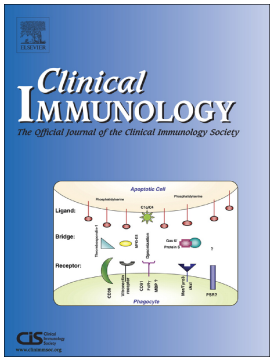


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PII: S1521-6616(20)30140-6

DOI: https://doi.org/10.1016/j.clim.2020.108512

Reference: YCLIM 108512

To appear in: *Clinical Immunology*

Received date: 27 February 2020

Revised date: 10 June 2020

Accepted date: 11 June 2020

Please cite this article as: M. Jokinen, S. Edelman, K. Krohn, et al., Neutralizing natural anti-IL-17F autoantibodies protect Autoimmune Polyendocrine Syndrome Type 1 (APS-1) patients from asthma, *Clinical Immunology* (2020), https://doi.org/10.1016/j.clim.2020.108512

# Neutralizing natural anti-IL-17F autoantibodies protect Autoimmune Polyendocrine Syndrome Type 1 (APS-1) patients from asthma

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This investigation was supported by Helsinki University Hospital Research funds.

Declaration of interest: none.

Keywords: Autoimmune Polyendocrine Syndrome Type 1, Asthma, IL-17F

To the Editor,

Patients with Autoimmune Polyendocrine Syndrome Type 1 (APS-1) provide a fascinating setting to study the association of human neutralizing autoantibodies against cytokines and allergy symptoms. This rare monogenic autosomal recessive disease, caused by mutations in the autoimmune regulator (AIRE) gene, classically presents with chronic mucocutaneous candidiasis and endocrine organ-specific autoimmunity. Typical features include accumulation of autoreactive T-cells and the presence of notable levels of high-affinity neutralizing autoantibodies, not only against tissue antigens but also against cytokines. Interestingly, some of the APS anti-cytokine autoantibodies have been claimed to be disease ameliorating [1] and could therefore contribute to the lack of reported classical asthma and allergy symptoms among APS-1 patients.

To advance our understanding on the role of human anti-cytokine antibodies on asthma or allergy, we enrolled 10 male and 19 female Finnish APS-1 patients and investigated the relationship between IgE sensitization, asthma and allergy symptoms and circulating cytokine autoantibodies (Figure 1). Our APS-1 patients had a mean age of 46 years, all encompassed the same Finn major AIRE mutation genotype and all but one had been diagnosed with chronic mucocutaneous candidiasis (CMC), as part of the classic diagnostic triad of APS-1 symptoms. The full clinical outline of our cohort is available in our previous publication [2].

IgE sensitization to the 112 most common allergen components was tested using ImmunoCAP immuno-solid phase allergen chip (ISAC) sIgE-microchip. Four of the 29 (14%) APS-1 patients none of which with asthma, were detected to be sensitized to one or more IgE chip allergen using a threshold of 0.3 ISAC standard unit. The positive results were for birch pollen (and cross-reactive PR-10 allergen components), common wasp, timothy and for domestic cat. One patient was triple positive for timothy, birch pollen and domestic cat while the three others were positive only for one

allergen: common wasp (one patient) or birch pollen (two patients). The relatively low rate of atopy is consistent with local prevalence of allergic rhinoconjunctivitis and asthma in Finnish middle-aged patients [3].

For allergy and asthma symptoms, peak expiratory flow (PEF), spirometry measurements and skin prick test results were inquired and information on the use of asthma medication and allergy symptoms (excluding drug allergies and atopic dermatitis) were collected with a structured questionnaire. Out of the 29 patients participating our study, four female patients (14%) had a diagnosed asthma (Figure 1). They all had been prescribed inhalant corticosteroids and sympaticomimetics and two of them used inhalants regularly. Of note, pulmonary autoimmunity has not been observed in this patient cohort during treatment and regular check-ups at university hospital level [2]. Regarding other allergy symptoms, 14 of the 29 patients reported symptoms. Eight patients reported conjunctival or nasal symptoms from birch pollen, three of them were validated by the IgE chip. Six patients reported symptoms from cat, but only one of these was validated by the IgE chip. Upper respiratory track symptoms from various fruit, nuts, fish and mold were also recorded, but no IgE sensitization data agreed with this information, reflecting low general specificity of self-reported symptoms. Notably, chronic mucocutaneous Candida infection may mimic food allergy symptoms. The one patient without history of CMC did not report allergy symptoms, nor tested positive on the IgE chip nor had asthma.

Serum autoantibodies against 81 cytokines and against three non-cytokine proteins (sclerostin, spartin and synphilin-1) were measured with enzyme-linked immunosorbent assay using recombinant antigens. Healthy sera from blood donors served as negative controls. Of the twelve inflammatory cytokines[4] and 17 chemokine ligands [4, 5] previously associated with asthma, our test panel included the following 12 inflammatory cytokines and 17 chemokine ligands: IL-1β, IL-3,

IL-4, IL-5, IL-6, IL-7, IL-13, IL-17A, IL-17F, IL-22, IL-23, IL-25 and CCL2, CCL3, CCL7, CCL11, CCL13, CCL17, CCL22, CCL24, CCL26 as well as CXCL1, CXCL5, CXCL9, CXCL10, CXCL11, CXCL12a, CXCL12b, CXCL13.

All patients typically had autoantibodies at an optical density (OD) level of 0.04 against several cytokines: all had autoantibodies against IFNα1b and IFNα5. The majority (28/29) of patients had autoantibodies against IFNα2b, IL-22 and IFNα4, while 25/29 patients had autoantibodies against IL-17F. In relation to the rest of the above mentioned asthma-associated chemokines, CCL13- and CXCL12a-specific autoantibodies were detected in one patient each. None of the patients had autoantibodies against IL-1β, IL3, IL-6, IL-13, IL-23, IL-25, CCL3, CCL7, CCL17, CCL24, CCL26, CXCL1, CXCL5, CXCL9 and CXCL12b. The potent neutralizing capacity of the above anti-cytokine autoantibodies has been previously reported [1].

Associations between clinical, symptom and assay variables were examined by means of T-tests followed by Benjamin-Hochberg false discovery rate multiple testing correction. Significance was defined using thresholds of 0.05 and 0.20 (Figure 2). Hierarchical clustering (Euclidean distance and Wards-linkage) was used to visualize data. These analyses revealed the distinct presence of three anti-cytokine antibodies IL-17F, IL-11 and IFN-ω1 between asthmatic and non-asthmatic patients (Figure 3). In particular, asthmatic APS-1 patients had a lower mean IL-17F antibody level compared to the non-asthmatic APS-1 patients (optical density 0.53 +/- 0.43 vs. 2.11 +/- 1.02, $Q<0.05$; Figure 3) suggesting that anti-IL-17F autoantibodies may provide protection against asthma.

The IL-17F pathway has a central role in the pathogenesis of asthma. It is involved in airway hyper reactivity, neutrophil infiltration, mucus hypersecretion and goblet cell hyperplasia, subepithelial fibrosis and smooth muscle cell hyperplasia as well as steroid resistance in asthmatic lung tissue [6]. IL-17F overexpression in bronchial and nasal tissue has been related to an increased number of

bronchial neutrophils, poorer lung function and more frequent exacerbation rates in asthmatics [7]. In murine models, blocking IL-17 has in turn been shown to ameliorate asthma phenotype. For example, the blockage of either IL-17F or IL-17A by both neutralizing antibodies or deficiency of the cytokine has been shown to prevent house dust mite -induced allergic asthma in mice [8]. Mice genetically lacking IL-17F have been noted to be more resistant to allergic bronchopulmonary aspergillosis upon intranasal challenge compared to wild type, and treatment with neutralizing IL-17Fab has been shown to reduce signs of respiratory allergy and lung inflammation in wild type mice [9]. In a Japanese case control study, a loss-of-function IL-17F mutation negatively correlates with asthma/COPD [10]. Moreover, a therapeutic anti-IL-17A monoclonal antibody is the subject of an ongoing clinical trial with severe asthmatics and a new trivalent monomeric neutralizing IL-17A/F Nanobody is studied for the treatment of psoriasis (clinicaltrials.gov) but no IL-17F targeting monoclonal antibody has yet been brought to clinical trials for treatment of asthma.

In conclusion, this study suggests a disease ameliorating role for IL-17Fab and indicates that IL-17F autoantibodies may provide protection against asthma, despite the limited sample size due to the rarity of APS-1. In future prospective studies, IL-17F levels could be verified using a larger study cohort as well as in bronchoalveolar lavage sampling. All in all, this study underlines the importance of disease ameliorating properties of APS-1 anti-cytokine autoantibodies.

We are indebted to Mrs. Alli Tallqvist and Mrs. Päivi Hanhinen for skillful technical assistance.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest related to this study.

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**Figure 1. The study design**. The study included altogether 19 female and 10 male APS-1 patients. All of the enrolled 29 APS-1 patients donated blood samples that were tested for autoantibodies against 81 cytokines as well as for 112 allergen-specific IgE antibodies with the ImmunoCAP ISAC sIgE-microchip. All 29 APS-1 patients also filled out a structured questionnaire about allergy and asthma symptoms.

**Figure 2. Analysis of serum cytokine autoantibody data in relation to allergy and asthma symptoms and findings.**

Hierarchical clustering analysis of serum autoantibody data. Cytokines against which none of the patients had autoantibodies were filtered prior to statistical testing. Top left column bar shows various patient classification variables for each patient marked with black and grey colors. Dark grey stands for missing information on one patient. Lower left corner highlights statistically significant differences of autoantibody values (FDR<0.2 marked in grey and <0,05 marked in black) between patients with or without IgE-mediated allergic sensitization, allergy symptoms, asthma, allergy or asthma medication, prick and/or PEF/spirometry tests performed, time point of symptom onset in childhood or adulthood, and between asthma and nonasthmatic/nonsensitized, and IgE-sensitized and nonasthmatic/nonsensitized patients.

Association between IL17F and asthma is shown on lines 3 and 12 (FDR<0.2 marked in grey and <0,05 marked in black).

**Figure 3. Box-plots of statistically significant serum cytokine autoantibodies between APS-1 patients without and with asthma.** Serum anticytokine autoantibody OD signal intensity box plot in comparisons that reached statistical significance at the threshold of Q<0.20 or Q<0.05.

- 14/29 (48%) APS-1 patients had allergy symptoms, only 4 (14%) verified by IgE chip
- 4/29 (14%) APS-1 patients had asthma
- The presence of anti-IL17F autoantibodies is associated with lack of asthma symptoms

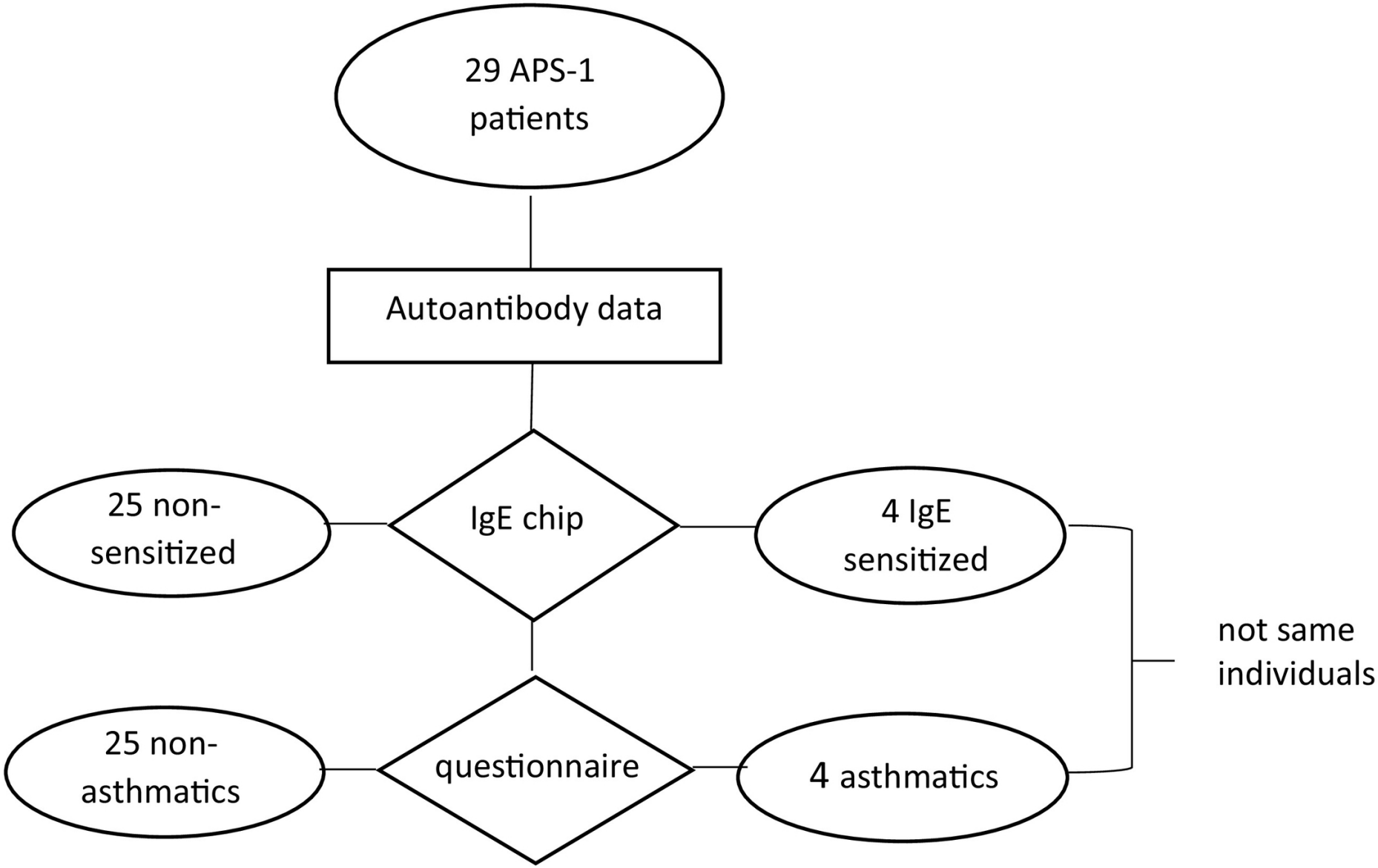
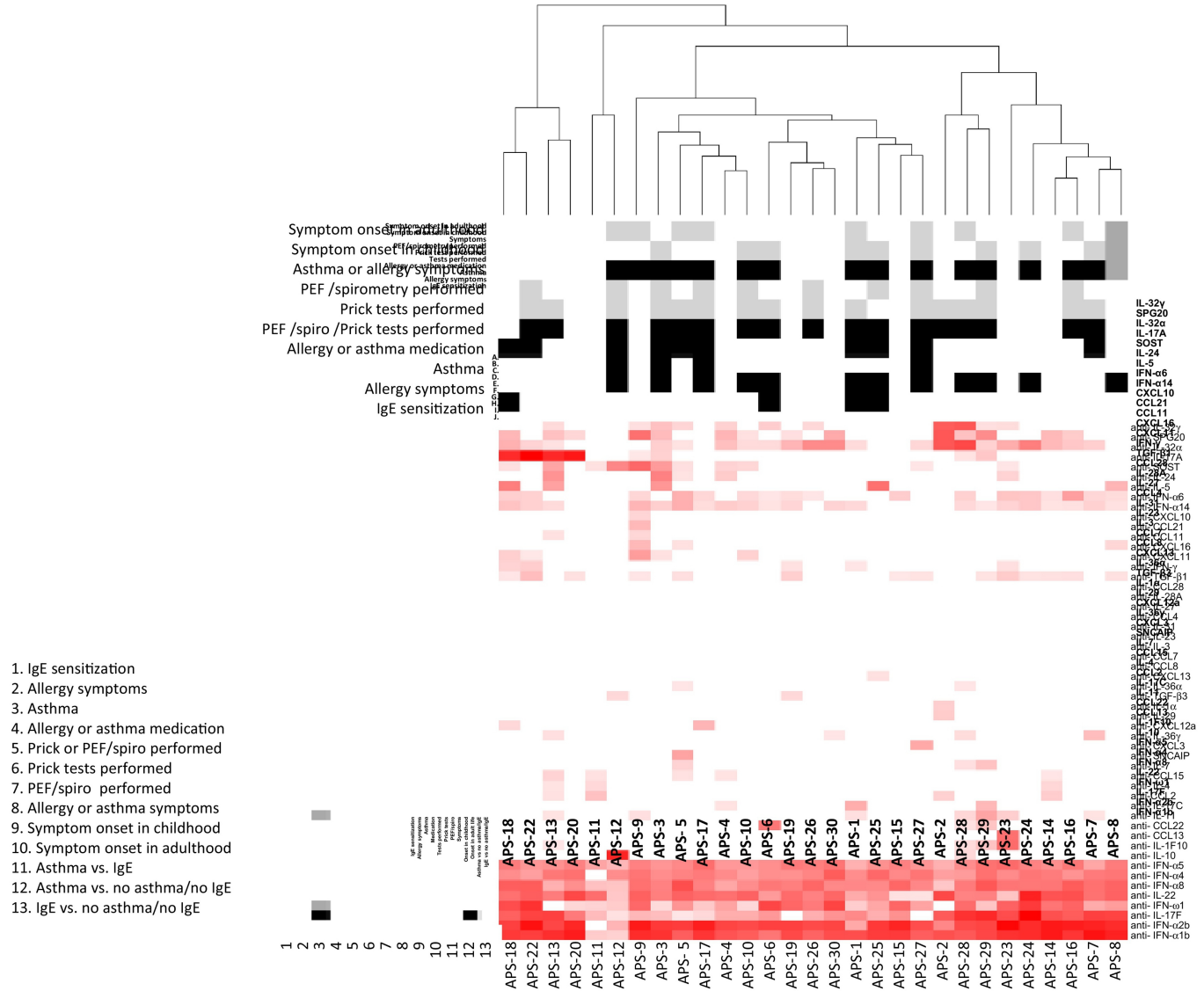

29 APS-1 patients
Autoantibody data
25 non-sensitized
IgE chip
4 IgE sensitized
not same individuals
25 non-asthmatics
questionnaire
4 asthmatics

Figure 1




1. IgE sensitization
2. Allergy symptoms
3. Asthma
4. Allergy or asthma medication
5. Prick or PEF/spiro performed
6. Prick tests performed
7. PEF/spiro performed
8. Allergy or asthma symptoms
9. Symptom onset in childhood
10. Symptom onset in adulthood
11. Asthma vs. IgE
12. Asthma vs. no asthma/no IgE
13. IgE vs. no asthma/no IgE

Figure 2

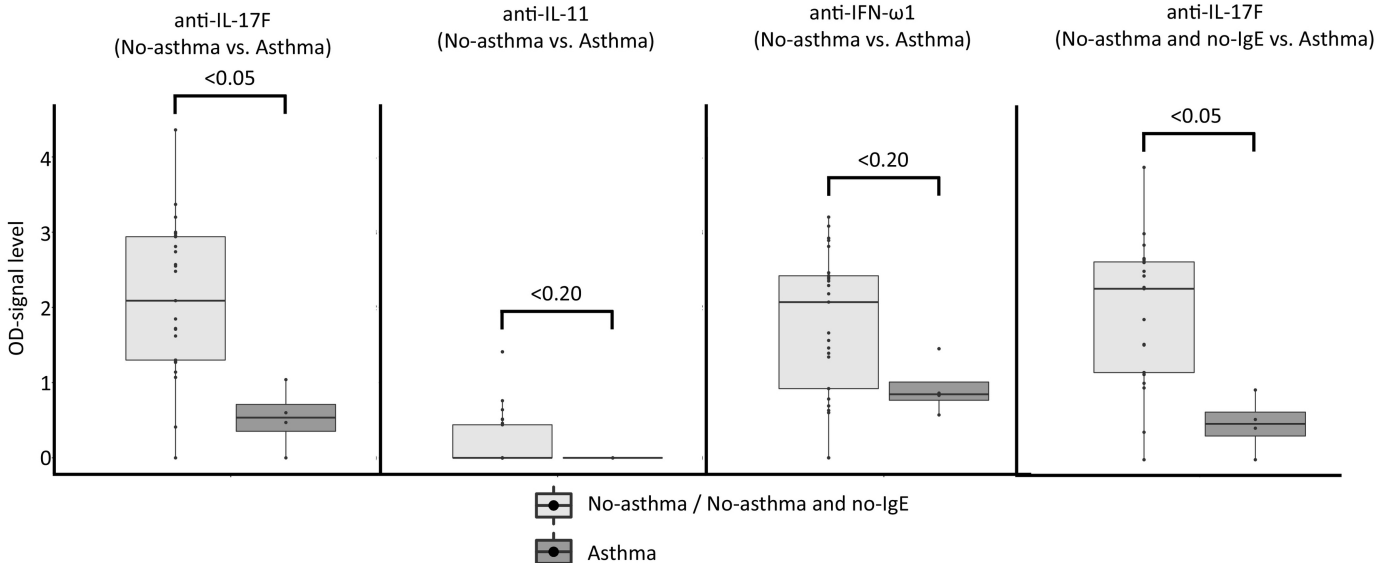

anti-IL-17F
(No-asthma vs. Asthma)
<0.05
anti-IL-11
(No-asthma vs. Asthma)
<0.20
anti-IFN-ω1
(No-asthma vs. Asthma)
<0.20
anti-IL-17F
(No-asthma and no-IgE vs. Asthma)
<0.05
OD-signal level
0
1
2
3
4
No-asthma / No-asthma and no-IgE
Asthma

Figure 3