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Transcriptomic and methylomic features in asthmatic and non-asthmatic twins

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To the editor,

Asthma is the most prevalent chronic lung inflammatory disorder characterized by reversible airflow obstruction, affecting 358 million people worldwide¹, with aggravating factors like obesity, attention-deficit/hyperactivity disorder, socioeconomic status like poor healthcare affordability and facility, smoking and alcohol intake². RNA sequencing (RNA-seq) in atopic asthma³, childhood asthma⁴, and adult-onset severe asthma⁵ have improved our understanding of cellular and molecular pathways involved. Genome-wide DNA methylation studies investigating 5-methylcytosine in CpG sites have linked methylation in lung cells with asthma endotypes and genetic risk⁶. Studies focusing on integration of genomics and interactomes have also been performed in asthma⁷. However, this multifaceted asthma phenotype together with genetic heterogeneity and environmental influences makes it challenging to fully understand the features that trigger and influence asthma development and progression. Towards addressing these challenges, we investigated transcriptomic and methylomic data in a twin cohort of asthmatic and non-asthmatic individuals.

In this exploratory study, the individuals were identified as asthmatics (GINA score ≥ 1) according to the Global Strategy for Asthma Management and Prevention guidelines (<http://>

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Conflict of interest:

ESB and FB are employees of Janssen Research and Development LLC, PA USA. RNB is an employee of Genentech, CA USA. CM is an employee of BioProcessing Solutions, NJ USA. KCN has received funding from, is currently funded by, or cofounded the following: the NIH, Food Allergy Research & Education, End Allergies Together, Before Brands, Alladapt Immunotherapeutics, Adare Pharmaceuticals, AstraZeneca, Novartis, Genentech, Astellas, DBV Technologies, ForTra, Aimmune Therapeutics, Regeneron, Nestle, Sanofi, ImmuneWorks, Cour, and the Environmental Protection Agency. All other authors declare that they have no relevant conflicts of interest.

Ethical statement:

All the biological samples were collected and studied under Stanford approved protocol (IRB 8629) in full compliance with CFR and GCP guidelines.

ginasthma.org). Participants with any viral or bacterial infections or immune disorder were excluded. All the participants with asthma were on a low dose of inhaled corticosteroid and rescue inhaler (albuterol) only. We collected PBMC from 16 female monozygotic twin-pairs (5 concordant asthmatic-pairs; 8 concordant non-asthmatic-pairs and 3 pairs discordant for asthma) among which 13 individuals were asthmatic (GINA score 1 n=4; 2 n=4; 3 n=5, Table 1). The transcriptomic RNA-seq was done on all 16 twin-pairs and the methylomic whole-genome bisulfite sequencing was done on 8 twin-pairs (3 concordant asthmatic-pairs; 5 concordant non-asthmatic-pairs) wherein 6 individuals were asthmatics (GINA score 1 n=1; 2 n=1; 3 n=4). Methods are detailed in supplementary Data S1.

Unsupervised hierarchical clustering of transcriptomic profiles shows 4/16 twin-pairs clustering together, and 2/8 twin-pairs cluster together in methylomic profiles. 2/3 twin-pairs discordant for asthma clustered together in transcriptomic profiles reflects similarity based on twinning (Figure 1A). The demographics age, BMI and weight are detailed in Table 1.

The transcriptomic analysis of total 1665 annotated genes (supplementary file) yielded 117 differentially expressed (46 up-regulated; 71 down-regulated, Table S1) genes in comparison to asthmatics over non-asthmatics (Figure 1B). The expression profiles of the top 10 differentially expressed genes are represented in Figure S2. The KEGG pathway enrichment analysis of these differentially expressed genes yielded pathways such as ribosome, glycosaminoglycan biosynthesis – chondroitin sulphate and SNARE interactions in vesicular transport to be significantly enriched (adj-pvalue < 0.05, supplementary Figure S1) which is linked with eosinophilic dysregulation in asthma⁸. Among 14562 CpGs identified by methylomics, 96 were found to be differentially methylated between asthmatics and non-asthmatics (Figure 1C, Table S2). Among the dysregulated features in our cohort, the genes *CTNNA3*, *SEMA3D*, *CDHR3*, *TGFB1*, *IRF3*, *CALM3*, *FCRL1*, *IFT22* and *IL4RA*, and the CpGs on *MUC20*, *SLC12A7*, *GSE1*, *DRAIC* and *SC5D* have been reported in asthma^{9,10}. The identification of functionally uncharacterized *ENSG00000231345* and *ENSG00000226143* genes in our cohort requires further investigations.

The integration of transcriptomic and methylomic significant features from the 8 twin-pairs with both RNA-seq and methyl-seq assays was done using regularized canonical correlation analysis (rCCA) to identify interactions between features. Among the total 13873 interactions, strong correlations (absolute $r > 0.7$; positive=813 and negative=2528, supplementary Figure S3) were noted. For visibility, only the top 100 interactions (positive=63, negative=37) are shown in Figure 1D. The expression of *CALM3* was found to be correlated with the CpG methylation status of *MUC20* ($r=-0.7421$) and *GSE1* ($r=-0.7240$), which were all previously reported to be asthma related¹⁰. Similarly, the expression of *IFT22* gene was found to be strongly correlated with the CpG methylation status of *DRAIC* ($r=0.8286$) in our cohort. The CpG methylation status of *ATXN2* ($r=-0.7188$) and *MADIL1* ($r=-0.7163$) were strongly correlated with *CALM3* and *MYL6* expression ($r=-0.7124$). The previously unreported *ZCCHC24* ($r=0.8554$) and *EVX1-AS* ($r=0.8424$) correlation with the CpG methylation status of *MUC20* requires further investigations in the context of asthma. The top 50 strongest correlated features from rCCA from our cohort are reported in Table S3.

The dysregulated features presented here reflect the differences between non-asthmatics and a range of asthmatics (mild to moderate) which is different from most prior studies that have focused on severe or acute asthma (GINA score 3+). In this study, the PBMC analyzed represents an overall disease rather than a cell-type specific phenotype, which needs to be considered while interpreting dissimilarities from previous studies. Although, monozygotic twins essentially share their genetic makeup and, if living together, also share much of their early life environmental exposure, we did not record information about twins living together. Twin-pairs discordant for asthma would be ideal for exploration of genetic vs environmental influence on asthma. But, with only 3 twin-pairs discordant for asthma (mild to moderate; GINA scores of 1 n=1; and 2 n=2) in our cohort, the sample size is too small to draw statistical conclusions. The inclusion of concordant twins as specific population but without sufficient number of discordant twin-pairs was not relevant for the current study. Hence, increasing the number of twin-pairs discordant for asthma is crucial for such future investigations. The analysis stratified by different GINA scores was not possible due to small sample size, hence only asthmatic (GINA score ≥ 1) and non-asthmatics were compared. Larger studies including male samples and sufficient number of participants with different GINA scores are needed to study specific asthma phenotypes which would greatly benefit the asthma specific signature identification and evaluation across asthma severities. Furthermore, the biological validation of identified differential features would strengthen our findings.

In conclusion, this exploratory study investigating transcriptomic and methylomic profiles in a twin cohort has enabled the identification of features of asthma vs non-asthmatics. While our specific cohort adds insight to our understanding of the phenotypes of mild to moderate asthma, the recognition of previously known *CALM3*, *DRAIC*, *IFT22*, *MUC20*, *SC5D* asthma features in our cohort supports our findings. Additionally, the multi-omic integration approach enables correlation-based identification of candidate features and their interactions not yet known in asthma disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References:

1. Burnett R, Chen H, Szyszkowicz M, et al. Global estimates of mortality associated with long-term exposure to outdoor fine particulate matter. *Proc Natl Acad Sci*. 2018;115(38):201803222. doi:10.1073/pnas.1803222115
2. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. *Lancet SEMINAR*. 2018;391(10122):7833–800.
3. Yick CY, Zwinderman AH, Kunst PW, et al. Gene expression profiling of laser microdissected airway smooth muscle tissue in asthma and atopy. *Allergy Eur J Allergy Clin Immunol*. 2014;69(9):1233–1240. doi:10.1111/all.12452

4. Yeh YL, Su MW, Chiang BL, Yang YH, Tsai CH, Lee YL. Genetic profiles of transcriptomic clusters of childhood asthma determine specific severe subtype. *Clin Exp Allergy*. 2018;48(9):1164–1172. doi:10.1111/cea.13175 [PubMed: 29758111]
5. Hekking PWP, Loza MJ, Pavlidis S, et al. Pathway discovery using transcriptomic profiles in adult-onset severe asthma. *J Allergy Clin Immunol*. 2018;141(4):1280–1290. doi:10.1016/j.jaci.2017.06.037 [PubMed: 28756296]
6. Nicodemus-Johnson J, Myers RA, Sakabe NJ, et al. DNA methylation in lung cells is associated with asthma endotypes and genetic risk. *JCI Insight*. 2016;1(20):e90151. [PubMed: 27942592]
7. Hwang S, Son SW, Kim SC, Kim YJ, Jeong H, Lee D. A protein interaction network associated with asthma. *J Theor Biol*. 2008;252(4):722–731. doi:10.1016/j.jtbi.2008.02.011 [PubMed: 18395227]
8. Adamko D, Odemuyiwa SO, Moqbel R. The eosinophil as a therapeutic target in asthma: Beginning of the end, or end of the beginning? *Curr Opin Pharmacol*. 2003. doi:10.1016/S1471-4892(03)00040-7
9. Park HW, Tantisira KG. Genetic Signatures of Asthma Exacerbation. *Allergy Asthma Immunol Res*. 2017 5;9(3):191–199. doi: 10.4168/aair.2017.9.3.191. [PubMed: 28293925]
10. Forno E, Wang T, Qi C, et al. DNA methylation in nasal epithelium, atopy, and atopic asthma in children: a genome-wide study. *Lancet Respir Med*. 2019;7(4):336–346. doi:10.1016/S2213-2600(18)30466-1 [PubMed: 30584054]

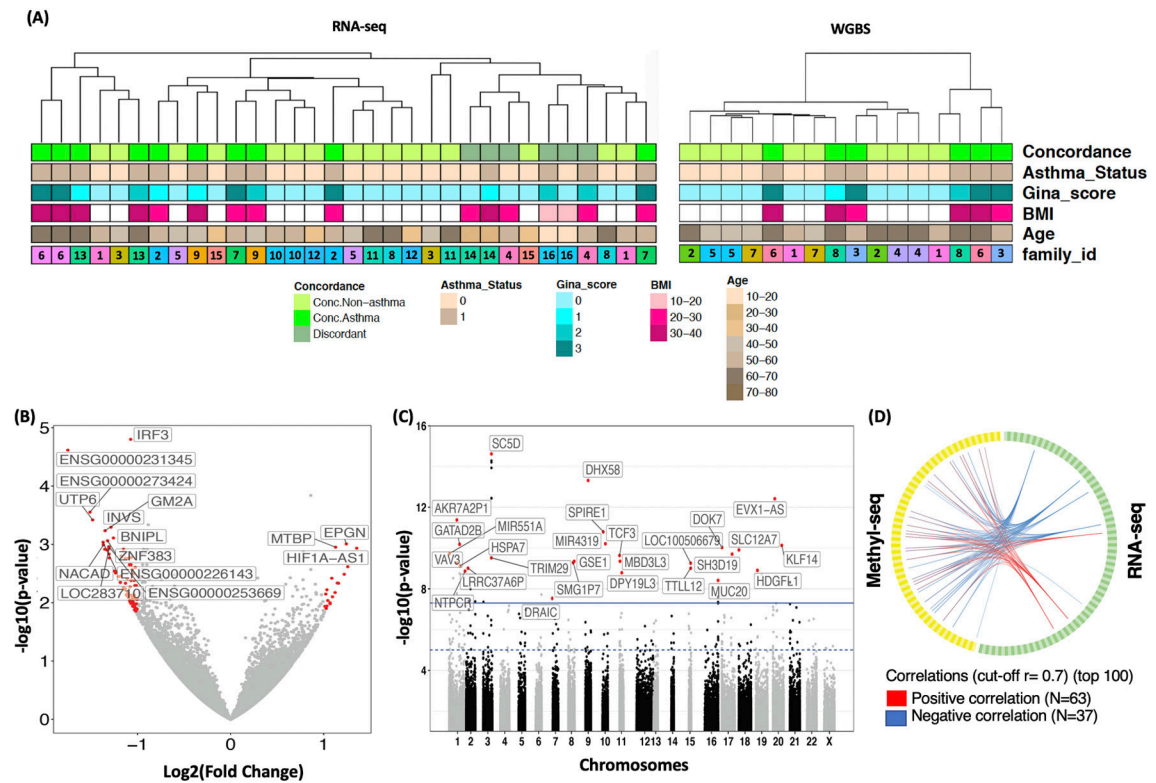


Figure 1.

(A) Unsupervised hierarchical clustering of RNA-seq and methyl-seq samples with complete linkage. (B) Volcano plot of differentially expressed genes (Grey = non-significant genes, Red = significant genes with absolute $\log_2(\text{Fold Change}) \geq 1$ and FDR-adjusted P -value < 0.01 , Top15 gene annotations are visualized). (C) Manhattan plot of CpGs across the chromosomes. (D) Circos plot showing the relation among all the features with strong Spearman's correlations (absolute $r > 0.7$; top 100 only) represented as links (red = positive; blue = negative). (Extended figure legends can be found in online Data S2).

Table 1:

Demographic information of participants

	Asthma Median [1st–3rd quartile] (N=13)	Non-asthma Median [1st–3rd quartile] (N=19)	P-value^{\$}
Age (y)	52 [46–62]	48 [31–58]	0.691
Weight (kg)	75.4 [66.70–93.40] (N=13)	62.6 [54.35–62.95] (N=3)	0.746
BMI (kg/m ²)	28.49 [22.25–32.31] (N=13)	24.72 [21.48–26.37] (N=3)	0.962
GINA score	1=4; 2=4, 3=5	0=19	0.019

^{\$}by generalized linear model with t-test.