eosinophils in the nasal epithelium of patients with eosinophilic chronic rhinosinusitis with nasal polyps [23], and has been proposed as a biomarker for seasonal allergic rhinitis as well as for eosinophilic chronic rhinosinusitis with nasal polyposis [24, 25]. However, findings regarding *CST1* are contradictory as the corresponding protein, Cystatin 1 has also been shown to inhibit allergen protease activity, suppress allergic rhinitis symptoms and protect the nasal epithelial barrier [26, 27].

We mainly observed pronounced symptoms from the lower airways among *CST1*-high children, suggesting that *CST1*-upregulation also plays a role in allergic disease of the lower airways. These findings are supported by George *et al.* [28], who found bronchial *CST1* upregulation among adults with eosinophilic COPD and asthma. Furthermore, upregulated *CST1* transcripts in nasal and bronchial epithelia has been patented as one of several IL13-dependent biomarkers that can identify patients with a high risk of asthma exacerbation [29]. It is known that pulmonary airway gene expression is reflected by gene expression in the upper airways, and recently, it has been shown that transcriptional variations associated with airway disease are present in both nasal and tracheal epithelium [30]. Gene expression is affected by epigenetic mechanisms, such as DNA methylation. Interestingly, DNA methylation differences associated to asthma, rhinitis and allergy have been shown in nasal epithelium from children, which may facilitate our understanding of gene–environment interactions [10].

The impact of exposure to dogs and other furry animals on allergic airway disease in sensitised individuals is well established [31]. Dog ownership was more common among the dog dander sensitised children than among the healthy controls, although we did not see differences in exposure between the *CST1*-high expressing group and other dog dander sensitised children, indicating that dog exposure *per se* cannot explain the increase in *CST1*-expression.

We found higher basophil allergen threshold sensitivity (CD-sens) towards dog dander among *CST1*-high children, whereas IgE levels against dog dander did not differ significantly between *CST1*-high and other children sensitised to dog. CD-sens expresses the *in vitro* effect of the allergen on basophils and thus provide a response that corresponds to the individual's clinical reaction upon allergen exposure [32]. Our

finding indicates that the *CST1*-high expressing children have a higher biological allergen sensitivity compared to *CST1*-low children. When investigating IgE to allergen extracts there is a risk of cross-reactivity of unknown clinical relevance, making the association between IgE levels and clinical presentation less reliable, which probably explain the lack of difference in IgE levels to dog dander between the two groups in our material. We have shown previously that children sensitised to dog, yet tolerant of dogs, had a lower CD-sens level than children with manifest dog allergy and that children with severe allergic asthma have increased basophil allergen sensitivity also compared to children with mild to moderate asthma [16, 33]. It has previously been shown that eosinophil recruitment in the nasal mucosa is enhanced by Cystatin 1 locally, but blood eosinophil levels were higher among individuals with high nasal *CST1* expression, suggesting systemic differences between individuals with high and low nasal *CST1* expression [23].

Lipocalins represent the most important group of inhaled dog allergen molecules. We have shown previously that multi-sensitisation to dog allergens and sensitisation to lipocalins is associated with increased morbidity [3, 13]. The pan-European research network MeDALL (Mechanisms of the Development of Allergy) has introduced the concept that mono- and polysensitised individuals represent different phenotypes, where polysensitisation is associated with multimorbidity and more severe disease [34].

Despite the absence of significant difference in IgE levels to dog dander, *CST1*-high expressing children showed more frequently polysensitisation to dog allergens and lipocalins, displayed more impaired lung function and higher inflammatory markers compared to those with lower *CST1* expression. Thus, increased nasal *CST1* expression could be one possible marker for this polysensitised phenotype.

Taken together, our study show that nasal *CST1* expression is associated with clinical and biochemical markers for asthma and airway allergy, suggesting that *CST1* has the potential of being a biomarker of the morbidity in individuals with allergic sensitisation.

Nevertheless, as this is a small study on children sensitised to dog dander, our findings need to be replicated in larger cohorts across all ages, sensitised to a wider range of allergens, to further elucidate the potential role of CST1 as a biomarker and therapeutic target in allergic patients.

Strengths and limitations

A major strength of this study is the use of nasal epithelial brushings, as many genes show cell-type specific expression patterns. Nasal epithelium is the first physical barrier against airborne allergens, and nasal brushings are therefore highly relevant to study in sensitised children. Importantly, the gene expression profiles in nasal airways have shown high similarity with gene expression profiles in the bronchial and small airways [8], indicating that the results also are applicable for children with asthma. An important limitation is the size of the study population, with only 10 individuals in the *CST1*-high group, making the analysis less robust so that minor differences between the groups might not be revealed. Although previous studies support our results, future studies evaluating the expression of these genes in children sensitised to dog, are required, specifically as we did not have the opportunity to replicate the results in a similar cohort. This is particularly important as differences in gene expression between the whole dog dander sensitised group and healthy controls were small.

Conclusion

CST1 was the most overexpressed gene in children sensitised to dog dander compared to healthy controls. Among children expressing higher levels of *CST1* we found a lower FEV_1, increased bronchial hyperreactivity, pronounced eosinophilia and higher basophil allergen threshold sensitivity towards dog dander, providing further evidence that *CST1* may be an important mediator and biomarker of allergic disease. Thus, the gene expression profile of the airway mucosa of children sensitised to dog may contribute to our understanding of the pathogenesis of dog allergy, as well as provide biomarkers and targets for the development of new treatments.

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Conflict of interest: U. Käck reports a lecture fee from Thermo Fisher outside the submitted work. E. Einarsdottir has nothing to disclose. M. van Hage reports lecture fees from Thermo Fisher Scientific and ALK, and consultancy fees from Biomay AG, Vienna, Austria and Hycor Biomedical LLC, CA, US, outside the submitted work. A. Asarnoj has nothing to disclose. A. James has nothing to disclose. A. Nopp has nothing to disclose. K. Krjutškov has nothing to disclose. S. Katayama reports grants from the Jane and Aatos Erkko Foundation during the conduct of the study. J. Kere has nothing to disclose. G. Lilja has nothing to disclose. C. Söderhäll has nothing to disclose. J.R. Konradsen has received material from Thermo Fisher Scientific to perform IgE analysis in this project.

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Online repository

Nasal gene expression profiling in children sensitized to dog dander identifies upregulation of *CST1* in more severe allergic disease

Figure E1: Principal component (PC) plot showing the first two PC components (PC1 and PC2), based on the ten most highly up and down-regulated genes. Cases are shown in red dots, controls in blue.

Table E1: Sequencing QC summary, including sequencing yield and mapping rates.

Table E2: Full list of significantly up- and downregulated genes based on normalized expression values for all samples. q-value <0.05 and FC > 1.2 (marked in red) or FC < 0.5 (marked in blue) were considered to be significantly differentially expressed.

Table E3: History of allergy and asthma among children with high expression of *CST1* and other dog dander sensitized children.

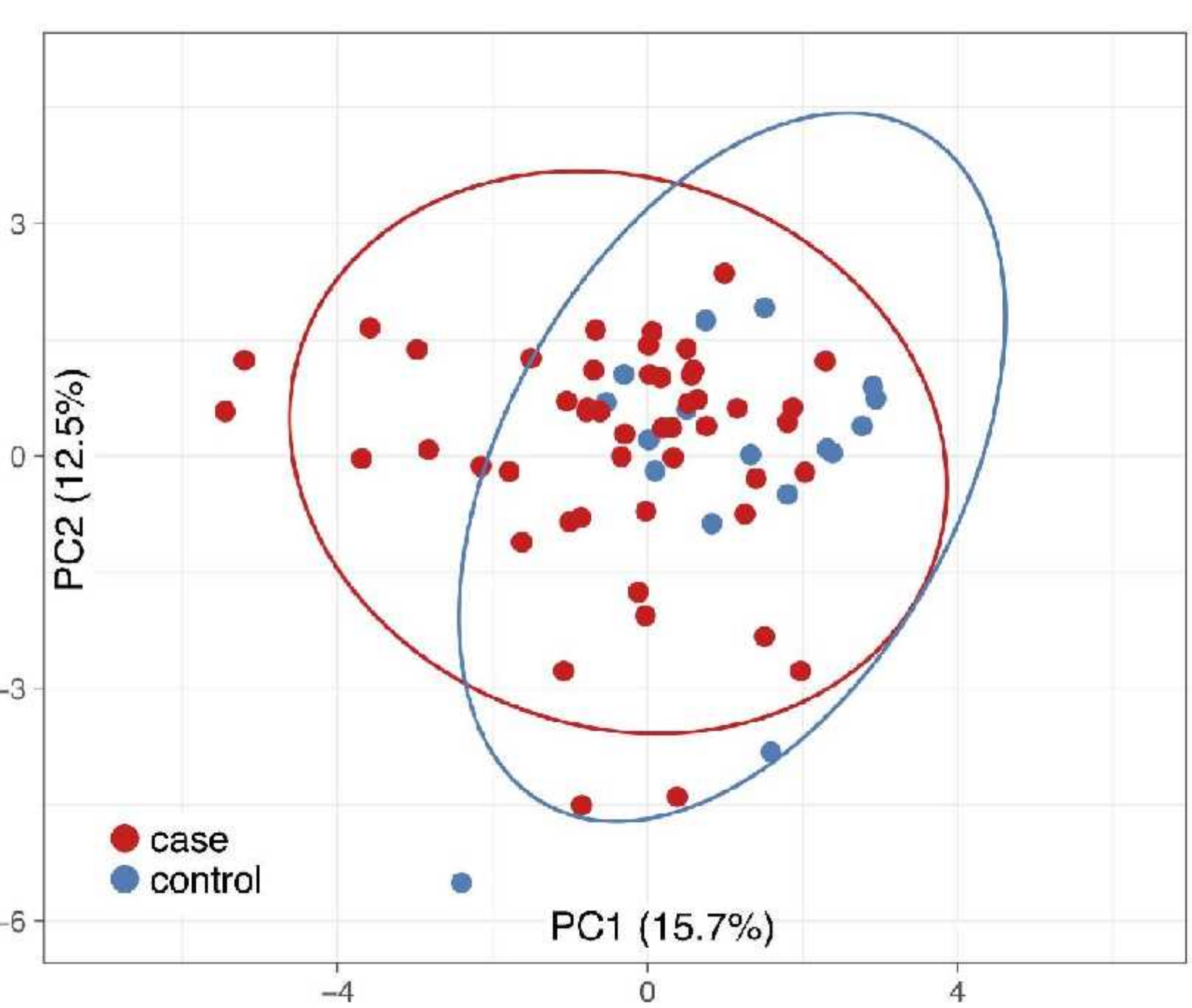
	CST1-high expressing (n=10)	Other dog dander sensitized (n=39)	p-value
HEREDITY n (%)			
Dog allergy, any parent	1/10 (5 %)	14/39 (36 %)	0.15
Asthma, any parent	2/10 (20 %)	18/39 (46 %)	0.17
Rhino conjunctivitis, any parent	7/10 (70 %)	27/39 (69 %)	1.00
EXPOSURE TO FURRY ANIMALS			
Dog at home	2	10	1.00
Dog in the catchment arera	6	33	0.18
Cat at home	0	2	1.00
Cat in the catchment arera	4	25	0.28
RHINITIS n (%)			
Reported rhinitis	9 (90 %)	39 (100 %)	0.20
Reported rhinitis at dog exposure	7 (70 %)	27 (69 %)	1.00
Nasal composite score Median points (IQR)	19 (14-22)	14 (8-21)	0.31
Age at onset (any rhinitis) Years, median (IQR)	2 (2-2)	4 (2-7)	**0.04**
ASTHMA n (%)			
Reported asthma	9 (90 %)	33 (84 %)	1.00
Reported asthma at dog exposure	5 (50 %)	20 (51 %)	0.94
Inhaled steroids regularly	6 (60 %)	23 (58 %)	1.00
Asthma control test Median (IQR)	22.5 (20-25)	22 (19-24)	0.52
Age at onset, years Median (IQR)	2.5 (1.5-9)	5 (2-8)	0.74
ECZEMA n (%)			
Ever eczema	5 (50 %)	25 (64 %)	0.48
Current eczema	4 (40 %)	17 (44 %)	1.0
Age at onset (months, IQR))	2 (1-3)	6 (3-12)	0.064

Table E4: Multi-sensitization to furry animal allergens and sensitization rates to cat and horse allergens, other airborne allergens and food among *CST1*-high expression cluster and the other dog dander sensitized cases.

Sensitization >0.1 kU/l	*CST1*-high expressing n=10	Other dog dander sensitized n=39	p-value
Multi-sensitization, Median (IQR)			
No. positive dog allergen molecules	5 (3-6)	2 (1-4)	**0.04**
No. positive lipocalins	6 (3-6)	3 (1-5)	**0.03**
Sensitization rates, n (%)			
Cat dander	9 (90)	38 (97)	0.37
Fel d 1	8 (80)	32 (82)	1.00
Fel d 2	5 (50)	9 (23)	0.12
Fel d 4	7 (70)	24 (61)	0.73
Horse dander	8 (80)	31 (79)	1.00
Equ c 1	7 (70)	23 (59)	0.72
D. pteronyssinus	8 (80)	30 (77)	1.00
D. farinae	7 (70)	28 (72)	1.00
Thimothy	8 (80)	36 (92)	0.27
Cladosporium	5 (50)	18 (46)	1.00
Birch	7 (70)	31 (79)	0.67
Mugwort	3 (30)	15 (38)	0.73
Egg white	8 (80)	18 (46)	0.08
Peanut	7 (70)	20 (51)	0.48
Cow's milk	8 (80)	20 (51)	0.16
Wheat	6 (60)	23 (39)	1.00
Soybean	7 (70)	18 (46)	0.29
Codfish	3 (30)	7 (18)	0.41
Fx5	9 (90)	36 (92)	1.00

Table E 5: IgE to other furry animals, airborne allergens and food allergens: Levels among sensitized. Levels above 100 kU_A/L were set at 100 kU_A/L for specific IgE and IgE to allergen molecules.

IgE-titer kU_A/l (IQR)	*CST1*-high expressing (n=10)	Other dog dander sensitized (n=39)	p-value (t-test on log values)
Total IgE	638 (250- 1169)	414 (198- 781)	0.66
Dog allergens			
Dog dander	46 (3.7-92)	8.9 (2.7-37)	0.12
Can f 1	14 (2.3-31)	4.7 (2.1-31)	0.57
Can f 2	20 (3.45-45.5)	2.9 (0.34-16)	0.08
Can f 3	1.8 (1.165-12.9)	0.95 (0.17-6.1)	0.62
Can f 4	3.29 (0.31-8.31)	0.99 (0.47-2.46)	0.15
Can f 5	4.8 (0.47-36)	2.3 (0.71-8.2)	0.58
Can f 6	0.76 (0.27-4.16)	0.72 (0.37-1.0)	0.54
Cat allergens			
Cat dander	9.53 (4.96-87.4)	7.32 (2.50-19.1)	0.11
Fel d 1	38.4 (5.29-83.6)	6.55 (2.32-24.5)	0.13
Fel d 2	0.33 (0.25-0.72)	0.65 (0.25-9.97)	0.40
Fel d 4	1.63 (0.50- 11.5)	3.39 (0.49- 13.6)	0.67
Horse allergens			
Horse dander	16.1 (7.05-43.5)	4.69 (0.58- 19.0)	0.07
Equ c 1	10.4 (1.36- 32.0)	5.85 (2.36- 15.8)	0.78
Other airborne allergens			
D. peronyssius	0.63 (0.25- 44.6)	0.52 (0.19- 3.60)	0.61
D. farinae	0.79 (0.33- 100)	0.76 (0.22- 2.73)	0.53
Thimothy	16.5 (3.88- 81.2)	5.42 (0.93- 53.0)	0.40
Cladosporium	3.15 (2.12- 4.22)	0.65 (0.15-3.10)	0.15
Birch	29.5 (0.15-100)	7.39 (1.06-43.2)	0.82
Mugwort	0.62 (0.29- 4.09)	0.75 (0.31-2.67)	0.91
Food allergens			
Egg white	0.82 (0.37- 6.68)	0.82 (0.34-1.88)	0.65
Peanut	10.8 (1.5-100)	1.46 (0.72-8.00)	0.09
Cow's milk	0.45 (0.22- 2.00)	0.32 (0.15-1.03)	0.45
Wheat	2.09 (0.78- 5.15)	0.57 (0.23- 2.01)	0.07
Soybean	2.88 (0.57-5.32)	0.53 (0.29- 3.07)	0.16
Codfish	0.22 (0.21- 0.30)	0.15 (0.15- 0.19)	**0.02**
Fx5	8.34 (1.04-55.6)	0.63 (0.25-5.28)	**0.03**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0
-3
-6
PC2 (12.5%)
case
control
PC1 (15.7%)
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