# Final research report

EAACI Long-term research fellowship 2019

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**Background**

AD and allergic diseases are affecting the lives of more than one billion people worldwide and approximately 3% of adults in the EU suffer from chronic or relapsing itching. AD is the most prevalent chronic disease in childhood and represents one of the highest financial burdens to the health care system. Approximately 5-10% of these diseases present with severe forms and can even cause deaths. AD is more of a systemic disease with several comorbidities presenting with a type 2 inflammation-driven systemic immune and tissue dysregulation.

**Objective**

This long-Term Fellowship aimed to investigate the novel biomarker and to perform extensive pathophysiological analyses in AD in the context of systemic and skin responses.

**Method**

We performed the experiments listed below:

1) Spatial RNA sequencing in skin biopsied samples

2) Targeted proteomics analysis in serum samples

3) Evaluation of skin barrier dysfunction by electrical impedance spectroscopy

1) We performed spatial transcriptomic analysis, named ‘Visium' established by 10X Genomics in human skin biopsied samples from LN and NL of AD and healthy controls. Spatial RNA-sequencing of skin was analyzed to demonstrate characteristics of cellular-infiltration in LN compared to NL and healthy controls.

2) The candidate used the biobanked samples from CK-CARE biobank and performed the Proximity extension assay (OLINK, Sweden). This novel technique enabled multiplex immunoassay that measures various biomarkers. We investigated the novel biomarker to evaluate the severity and barrier dysfunction of AD.

3) Electrical impedance spectroscopy (EIS) is a noninvasive device, which can detect the barrier dysfunction of AD (Rinaldi et al. *Allergy,* 2019 and 2021). We measured the EIS score on the skin of NL and LN of AD and healthy control.

**Results**

**Spatial gene profiling in the cellular -infiltrated area of atopic dermatitis patients**

We separate the spots into six clusters because of specific gene expressions. We identified significantly upregulated genes, such as CCL13, CCL17, CCL18, CCL19, and TNC in cellular-infiltrated/capillary cluster in LN compared to the same cluster in healthy control and NL.

Interestingly, the ligand-receptor analysis showed a significant correlation in the gene expression of CCL19 and its receptor CCR7.

**The EIS score and severity of AD correlate with the serum biomarkers.**

We investigated biomarkers that were significantly upregulated in the serum of AD patients correlated with severity and barrier dysfunction. Serum biomarkers levels, such as CA12, REG1A, TNC, and CCL18, showed a correlation with the severity of AD and skin barrier dysfunction. Serum levels of CCL19 were also significantly upregulated in AD patients, but there was no significant correlation with severity and barrier dysfunction.

**Discussion**

Here, we investigated the detail of the pathogenesis of AD and biomarkers related to severity and skin barrier dysfunction of AD.

Recently, we reported that Electrical impedance spectroscopy (EIS) is a novel and noninvasive method to measure the skin barrier condition (Rinaldi et al. *Allergy*, 2019 and 2021). The score of EIS clearly reflected the barrier dysfunction of AD patients and EIS score was more sensitive than trans-epidermal water loss. In this study, we found that serum levels of CCL18 and TNC are significantly correlated with the severity and their barrier dysfunction. Spatial transcriptomics of human AD skin showed that gene expression of CCL18 and TNC are localized in cellular-infiltrated/capillary cluster and significantly upregulated in the same cluster compared to healthy control. Moreover, the serum level of CCL19 was significantly upregulated in the serum from AD patients. CCR7, which is known as a chemokine receptor for CCL19, is expressed on naïve T cells, central memory T cells, regulatory T cells, naïve B cells, semi-mature/mature DCs and NK cells, and a minority of tumor cells. It has been reported that CCL19-CCR7 interactions act as a key regulator guiding homeostatic lymphocytes to secondary lymphoid organs. Recently, He et al. performed the single-cell analysis of human skin biopsy samples from AD patients and reported LAMP3+, CCR7+ activated DC subsets and CCL19+ fibroblast subsets in AD (He et al. *J Allergy Clin Immunol* 2020). We found a significant correlation of the gene expression of CCL19 and CCR7 by ligand-receptor analysis in the cellular-infiltrated areas of LN. These results suggest that there are CCL19 producing cells, such as fibroblast, in the cellular-infiltrated area in LN and CCR7+ immune cells migrate into LN.

**Conclusion**

We identified CCL18 and TNC as serum biomarkers related to severity and skin barrier dysfunction of AD. Spatial gene expressions showed the correlation of gene expression of CCL19-CCR7 pair in LN. To investigate the correlation with spatial transcriptomics, proteomics, and EIS has the potential to shed light on novel pathogenesis of AD.

**Expected publication**

In this project, it is expected to identify role of immune cells in the skin, better characterize the nonlesional skin and lesional skin of AD, and discover the novel biomarkers. Our result will have an impact not only on research of skin, but also other allergic diseases, because AD initiates allergic march, such as food allergy, bronchial asthma, and allergic rhinitis. Our preliminary data demonstrate that the study is prone to several high-level publications.

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