Peripheral Blood Transcriptome Profiles of Adults with Type I Hypersensitivity

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Abstract

Allergy of type I hypersensitivity affects about 150 million people in Europe. It is clinically manifested as atopic dermatitis, conjunctivitis, rhinitis, rhinoconjunctivitis, and allergic asthma. However, the underlying mechanisms occurring at the gene expression level remain poorly understood. To address this gap, the transcriptome of peripheral blood cells from participants with type I hypersensitivity symptoms was measured to gain insights into mechanisms underlying the disease. We examined immunological pathways of observed transcriptomic profiles to examine immune-related alterations in participants with atopic disorders. A diverse array of enriched pathways and cellular processes associated with type I hypersensitivity reactions were identified within domains such as antigen-presenting cells (APCs), interleukins, mast cells, CD molecules, T helper (Th) and T regulator (Treg) cells, and B cells. These findings collectively suggest that disturbances at the gene expression level contribute to immunological disorders in individuals experiencing allergic manifestations.

Peripheral Blood Transcriptome Profiles of Adults with Type I Hypersensitivity Running title (max 50 characters): Transcriptomic Profiles of Adults with Allergies

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Abstract

Allergy of type I hypersensitivity affects about 150 million people in Europe. It is clinically manifested as atopic dermatitis, conjunctivitis, rhinitis, rhinoconjunctivitis, and allergic asthma. However, the underlying mechanisms occurring at the gene expression level remain poorly understood. To address this gap, the transcriptome of peripheral blood cells from participants with type I hypersensitivity symptoms was measured to gain insights into mechanisms underlying the disease. We examined immunological pathways of observed transcriptomic profiles to examine immune-related alterations in participants with atopic disorders. A diverse array of enriched pathways and cellular processes associated with type I hypersensitivity reactions were identified within domains such as antigen-presenting cells (APCs), interleukins, mast cells, CD molecules, T helper (Th) and T regulator (Treg) cells, and B cells. These findings collectively suggest that disturbances at the gene expression level contribute to immunological disorders in individuals experiencing allergic manifestations.

Keywords: *Allergic asthma, Atopic dermatitis, Immune disorders, Transcriptomic profiling, Type I hypersensitivity*

Introduction

Allergy of type I hypersensitivity is a severe condition experienced by about 150 million people in Europe[1]. Type I hypersensitivity can be manifested as atopic dermatitis, rhinitis, conjunctivitis, rhinoconjuctivitis or allergic asthma. These manifestations very often appear together[2]. In addition, they usually occur gradually from atopic dermatitis to allergic asthma, collectively known as the atopic march[3, 4]. All of these clinical signs, even if they are distinct, come from a single intrinsic process: IgE mediated (type I) hypersensitivity. In type I hypersensitivity, T helper type 2 (Th2) cells play a key role in the patient response to antigens by activating immunoglobulin (Ig) E sensitization[3, 5].

The type I hypersensitivity reaction occurs when a mucosal surface (i. e. respiratory or gastrointestinal tract) or the skin is exposed to an antigen, which causes an allergy (allergen)[6, 7]. The immune response to the antigen begins with the sensitization phase[7]. During this phase, the antigen is captured by antigen-presenting cells (APCs), and subsequently presented to naive T cells[7, 8]. This interaction leads to the activation of naive T cells, which then secrete interleukin-4 (IL-4), which together with IL-4 produced by basophils and mast cells, initiate their differentiation into Th2 cells[7, 9]. Additionally, differentiated Th2 cells further secrete IL-4 and IL-13 which stimulate B cell activation, leading to class-switching and production of IgE antibodies by activated B cells[7, 8, 10]. Consequently, the binding of IgE antibodies to high-affinity IgE receptors (FcεRI) on mast cells and basophils cause their sensitization, and this leads to the subsequent effector phase. The effector phase of type I hypersensitivity starts with cross-linking of the antigen and IgE antibodies bound to FcεRI on mast cells and basophils[10, 11]. The cross-linking triggers the activation of mast cells and basophils leading to their degranulation. During degranulation, inflammatory mediators in granules, such as histamine, leukotrienes, and cytokines, are rapidly released, which induce characteristic hypersensitivity symptoms, such as itching, sneezing, nasal congestion, wheezing, dermatitis, or gastrointestinal symptoms[7, 8, 11, 12]. When re-exposure to the allergen occurs, the type I hypersensitivity response is immediate[8].

Although the general progression of type I hypersensitivity reaction is well known, the underlying mechanisms at the transcript level remains understudied. To understand the complex transcriptomic mechanisms underscoring patients with type I hypersensitivity symptoms, we investigated the blood transcriptome of adult participants from Central European Longitudinal Study of Parents and Children: Young Adults (CELSPAC: YA) cohort study. We performed differential gene expression analysis (DGEA) and compared transcriptomic profiles of participants with allergic reaction to population without any of allergic triggers or symptoms. By analysing differences in gene expression and identifying key biomarkers, we aim to better understand how these conditions develop and potentially predict their future progression. The analysis of the blood transcriptome not only serves as a non-invasive and accessible source of information, but also offers a comprehensive

view of the systemic changes occurring in response to antigens. Such knowledge can pave the way for more targeted and effective therapeutic interventions, leading to improved patient outcomes and ultimately, better management of type I hypersensitivity.

Materials and methods

Study population and sample collection

The data analysed in this study is derived from the CELSPAC: YA cohort study (ethical approval no. ELSPAC/EK/2/2019), a follow-up study of European Longitudinal Study of Pregnancy and Childhood (ELSPAC-CZ) performed in Czech Republic (for more detailed description of ELSPAC-CZ, see Piler et al. (2017)[13]). Participants underwent blood sampling and completed comprehensive questionnaires. The questionnaire data contain information about participants such as their age, BMI, education, smoking status, alcohol consumption, and health outcome. The health outcome information includes information on immune-related diseases, such as atopic dermatitis, asthma, allergies to food, drugs, insects and pollen, dust, or mite, contact dermatitis, celiac disease, psoriasis, or lupus. The detailed information of CELSPAC: YA cohort is provided in our recent study by Rudzanova et al. (2023)[14].

All participants with non-allergic triggers or symptoms (e.g. contact dermatitis) were excluded from the analysis to avoid coincidence with inflammation other than type I hypersensitivity. To analyse the underlying mechanisms of type I hypersensitivity, we formed 3 test groups: 1) subjects with allergy: All participants who answered "yes" to the question "Have you ever been diagnosed with an allergy (pollen, dust mite, food, insects), asthma or atopic dermatitis by a doctor? " in a questionnaire; 2) Subjects with allergy without asthma and atopic dermatitis: All whose answered "yes" to the questionnaire question "Have you ever been diagnosed by a doctor with an allergy (pollen, dust mite, food, insect)" and simultaneously answered "no" to the question "Have you ever been diagnosed by a doctor with asthma or atopic dermatitis". 3) Subjects with allergic asthma and atopic dermatitis: All those who answered "yes" to the question "Have you ever been diagnosed by a doctor with asthma and atopic dermatitis at the same time?". The questionnaires were completed under the supervision of health professionals.

The collected whole blood samples (9 mL) were immediately centrifuged, and the buffy coat fraction (i.e., white blood cell fraction) was separated by Ficoll-Paque to isolate peripheral blood mononuclear cells (PBMC). The PBMC fraction was suspended in RNAprotect Cell Reagent and frozen (-80 °C) in 300 µL aliquots containing ˜13 million cells until use for analysis. RNA was then extracted from PBMC in RNAprotect Cell Reagent with a Zymo Research: Quick-RNA Whole Blood (R1201) extraction kit according to manufacturer´s instructions. Quality parameters like concentration, purity (Nanodrop, Thermo Fisher Scientific) and integrity (Agilent 5200 Fragment Analyzer system) of extracted RNA were determined. For the library preparation and sequencing, 1 µg of high-quality RNA per sample was used. The mean RNA Integrity Number (RIN) for samples was 9.0 (min - max: 7.3 – 10.0).

Library preparation and Sequencing

Genome-wide analysis of gene expression was conducted using the Next Generation Sequencing (NGS) platform with the QuantSeq library preparation step. cDNA libraries for each sample (RNA) were generated from 1 µg of total RNA using the Quantseq 3' mRNA-Seq Library Prep kit for Illumina (Lexogen) following the manufacturer's instructions. QuantSeq generates strand-specific NGS libraries close to the 3' end of poly-A RNA[15]. Standard external barcodes were ligated to allow for multiplex sequencing. After PCR amplification, the libraries were size-selected with Agencourt AMPure XP magnetic beads (Beckman Coulter). Libraries were quantified by Qubit (Life Technologies), and their size (250 bp) was determined using an Agilent 2100 Bioanalyzer. Libraries were sequenced (Illumina NovaSeq platform) and quality checked (110 bp single read) to obtain min. 20–25 million reads per sample. Following this, NGS data were demultiplexed.

Data preprocessing

After sample demultiplexing, UMI tags (6 bp) were removed from all reads using umi_tools (1.0.0). Afterwards, TruSeq adapters and low quality 3'-ends of reads (treshold: 13) were trimmed using bbmap (38.42).

Short reads (length < 30 bp) and reads with low quality (treshold: 24) were filtered out. Samples were mapped against reference human genome (Genome Reference Consortium Human Build 38) using STAR (2.7.7a) and deduplicated using umi_tools (1.0.0). Transcript features were summarized using htseq-count (0.11.1). During data pre-processing, quality of samples was continuously controlled using fastqc (0.11.5), qualimap (11_12-16) and multiqc (1.8, 1.12).

Data processing and statistical analysis were performed using R programming software (version 4.2.2)[16]. Only samples with allergies to food, insect, pollen, dust, or mite were included in the analysis; and participants without any other immune-related diseases (i.e., celiac disease, psoriasis, or lupus) were assigned as a control group. Samples with any missing data were filtered out. Genes with at least 5 CPM (counts per million) in at least 20% of samples were kept in the data set. The data was normalized using TMM (trimmed mean of M values) normalization and transformed to continuous log2 scale using limma-voom[17, 18]. Batch effects were accounted for using principal component analysis (PCA) plots and by correlation of principal components with potential confounders. Surrogate variable analysis was performed to the data and the first 10 surrogate variables were used to adjust the unknown cell blood composition[19, 20, 21].

Statistical analysis

Differentially expressed genes were identified using limma lmFit model and p-values were corrected for multiple testing using Benjamini-Hochberg false discovery rate (FDR)[22]. The model was adjusted for biological, socio-economic, and technical covariates (sex, age, BMI, education, smoking status, alcohol consumption and library preparation batch). Genes were annotated using GeneCard22, DAVID and GO databases[23, 24, 25]. Functional analysis, namely Gene Set Enrichment Analysis (GSEA) and Sub-Network Enrichment Analysis (SNEA) was performed using Pathway Studio (version 12.0)[26].

Results

Study population

The final dataset included 218 participants from CELSPAC: YA cohort study between 20–37 years old with an average BMI of 23.41. Overall, 114 participants had at least one allergic manifestation and 104 participants did not suffer from any of allergic triggers or symptoms. The detailed description of the population is presented in Table 1.

Table 1 : Description of study population participants (subset derived from CELSPAC: YA cohort).

Study population	**Study population**	**Total**	**Men**	**Women**
N of participants	N of participants	218	115 (52.7%)	103 (47.3%)
Age[1] (years)	Age[1] (years)	27 (20–37)	27 (26–37)	27 (20–32)
BMI[1]	BMI[1]	23.5 (17.6–36.5)	23.5 (19.2–36.5)	22.9 (17.6–30
Smoking	No	155 (71.1%)	83 (72.2%)	72 (69.9%)
	Yes	63 (28.9%)	32 (27.8%)	31 (30.1%)
Alcohol consumption	Low ([?] 1 X a week)	116 (53.2%)	55 (47.8%)	61 (59.2%)
	High (> 1 X a week)	102 (46.8%)	60 (52.2%)	42 (40.8%)
Education	Primary and high school	49 (22.5%)	29 (25.2%)	20 (19.4%)
	University	169 (77.5%)	86 (74.8%)	83 (80.6%)
Population	With no allergic symptoms	104 (47.7%)	52 (45.2%)	52 (50.5%)
	With allergy symptoms	114 (52.3%)	63 (54.8%)	51 (49.5%)
Triggers	Food allergy	16 (7.3%)	4 (3.5%)	12 (11.7%)
	Insect allergy	12 (5.5%)	2 (1.7%)	10 (9.7%)
	Pollen, dust and mite allergy	87 (39.9%)	53 (46.1%)	34 (33.0%)
Symptoms	Allergic asthma	30 (13.8%)	15 (13.0%)	15 (14.6%)
	Atopic dermatitis	28 (12.8%)	14 (12.2%)	14 (13.6%)
	Other symptoms	56 (27.9%)	34 (29.6)	22 (21.4)

Study population	Study population	Total	Men	Women
Tested groups	Subjects with allergy	114 (52.3%)	63 (55.8%)	51 (49.5%)
	Subjects with allergy without asthma and AD[2]	65 (29.8%)	37 (32.2%)	28 (27.2%)
	Subjects with allergic asthma and AD	9 (4.1%)	3 (2.6%)	6 (5.8%)

[1]median (min–max)[2]AD – atopic dermatitis

Differential gene expression analysis

We observed 165 differentially expressed gene transcripts (FDR < 0.2) in group of participants with allergic asthma and atopic dermatitis (AD), and 50 of these transcripts were up-regulated , and 115 down-regulated in relation to the control group (Table 1, Supplementary Material 1: Figure S1). The number of up- and down-regulated genes at different FDR levels are shown in Table 2. Additionally, other 2083 transcripts (p value < 0.05) have been found altered across all tested groups (subjects with allergy, allergy without asthma and AD, and subjects with allergic asthma and AD), from which 254 were identified to be directly involved in immune pathways using GO database with 7 genes commonly shared in all groups (Figure 1 and 2, Supplementary Material 2).

Table 2 . Summary of differentially expressed genes in subjects with allergic asthma and AD at different FDR levels.

Subjects with allergic asthma and AD	N of genes < FDR 5 %	N of genes < FDR 10 %	N of genes < FDR 20 %
all	5	22	165
up-regulated	0	9	50
down-regulated	5	13	115

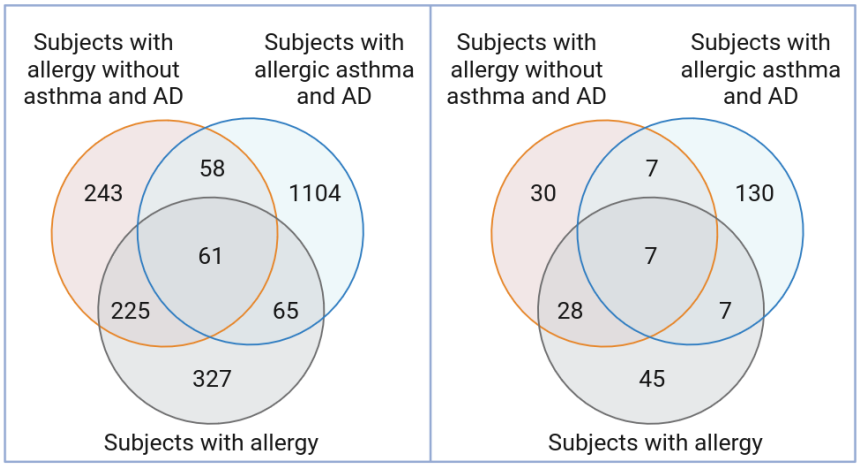

Figure 1 . Altered transcripts in each experimental group (subjects with allergy, subjects with allergy without asthma and AD, and subjects with allergic asthma and AD) in comparison to control group. Left venn diagram: all altered transcripts (p value < 0.05); right venn diagram: altered transcripts related with immune processes identified by GO database. Overlapped genes between groups (N=7) are shown in Figure 2 in detail.

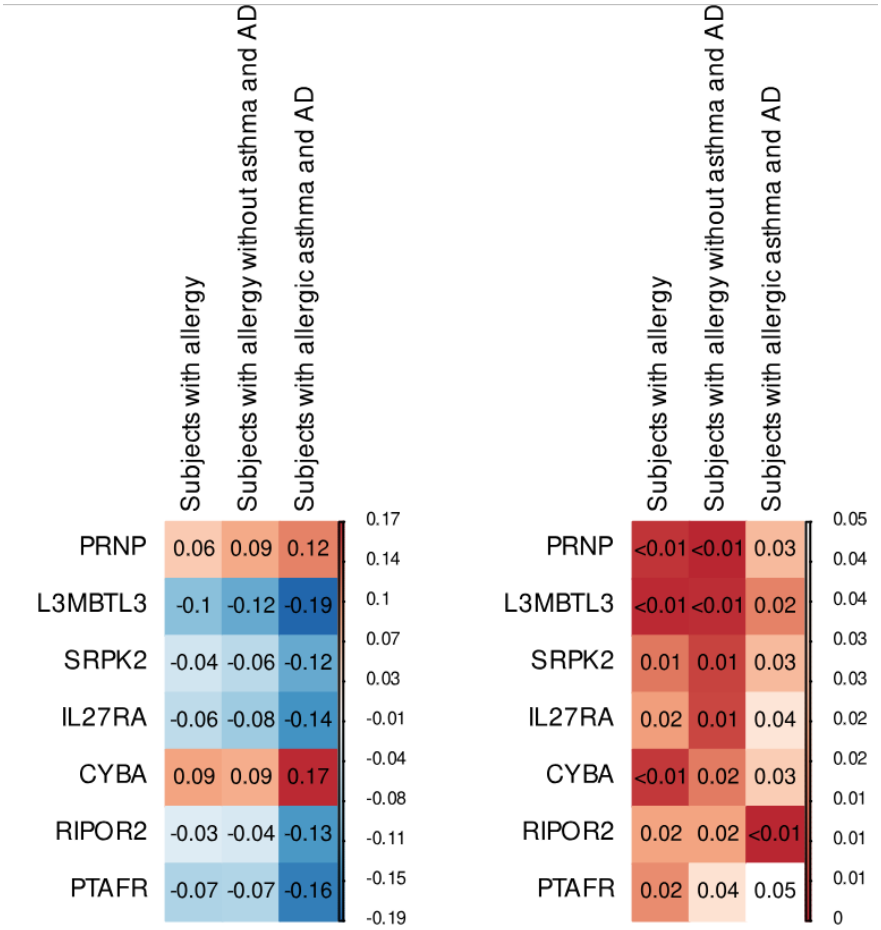

Figure 2 . Altered transcripts involved in immune processes identified by GO, across all tested groups (subjects with allergy, subjects with allergy without asthma and AD, and subjects with allergic asthma and AD) in comparison to control group. Individual cells show log2 fold changes (left panel), and p-values (right panel).

Functional analysis

Using GSEA, we observed 60 altered entities (including biological processes, cell processes, biomarkers, diseases, metabolic pathways, pathological processes, and signal processing) in participants with allergy, 186 entities in participants with allergic asthma and AD, and 115 entities in participants with allergy without asthma and AD (Supplementary Material 3). Enriched entities for each of these categories are catalogued into five main clusters: immunoglobulins; interleukins; CD molecules; mast cells; chemokines; and receptor tyrosine kinases (Table 3). Other significant pathways related mainly to tumor necrosis factors (TNFs) and TNF receptors (TNFRs), toll-like receptors (TLRs), or chemokines are presented in Supplementary Material 3.

Further, applying SNEA on cell processes, we identified 484 altered cell processes in participants with allergy, 288 cell processes in participants with allergic asthma and AD, and 514 cell processes in participants with allergy without asthma and AD (Supplementary Material 4). Chosen cCell processes altered in at least two of the three tested groups with corresponding p-values are depicted in Table 4. These processes are clustered into five main domains, namely APCs; Th cells; Th2 cells, B cell activation and IgE production; mast cells and eosinophils; and T cytotoxic cells. Among the processes, APC survival, eosinophil chemotaxis, and

CD8+ T-cell lymphocyte anergy have been found significantly altered in all three tested groups (Figure 3).

Finally, applying SNEA on clinical parameters (i.e., identification of parameters that would be impacted), we observed 297 clinical parameters in subjects with allergy, 136 clinical parameters in participants with allergic asthma and AD, and 304 clinical parameters in participants with allergy without asthma and AD. The altered clinical parameters are dominantly associated with pulmonary and skin problems, but also include networks related to mast cells, Th cells, eosinophils, or parasite infection (Supplementary Material 5).

Table 3 . Deregulated pathways identified in participants with allergy, subjects with allergy without asthma and AD, and subjects with allergic asthma and AD in comparison to control group using GSEA.

	Enriched entity name	**Hit type**
Immunoglobulins	IgE Induces Airway Smooth Muscle Cell Proliferation	Disease
	V(D)J Recombination Activation	Biological process
	IgE Receptors -> Targets in Lymphoid System and Blood	Biomarkers
Interleukins	IL3 Expression Targets	Biomarkers
	IL17F Signaling in Bronchial Epithelial Cell in Asthma	Disease
	IL4 Expression Targets	Biomarkers
	IL10/STAT3 Signaling in M2 Macrophage and Retinal Angiogenesis	Disease
	IL17 Signaling in Psoriasis	Disease
CD molecules	CD80 -> STAT Expression Targets	Biomarkers
	CD86 -> STAT Expression Targets	Biomarkers
	CD80 -> NF-kB Expression Targets	Biomarkers
	CD38/CD19 -> JUN/FOS/NF-kB Signaling in B-cell Proliferation	Signal processing
	CD38 -> cADPR/Calcium Signaling	Signal processing
	CD157 -> cADPR/Calcium Signaling	Signal processing
Mast cells	Mast-Cells Activation in Atopic Dermatitis	Disease
	Mast-Cell Activation without Degranulation through CRHR1 Signaling	Biological process
Receptor tyrosine kinases	VEGFA -> FOXO3A Expression Targets	Biomarkers
	VEGFR -> FOXO3A Signaling	Signalling process
	TGFA -> FOXO3A Expression Targets	Biomarkers
	HBEGF -> FOXO3A Expression Target	Biomarkers
	EREG -> FOXO3A Expression Target	Biomarkers

Table 4. Enriched cell processes identified in at least two out of three tested groups (subjects with allergy, subjects with allergy without asthma and AD, and subjects with allergic asthma and AD) in comparison to control group using SNEA.

	Cell process name	**Subjects with allergy (p-v**
APCs [1]	antigen-presenting cell survival	0.05
	antigen-presenting cell function	0.03
	antigen-presenting cell phenotype	0.01
Th cells [2]	helper T-cell ratio	0.03
	CD4+ T-cell death	<0.01
	CD4+ T-cell lymphocyte anergy	0.01
Th2 cells, B cell activation and IgE[3] production	Th2 cell population	>0.05
	Th2 cell death	0.02
	germinal center B-cell apoptosis	0.01
	germinal center B-cell phenotype	0.01
	marginal zone B-cell function	0.03

	Cell process name	Subjects with allergy (p-v
	B-cell transendothelial migration	0.05
Mast cells, eosinophils	mast cell migration	0.01
	eosinophil chemotaxis	<0.01
	eosinophil survival	0.03
T cytotoxic cells	CD8+ T-cell lymphocyte anergy	0.04
	CD8+ T-cell migration	0.03
	T-cell cytotoxicity	0.03
	CD8+ T-cell adhesion	0.03
	CD8+ memory T-cell formation	0.03
	CD8+ T-cell cytotoxicity	>0.05
	CD8+ T-cell division	<0.01

[1] APCs (Antigen presenting cells),

[2] Th cells (T helper cells),[3] IgE (Immunoglobulin E)

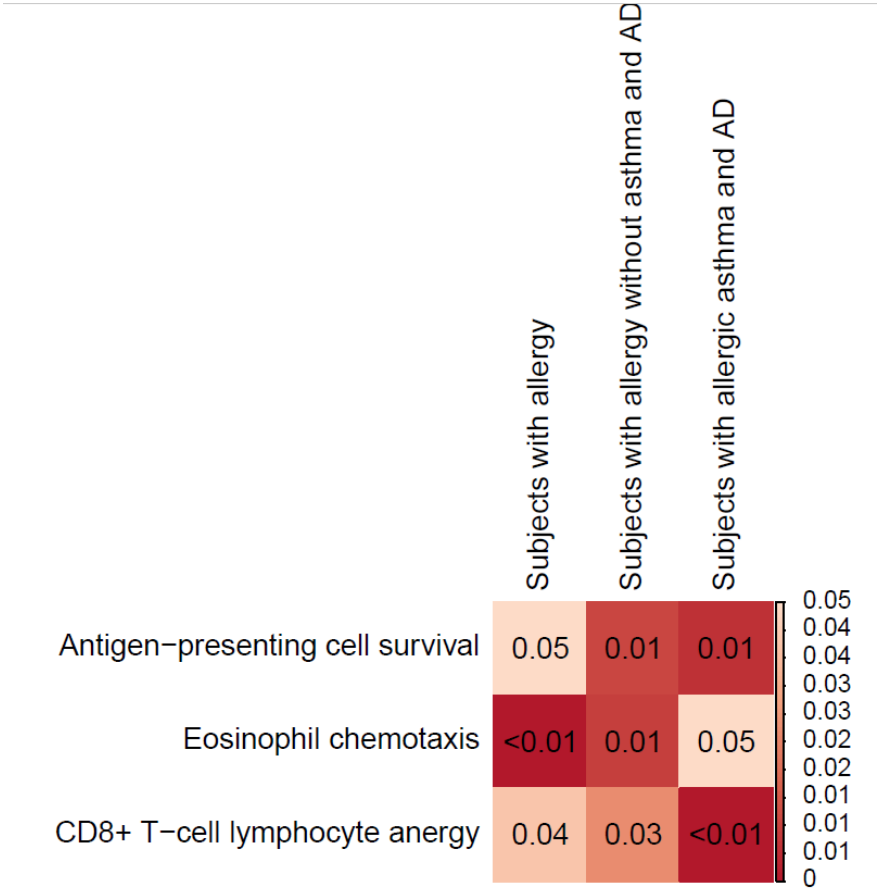

Figure 3 . Significantly enriched cell processes identified in all three tested groups (subjects with allergy, subjects with allergy without asthma and AD, and subjects with allergic asthma and AD) in comparison to control group using SNEA. Individual cells present a p value of selected pathways.

Discussion

As the type I hypersensitivity can manifest as atopic dermatitis, conjunctivitis, rhinitis, rhinoconjuctivitis, or allergic asthma, we compared gene transcripts common for participants with these conditions (between allergy group, allergy without asthma and AD group, and allergic asthma and AD group) and confirmed that there are shared genes among these groups. There were 61 altered transcripts identified commonly for all three tested groups (Figure 1, Supplementary Material 1: Figure S2). Among them, 7 genes were found to be involved in immune-related pathways in GO database (Figure 1 and 2). These seven genes have established roles in immunity; however, it is important to emphasize that these genes often have multiple functions beyond immunity, and their roles can be complex and context-dependent. With respect to immune processes, this cluster of genes is associated with T cell activation, differentiation, and migration (PRNP, IL27RA, RIPOR2)[27 ,28 ,29], immune cell regulation (L3MBTL3, IL27RA)[30, 31], and is linked to the severity of immune mediated diseases such as asthma and dermatitis (IL27RA) and anaphylactic response (PTAFR)[28, 32]. When assessing gene function by Gene Ontology (GO)[33], the top 15 biological processes (FDR <0.05) indicate pro-inflammatory machinery as the biological processes include T cell migration, leukocyte activation and production of pro-inflammatory signals IL-6 and IL-17.

By employing both GSEA and SNEA on a whole genome profile, we identified cell processes and other functional entities enriched by estimated gene profiles related with type I hypersensitivity. During both the sensitization phase and effector phase, many signaling molecules are employed, e.g., interleukins, immunoglobulins, or CD molecules, whose signaling pathways were revealed by GSEA analysis (Table 3). Cell processes identified by SNEA were shared among at least two of the three groups. These shared pathways were involved in APC function, Th cell signaling, including Th2 cell's function leading to IgE production. These processes are crucial in the sensitization phase of the type I hypersensitivity. Further, mast cells, basophils, and eosinophils, whose signaling pathways were also enriched in our analyses, are important for the process of atopy, namely for the effector phase.

SNEA identified various cell processes belonging to cell groups such as APCs, Th cells, and B cells (Table 4), that are involved in sensitization phase. The sensitization phase starts by reaction of naive T cells to an antigen (allergen) by APCs[8]. At this stage, APCs, namely dendritic cells capture and process the antigen and, through major histocompatibility complex (MHC) trigger T-cell receptors (TCRs) presented on naive T cells[34]. Engagement of MHCs class II with TCRs activates the proliferation of naive T cells and promotes inflammatory signaling cascades, resulting in differentiation of T cells into Th cells and regulatory T cells (Tregs)[35].

GSEA revealed roles for many immune-related molecules, including CD80, CD86, IL-3 and IL-4. Specifically, CD80 and CD86 are membrane proteins expressed by APCs during sensitization/regulation. Engaging with CD28 receptor, CD80 and CD86 act as co-stimulators in APC:T cell interaction and enhance TCR signaling[36, 37]. TCRs and CD28 downstream signaling is associated with activation of NF-ϰB transcription factor, one of crucial regulators of IL-4 expression, that plays an essential role in Th2 cell differentiation[38]. Beside IL-4, IL-25, IL-33, and Thymic Stromal Lymphopoietin (TSLP) cytokines, IL-3 has also been found to be involved in Th2 cell differentiation. While IL-4 directly drives Th2 cell differentiation, IL-3 regulates the process indirectly by enhancing the production of IL-4[39, 40].

In addition to ACPs, Th cells, and B cells, SNEA identified enriched cell processes related with Th2 cells and germinal centers (GC). B cells interact with Th cells. Th2 cells play a crucial role in the entire inflammatory process. Th2 cells stimulate activation of B cells via production of IL-4 and IL-13. The interaction induces B cell differentiation and isotype switching of Ig heavy chain, and therefore, differentiated B cells migrate into secondary lymphoid organs where they induce GC formations. In germinal centers, B cells undergo somatic hypermutation and proliferation, and start to produce IgE antibodies[41, 42, 43, 44]. Consequently, IgE antibodies bind to IgE receptors presented on mast cells and basophils whereby trigger the effector phase[8, 45, 46].

Other enriched entities provided by GSEA include receptor tyrosine kinases, specifically VEGFA, VEGFR,

EREG, TGFA and HBEGF, in conjunction with the forkhead box O3a (FOXO3A). In addition to these expression targets, enriched entities related to IL-17 were observed in our study. Both FOXO3A and IL-17 are involved in the IgE production network. Moreover, Tgfb expression has an impact on many cell processes related with T cells and B cells (for more details of the genes regulating these pathways, see Overlapping genes in Supplementary Material 4). Production of IgE antibodies is indirectly controlled by Tregs. Tregs, together with regulatory B cells (Bregs), act as an inhibitor of production of IgE antibodies through regulation of transforming growth factor β (TGFβ) and IL-10. Therefore, this cytokine (TGFβ) plays a dominant role in IgE regulation during inflammation. TGFβ is known as an inhibitor of B cell differentiation and proliferation and can induce Ig isotype switching into IgA antibodies[47].

Simultaneously, TGFβ induces conversion of CD4+ T cells to Tregs under regulation of FOXO3A protein[48]. FOXO3A protein was examined as a suppressor of both pro-inflammatory cytokines, such as IL-4 and IL-13, and anti-inflammatory cytokines, namely IL-10[48, 49, 50]. Another member of the FOXO family, the FOXO1 transcription factor, negatively regulates the generation of Th17 cells and, consequently, the secretion of IL-17, which also promotes IgE production[48, 51]. Although, the regulatory network involving FOXO transcription factors underscores its role in regulating cytokine expression, which in turn affects IgE production and contributes to immune homeostasis, the impact of the observed tyrosine kinases on the FOXO3A transcription factor remains understudied.

Lastly, both GSEA and SNEA identified enriched processes related to mast cells that are involved in effector phase of type I hypersensitivity reaction. The effector phase starts by degranulation of activated basophils and mast cells[8, 45]. Activated mast cells secrete their mediators, namely cytokines (IL-4, IL-5, IL-13, and TNFs), leukotrienes, and during degranulation, histamine, heparins, tryptases or chymases. The degranulated mediators provoke eosinophil adhesion, accumulation, and migration, and neutrophil accumulation52. The cytokines produced by mast cells, especially TNFs, promote the mast cell development and migration that support the recruitment of eosinophils, neutrophils, and mast cells (themselves), and overall allergic reaction[53, 54]. Moreover, IL-3 stimulates TNF production that is crucial for mast cell development[55].

In conclusion, expression profiling blood in participants with allergic manifestations including allergic asthma, atopic dermatitis and other symptoms provided insights into immune-related pathways. Crucial processes involved antigen presentation, Th2 cell signalling leading to IgE production, and mast cell, basophil, and eosinophil involvement in the effector phase. Moreover, the 7 observed common genes may play an important role in immune processes related to type I hypersensitivity and may be candidates for new potential biomarkers. However, these genes should be confirmed by further clinical or focused *in vivo*studies. Understanding these mechanisms is vital for targeted therapies and improved management of atopic diseases.

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