Sputum transcriptome analysis of co-regulated genes related to arachidonic acid metabolism in N-ERD

Lucyna Mastalerz[1], Radosław Kacorzyk[1], Bogdan Jakiela[1], Adam Ćmiel[2], and Marek Sanak[1]

[1]Uniwersytet Jagiellonski w Krakowie Wydzial Lekarski
[2]Department of Applied Mathematics AGH University of Science and Technology Krakow Poland

July 4, 2022

Title: **Sputum transcriptome analysis of co-regulated genes related to arachidonic acid metabolism in N-ERD**

Short title: **Cluster analysis of sputum transcriptome in N-ERD**

To the Editor

Nonsteroidal anti-inflammatory drug–exacerbated respiratory disease (N-ERD) is a disease characterized by alteration of arachidonic acid (AA) metabolism. The disease has variable pattern of lipid biomarkers and bronchial inflammation.[1-3] How this heterogeneity is reflected by expression of AA-related genes before and after aspirin therapy remains unknown. We collected induced sputum from 27 participants before aspirin desensitization and after 52-week high-dose aspirin therapy (650 mg/d).[2] Abundance of 87 genes mRNAs, including 15 AA-related ones was assessed in a total RNA from the sputum cells. By a method of unsupervised cluster analysis the genes expression was used to find co-regulated transcripts, based on similar expression patterns before and after aspirin therapy. We hypothesized that clustering of co-expressed AA-related genes might elucidate the genetic heterogeneity of N-ERD at baseline. Thereafter, we determined effect of a high-dose aspirin therapy on the aggregation of co-regulated AA-related genes into the clusters. Gene expression was assessed using a quantitative real-time polymerase chain reaction. The cluster analysis was performed using distances calculated on correlations between the expression of individual genes. Similar expression patterns were classified together as co-regulated genes, thus in separate clusters mRNA expression was independent from one another. The study group characteristics, data collection, and statistics are described in Supplementary Material (**Methods, Table E1, Fig.E1**).

At baseline, five expression clusters were identified (**Fig.1, Supplementary material Fig.E2**). Each cluster contained genes encoding AA metabolic enzymes and/or receptors for eicosanoids. The first cluster included COX-2–pathway co-regulated genes of AA metabolism (*PTGS2* and *PTGES*). Cluster 2 included *PTGER2* encoding the EP2 receptor for PGE_2. Absence of genetic co-expression for PGE_2 biosynthesis and the type-2 receptor indicated their transcriptional independence. The best grouped Cluster 3 consisted of lipoxygenases *ALOX5* , *ALOX12* ,*ALOX15, HPGDS* encoding hematopoietic prostaglandins D synthase and its inactivating hydroxyprostaglandin dehydrogenase *HPGD* . Interestingly, we recently reported that high sputum *HPGD*expression can predict better response to a high-dose aspirin therapy in N-ERD.[2] Cluster 4 included genes encoding receptors for eicosanoids *PTGDR2, CYSLTR1, CYSLTR2* , and *PTER4* . The fifth cluster included COX-1 mRNA co-expressed with *LTC4S* and*ALOX15* transcripts (**Fig.2**).

After 52 weeks high-dose aspirin therapy, only two clusters were identified: cluster 1 containing, *LTC4S* and *ALOX15* ; and cluster 2 containing the remaining 13 genes of AA-related group (**Fig.2** , **Supplementary**

material Fig.E3-E4).

Grouping *LTC4S* and*ALOX15* together as co-regulated was associated with clinical improvement.[2] This supports that cells expressing synthase for cysteinyl leukotrienes and 15-HETE(S) are mandatory for beneficial response to aspirin therapy in N-ERD. N-ERD patients with symptom improvement on aspirin had higher plasma 15-HETE levels at baseline than those with symptom worsening.[4] Moreover, high sputum *HPGD* expression may predict benefits from subsequent aspirin therapy.[2] Interestingly, HPGD is required for 15-oxo-eicosatetraenoic acid (15-Oxo-ETE) synthesis, and 15-Oxo-ETE levels were markedly higher in N-ERD. Thus, epithelial and mast cell interactions leading to 15-Oxo-ETE synthesis could contribute to AA metabolism alterations via the 15-LOX pathway.[5] We suggest that this can be prevented by long-term high-dose aspirin therapy in patients with N-ERD. In a physiological sputum macrophages are predominant cells. In N-ERD macrophages seems to be reprogrammed to produce proinflammatory mediators.[6] This involves epigenetic DNA changes leading to enhanced fatty acids metabolism and release of lipid mediators. Presented clusters after high dose aspirin therapy may reflect reversal of these alterations. In conclusion, this is the first study presenting an aggregation of co-regulated genes related to AA metabolism and signaling in N-ERD patients before and after aspirin therapy. On aspirin, almost all AA-related genes became grouped together, indicating aspirin's ability to regulate the expression of these genes in N-ERD.

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Authors : Lucyna Mastalerz[a], Radosław Kacorzyk[a], Bogdan Jakieła[a], Adam Ćmiel[b], Marek Sanak[a]

[a]II Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland

[b]Department of Applied Mathematics, AGH University of Science and Technology, Krakow, Poland

Address for correspondence: Prof. Marek Sanak

Department of Internal Medicine, Jagiellonian University Medical College,

ul. Skawińska 8, 31-066 Krakow, Poland

E-mail: marek.sanak@uj.edu.pl

Telephone number: 12 430 52 66

Word count (body): 599

Number of tables: 0

Number of figures: 2

Conflict of Interest: None declared.

Funding information: This work was supported by the National Science Centre, Poland (grant number UMO-2015/19/B/NZ5/00096; NCN).

ORCiD

Lucyna Mastalerz:*0000-0002-8994-0036*

Marek Sanak:*0000-0001-7635-8103*

Author's contribution statement:

1. Lucyna Mastalerz: **50%**
2. Designed the study, conceived, and designed the analysis, wrote the paper.
3. Radosław Kacorzyk: **10%**
4. Collected the data, prepared graphics.
5. Bogdan Jakieła: **10%**
6. Contributed to the interpretation of the results.
7. Adam Ćmiel: **10%**
8. Performed statistical analysis, prepared graphics.
9. Marek Sanak: **20%**
10. Contributed to the interpretation of the results, supervised the preparation of the manuscript.

All authors provided critical feedback and helped shape the research, analysis, and manuscript.

Figure 1. Dendrogram showing a hierarchical cluster analysis of 87 genes in sputum cells from patients with NSAID-exacerbated respiratory disease at baseline. Cluster analysis was performed using the Ward method on Spearman's correlation. Each color represents different cluster: blue – cluster number 1, yellow – cluster number 2, green – cluster number 3, red – cluster number 4, and violet – number 5. Genes related to arachidonic acid pathways are in bold.

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Figure 2. Clusters at baseline and at 52 weeks of high-dose aspirin therapy