Molecular clustering of genes related to the atopic syndrome: towards a more tailored approach and personalized medicine?

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ABSTRACT

Background

The atopic syndrome consists of heterogeneous manifestations, in which multiple associated genetic loci have recently been identified. It is hypothesized that immune dysregulation plays a role in the pathogenesis. In primary immunodeficiency diseases (PIDs), which are often monogenic immunodysregulation disorders, the atopic syndrome is a frequently occurring comorbidity. Based on the genetic defects in PIDs, novel gene/pathway-targeted therapies have been evaluated, which could be relevant in the atopic syndrome as well.

Objective

We aimed to define subclasses within the atopic syndrome based on the expression profiles of immune cell lineages of healthy mice.

Methods

Overlap between known atopy-related genes as described in the Human Gene Mutation Database and disease-causing genes of monogenic PIDs was evaluated. Clusters of atopy-related genes were based on the overlap in their co-expressed genes using the gene expression profiles of immune cell lineages of healthy mice from the Immunological Genome Project. We analyzed pathways involved in the atopic syndrome using Ingenuity Pathway Analysis.

Results

Twenty-two (5.3%) genes were overlapping between the atopy-related genes (n=160) and PID-related genes (n=278). We identified seven distinct clusters of atopy-related genes. Functional pathway analysis of all atopy-related genes showed relevance of T helper cell-mediated pathways.

Conclusion

This study shows a model to define clusters within the atopic syndrome based on gene expression profiles of immune cell lineages. Our results support the hypothesis that both genetic mechanisms and immune dysregulation play a role in the pathogenesis. It also opens up the possibility for novel therapeutic targets and a more tailored approach towards personalized medicine.

INTRODUCTION

Atopy is the genetic predilection to produce specific immunoglobulin (Ig) E following exposure to allergens. This predisposition results in the development of atopic dermatitis (AD), food allergy (FA), asthma, and allergic rhinitis (AR): the atopic syndrome.[1] The worldwide prevalence of these manifestations in children varies between 15-20%, 1-10%, 3-29%, and 9-15%, respectively, and in adults from 1-3%, 3-4%, 2-12%, and 7-42%, respectively.[2-6] Atopic manifestations share a common mechanism involving allergen-specific IgE, which triggers the release of inflammatory mediators, like histamine, in the skin, gastrointestinal tract, lungs and nose. The course of these manifestations over time is characterized by the atopic march, generally starting with AD in infancy and followed by FA, asthma, and AR later in childhood.[7] However, it is known that the atopic march not always follows the classic sequence and may occur at any age.[8,9] Furthermore, not all atopic patients will develop the complete spectrum of atopic manifestations.[7] Despite its heterogeneous presentation, patients with atopic manifestations are mostly uniformly treated with topical or systemic immunosuppressive agents and/or antihistamines resulting in varying therapeutic responses as well.[10-13]

Subgroups of the atopic phenotype, termed endotypes, are possibly responsible for the differences in disease manifestations and treatment responses. These endotypes are the result of variations in physiologic, biologic, immunologic and/or genetic mechanisms.[14] Various genetic loci associated with both inflammation and multiple atopic manifestations have been identified in recent years based on Genome-Wide Association Studies (GWAS), showing common genetic mechanisms involved in the atopic syndrome.[15-24] Nevertheless, the genetics of the atopic syndrome remain complicated for different reasons. For example, gene polymorphisms in different genes might cause the atopic syndrome independent of each other, and bearing a predisposing gene polymorphism does not necessarily result in development of the atopic syndrome.[24] The genetic complexity in the atopic syndrome possibly results in its heterogeneous clinical phenotype. Defining the endotypic profile of atopic patients in more detail contributes to determination of more homogeneous subclasses of patients. Subclasses are currently defined based on clinical and immunological characteristics, like the type of immune response involved.[25] However, stratification of atopic patients based on their genetic defect or polymorphism linked to their expression profile of immune cell lineages has not yet been investigated. This endotyping approach could be of interest as immune dysregulation may play an important role in the pathogenesis of the atopic syndrome. Interestingly, the atopic syndrome is a prevalent comorbidity in primary immunodeficiency diseases (PIDs), for example in hyper IgE syndrome (HIES), Comèl Netherton syndrome, and immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, which suggests that the atopic syndrome could be caused by a genetic

defect in pathways that are also involved in these monogenic PIDs.[26,27] This is supported by the hypothesis of autoallergy, in which atopy seems to stand at the boundary between allergy and auto-immunity, given the presence of IgE antibodies against self-proteins.[28-30]

Several gene-targeted and/or pathway-targeted treatment strategies for PIDs have recently been under clinical evaluation, which could be of clinical benefit in atopy as well. Identification of genetic pathways for these targeted and personalized treatment modalities is therefore essential.

We hypothesized that subclasses within the atopic syndrome exist based on genes that act in the same molecular pathway. Additionally, genetic defects in pathways that cause a PID might also be involved in the atopic syndrome.

Therefore, the aim of this study was to define subclasses within the atopic syndrome via molecular clustering of atopy-related genes based on their expression profiles of immune cell lineages. We first evaluated the overlap between atopy-related genes and monogenic PID genes. Secondly, we clustered the atopy-related genes based on their expression profile of immune cell lineages of healthy mice. Finally, we analyzed the pathways in which the atopy-related genes are involved.

MATERIALS AND METHODS

Data collection and content – overlap atopy/primary immunodeficiency disease genes

We obtained a complete list of all mutated genes responsible for atopic manifestations by performing a comprehensive search in the Human Gene Mutation Database (HGMD, HGMD® Professional, https://portal.biobase-international.com) up to August 21th 2018.[31] Genes were searched using the phenotype terms "atopy", "increased IgE", "atopic dermatitis", "eczema", "food allergy", "allergy", "asthma", and "allergic rhinitis" (Table S1). Atopy-related genes and the number of mutations per gene were extracted. Additionally, disease-causing genes of monogenic PIDs were obtained from the phenotypic classification for PIDs of the International Union of Immunological Societies (IUIS).[32] We performed a cross-check on atopy-related mutations in PID genes using HGMD. Overlapping genes between both the HGMD and PID lists were identified to select atopy-related with a defect in the same gene as a PID.

Clustering and visualization of atopy-related genes

The atopy-related genes were clustered to identify more homogeneous subclasses of the atopic syndrome. Clusters were made based on their gene expression profiles of immune cell lineages. Therefore, gene expression data from the Immunological Genome Project (ImmGen, http://www.immgen.org) was downloaded from the Genome Expression Omnibus (GEO) database accession number GSE15907 and GSE37448. The ImmGen datasets comprise the gene expression of a large amount of immune cell lineages (both hematopoietic and mesenchymal), that were grouped into 12 cell-populations. Currently, there is limited data on the gene expression signatures of human immune cell types. Therefore, immune cell lineages of healthy mice were used, which might give insights in atopic processes also applicable in human. All atopy-related genes selected via the HGMD query were searched in the ImmGen dataset. The top 40 co-expressed genes in mice were extracted per atopy-related gene. These genes are of biological interest as co-expressed genes are controlled by the same transcriptional regulatory program, functionally related, or members of the same pathway or protein complex as our atopy-related genes of interest.[33] We overlaid the co-expressed genes to identify genes that occurred in the top 40 lists of multiple atopy-related genes. Based on the overlap in co-expressed genes, indicating the degree of similar expression of atopy-related genes, the atopy-related genes were clustered in an unsupervised manner. Accordingly, it is likely that the clustered atopy-related genes act in the same molecular pathway. The clusters were visualized by constructing a correlation network plot using the "qgraph" package in RStudio version 3.4.1.[34] The lines between the genes were weighted and only correlations with a minimum correlation coefficient of 0.65, indicating a strong (positive) relationship, were visualized. If the top 40 list of an atopy-related gene did not contain a single overlapping gene, this atopy-related gene was labeled as an unclustered "bin" gene.

To visualize the gene expression profiles of the clusters, a heat map of the gene expression per cell lineage was constructed. Therefore, gene expression data were imported into Omniviz software version 6.1.13.0. Using Omniviz, the geometric mean of each probeset was calculated and transcriptomic data was log2 transformed to normalize the data. Changes in gene expression were constituted by deviations from the geometric mean to visualize whether genes of immune cell lineages were higher or lower expressed. These deviations are visualized in a heat map by a gradient from red (high expression) to blue (low expression) and ordered per cluster.

Functional pathway analysis

We validated whether the extracted genes from HGMD were atopy-related through analysis of the pathways containing these atopy-related genes. As the separate clusters included small numbers of genes, all clustered atopy-related genes from HGMD with and without unclustered "bin" genes were analyzed using Ingenuity Pathway Analysis (IPA,

Qiagen©) software.[35] The most important pathways, in which the atopy-related genes were involved, were extracted from IPA. The pathways were ranked according to their *p*-value (-log transformed) and the ratio of the atopy-related genes found in each pathway over the total number of molecules in that pathway, indicating the significance of the association between the atopy-related genes and the identified pathways. A *p*-value was calculated using a Fisher's Exact test to determine the probability of the association between the atopy-related genes and the pathways is explained by a random chance alone. A –log (*p*-value) equal to or greater than 1.3, corresponding to a *p*-value of 0.05, was considered statistically significant.

RESULTS

Content of data

The search in HGMD on atopic manifestations retrieved 159 atopy-related genes known in human (Table S1). Based on the overview of the IUIS, 278 disease-causing genes of monogenic PIDs were obtained.[36] During the cross-check on atopy-related mutations in PID genes, *TRAF3IP2* was identified in which mutations were described that might result in an eczema phenotype. This gene did not appear in the search results of HGMD and was therefore added to the list of atopy-related genes, resulting in a total of 160 genes for further analysis. The top three genes with the highest number of atopy-related mutations included *STAT3* (n=107), *FLG* (n=62) and *DOCK8* (n=45). Other genes had six or less atopy-related mutations per gene (Table S1). Twenty-two (5.3%) genes of the atopy (n=160) and PID (n=278) lists were overlapping, including *ARPC1B*, *BTK*, *CASP8*, *CFTR*, *CTLA4*, *DOCK8*, *ICOS*, *IL10*, *IL12B*, *IL12RB1*, *IL17F*, *IL21*, *IL21R*, *IL7R*, *ITK*, *ORAI1*, *PGM3*, *SPINK5*, *STAT3*, *TNFRSF13B*, *TRAF3IP2* and *TYK2* (Figure 1 and Table S1).

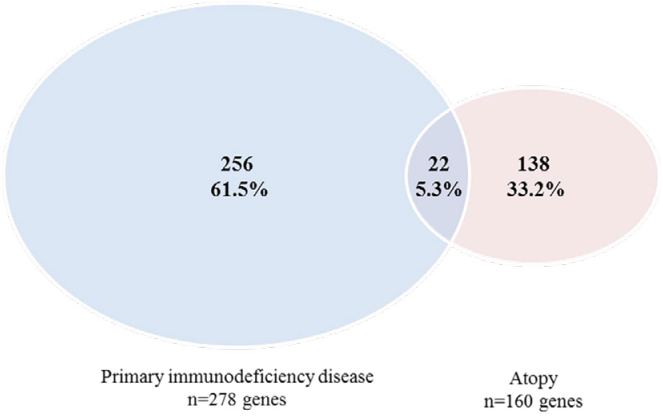

Figure 1. Venn diagram illustrating the overlap of the primary immunodeficiency disease-related genes and the atopy-related genes identified in the Human Gene Mutation Database

Clustering of genes

Fifteen (9.4%) of the 160 atopy-related genes were not expressed in the mouse immune system, of which immune cell lineages were used in the ImmGen dataset, and were therefore excluded from further analysis. As some genes had multiple transcripts and appeared more than once in the gene expression dataset, the complete list for clustering resulted in 153 probes. Eleven clusters were identified, of which seven clusters included five or more genes (clusters A, C, D, F, H, J and K), and 37 non-correlated genes remained as "bin" (Figure 2 and 3, Table S1). Based on the gene expression profiles, we identified one pair of anti-correlated clusters (clusters D and F), i.e. opposite expression profiles between clusters D and F (Figure 3). The 22 overlapping genes between the atopy-related genes and monogenic PID genes were localized in two of the seven atopy-related gene clusters, including cluster F (n=8) and cluster D (n=3) (Table S1).

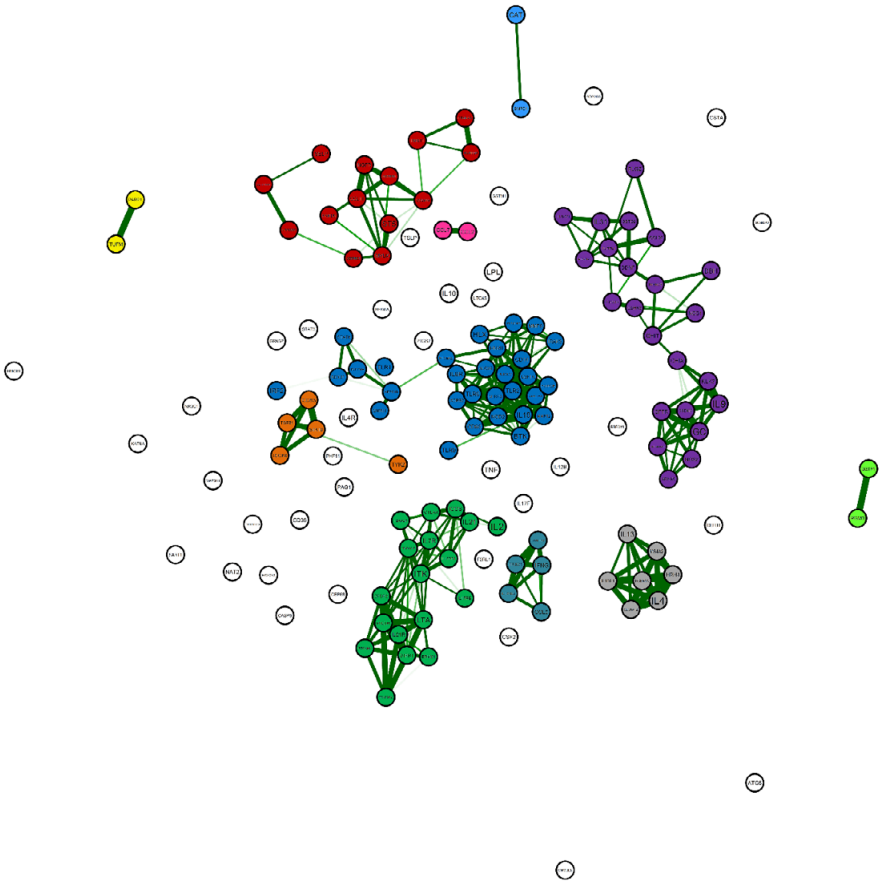

Figure 2. Genetic correlation network plot of atopy-related genes
The line width between the atopy-related genes indicate the overlay in the top 40 co-expressed gene lists per atopy-related gene and is proportional to the strength of correlation within the clusters.

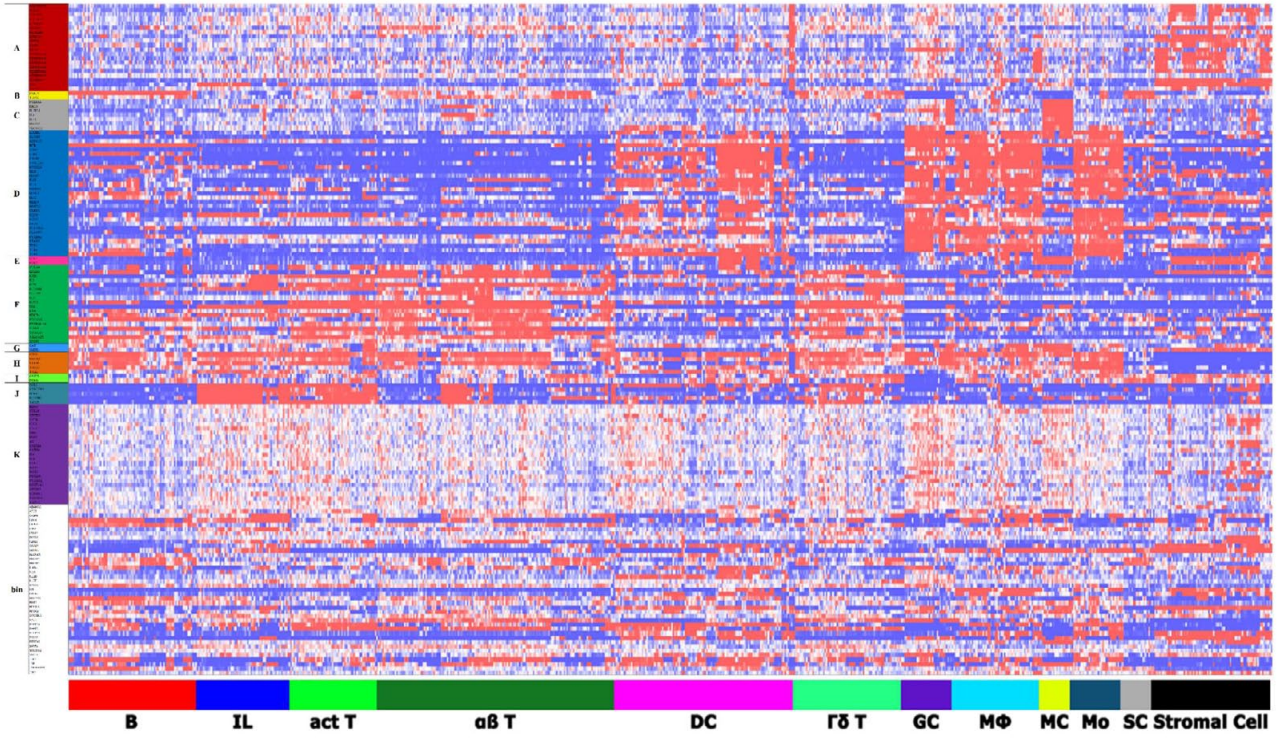

Figure 3. Heat map representing the atopy-related gene expression across the immune cell lineage of healthy mice ordered according to the identified clusters within the atopic syndrome

Data on the expression of atopy-related genes across the immune cell lineages was constructed using the Omniviz software, in which changes in gene expression were visualized by a gradient from red (high expression) to blue (low expression). Genes were alphabetically ordered according to the identified genetic clusters within the atopic syndrome. Abbreviations: B, B lymphocyte; IL, innate lymphocyte; act T, activated T lymphocyte; αβ T, αβ T lymphocyte; DC, dendritic cell; Γδ T, Γδ T lymphocyte, GC, granulocyte; MΦ, macrophage; MC, mast cell; Mo, monocyte; SC, stem cell.

Functional pathway analysis

Functional pathway analysis in IPA of the atopy-related genes both with and without taking unclustered "bin" genes into account resulted in T helper (Th) lymphocyte-mediated pathways. Taking all atopy-related genes (n=160) into account, it resulted in the specific pathways "T helper lymphocyte differentiation", "Th1 and Th2 activation pathway", and "Th2 pathway", in which respectively 22, 28, and 24 atopy-related genes were involved (Table S2a). Additionally, pathway analysis of the clustered atopy-related genes only (n=108) resulted in the specific pathways "Th1 and Th2 activation pathway" (n=22 genes), "T-helper lymphocyte differentiation" (n=16 genes), and "Th2 pathway" (n=19 genes) (Table S2b).

DISCUSSION

This is the first study that describes clusters in the clinically heterogeneous phenotype of the atopic syndrome based on gene expression profiles of immune cell lineages of healthy mice. The overlap between atopy-related genes (n=160) and monogenic PID genes (n=278) was limited to 22 (5.3%) genes. We identified seven distinct clusters within the atopic syndrome based on the expression profiles of atopy-related genes. Functional pathway analysis of all known atopy-related genes resulted in identification of Th lymphocyte-mediated processes underlying the atopic syndrome.

The atopic syndrome is a prevalent comorbidity in a number of PIDs, suggesting that the atopic syndrome can be a symptom of PIDs and that immune dysregulation plays a role in the pathogenesis. Interestingly, the number of overlapping genes in this study was limited (5.3%) and did not belong to one PID category according to the IUIS phenotypic classification or immunologic component.[32] Nonetheless, the overlapping genes were bundled in just two of the seven atopy-related gene clusters (cluster D and F), which suggests that these endotypes of the atopic syndrome are associated with the predisposition to develop a PID. However, atopy-related mutations in these genes might differ from the disease-causing mutations of the PIDs.

Current literature reports nine PIDs to be possibly related to the atopic syndrome, including autosomal dominant HIES (AD-HIES; *STAT3*), autosomal recessive HIES (AR-HIES; *DOCK8*), Comèl Netherton syndrome (*SPINK5*), hypogammaglobulinemia, selective IgA deficiency (SIgAD), IgM deficiency, IPEX (*FOXP3*), chronic granulomatous disease (CGD; *CYBA, CYBB, NCF1*, *NCF2* and *NCF4*), and phospholipase C gamma 2 (PLCG2) gene associated antibody deficiency and immune dysregulation (PLAID; *PLCG2*), and 28 additional genetic PID conditions.[27,37] Only eight genes (*STAT3, DOCK8*, *SPINK5*, *FLG*, *ARPC1B*, *PGM3*, *ERBIN*, and

TYK2) were extracted from HGMD using the atopy phenotype search. Furthermore, only two of the 22 overlapping atopy-related and PID-related genes identified in this study were reported in literature to be involved in PIDs and the atopic syndrome.[27] The discrepancy between literature and HGMD could firstly be explained by the recent expansion of novel mutations derived from next generation sequencing (NGS). Secondly, the atopic manifestations in PIDs, as described in literature, might be an occasional finding and not related to the disease causing genes of PIDs. Thirdly, the heterogeneous course and presentation of the atopic syndrome may make it difficult to associate genetic mutations with atopic manifestations. Moreover, the infectious symptoms in PIDs might be a more prominent clinical feature than the atopic manifestations, which therefore could have resulted in a registration bias.

We found a low number of mutations in most atopy-related genes in human (six or less mutations in 157 of the 160 genes), suggesting that other phenomena contribute to the disease such as post-translational modifications. Alternatively, various genes that interact with environmental factors might be involved in the atopic syndrome, in which each gene contributes only to a small amount of the overall disease risk.[38] Furthermore, the differences between the clusters could indicate that immune regulation plays a role in the atopic syndrome next to underlying genetic mechanisms.

Strikingly, two of the identified clusters (D and F) have a completely opposite expression profile, both in lymphoid and myeloid cell lineages. An explanation for this phenomenon may be that both clusters share the same upstream regulator. Depending on a gain or loss of function mutation in this enhancer, the gene expression profile can be influenced by an agonist or antagonist of this regulator. By performing a functional pathway analysis of the atopy-related genes in only clusters D and F, we would explore the functional significance of these clusters. The analysis resulted in the pathways "T helper cell differentiation", "TREM1 signaling" and "Th1 and Th2 activation pathway", which is completely corresponding with the pathways involved in all atopy-related genes (data not shown). Therefore, we could unfortunately not differentiate between the functional significance of all atopy-related genes and those included in clusters D and F.

The identified Th lymphocyte-mediated pathway supports the hypothesis that changes in the immune system underlie and could be involved in the pathogenesis of atopy. In AD it has been previously described that acute skin lesions are characterized by Th2 lymphocyte infiltration with a shift towards predominantly Th1 lymphocytes in the chronic phase.[39-41] In addition, asthma was reported as a Th2 lymphocyte -mediated diseases driven by allergen exposure.[42] Moreover, patients with FA and AR are characterized by allergen-specific Th2 lymphocyte-mediated responses showing that the obtained Th lymphocyte-pathways

involved in atopy are in agreement with these of the individual atopic diseases.[43-45] In most of our identified clusters (except clusters F, G, H, I and J) the atopy-related genes do not show increased expression in T lymphocytes (Figure 3). Therefore, genes in these clusters might be expressed in immunologic cells that co-interact with T lymphocytes, including Th lymphocytes, or in cells that are progenitors of Th lymphocytes.

This study has some limitations. Firstly, as we could not include terms concerning the skin barrier in the search, we might have missed gene expression profiles of barrier cells. However, by using the terms "atopic dermatitis" and "eczema" we have identified important barrier genes, like *COL6A5*, *FLG* (subtypes), *FLG2*, and *KLK7*. Secondly, some discrepancies were found in the HGMD database. The genes from the atopic phenotype search did not completely overlap with the results from the search on atopy-related mutations per gene. Therefore, we identified atopic phenotypes per gene on the results of both searches. Thirdly, we clustered genes based on their expression profiles in the ImmGen dataset, which uses characterized immune cells of mice. The gene expression profiles of immune cell lineages in healthy mice may not be identical to these in (atopic) humans. This explained why we could not cluster all human atopy-related genes including *FLG*, which is an important atopy gene based on the number of atopy-related mutations (n=62). Furthermore, the data from mice cannot directly be applied for subgrouping of the atopic syndrome in humans. Therefore, large cohorts of patients with the atopic phenotype should be sequenced using NGS to investigate whether atopy clusters could be generated based on the gene expression profiles of immune cell lineages of atopic human. Identification of clusters of atopy-related genes by NGS potentially opens novel ways to select eligible patients for pharmaceutical studies and could predict therapeutic responses.

CONCLUSION

This study shows a model, using data of healthy mice, to define clusters of the atopic syndrome based on gene expression profiles of immune cell lineages. We identified seven distinct clusters within the atopic syndrome in which Th lymphocyte-mediated pathways were most often involved. This supports the hypothesis that both genetic mechanisms and immune dysregulation have a role in the pathogenesis of the atopic syndrome. Our results also opens up the possibility for identification of novel therapeutic targets towards a more tailored approach and personalized medicine.

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SUPPLEMENTARY MATERIAL

Table S1. Atopy-related genes from the Human Gene Mutation Database

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
ADCYAP1R1	Adenylate Cyclase Activating Polypeptide 1 Receptor Type I	Asthma	NA	A
CCL11	C-C Motif Chemokine Ligand 11	Asthma	NA	A
COL6A5	Collagen Type VI Alpha 5 Chain	Dermatitis	4	A
CTNNA3	Catenin Alpha 3	Food allergy	2	A
FRMD6	FERM Domain Containing 6	Asthma	1	A
KCNMB1	Potassium Calcium-Activated Channel Subfamily M Regulatory Beta Subunit 1	Asthma	1	A
LRRC32	Leucine Rich Repeat Containing 32	Dermatitis	6	A
MYLK	Myosin Light Chain Kinase	Asthma	1	A
NGFR	Nerve Growth Factor Receptor	Asthma	NA	A
SELP	Selectin P	Atopy	1	A
SERPINA1	Serpin Family A Member 1	Asthma	1	A
SERPINE1	Serpin Family E Member 1	Asthma	NA	A
ST5	Suppression Of Tumorigenicity 5	Asthma	1	A
TWIST1	Twist Family BHLH Transcription Factor 1	IgE	NA	A
PARP1	Poly(ADP-Ribose) Polymerase 1	Asthma	NA	B
TUFM	Tu Translation Elongation Factor, Mitochondrial	Asthma	1	B
FCER1A	Fc Fragment Of IgE Receptor Ia	Dermatitis	1	C
HRH4	Histamine Receptor H4	Dermatitis	NA	C
IL1RL1	Interleukin 1 Receptor Like 1	Dermatitis	2	C
IL4	Interleukin 4	IgE Asthma	3	C
IL13	Interleukin 13	Atopy IgE Asthma Rhinitis	2	C
MS4A2	Membrane Spanning 4-Domains A2	Atopy IgE Asthma Rhinitis	5	C
SLC6A12	Solute Carrier Family 6 Member 12	Asthma	1	C
ADRB2	Adrenoceptor Beta 2	Dermatitis Asthma	2	D
ALOX5	Arachidonate 5-Lipoxygenase	Asthma	1	D
ARPC1B[†]	Actin Related Protein 2/3 Complex Subunit 1B	Allergy	1	D
BTK[†]	Bruton Tyrosine Kinase	Asthma	1	D
CD14	Cluster of Differentiation 14 Molecule	Asthma Rhinitis	NA	D
CD86	Cluster of Differentiation 86 Molecule	Asthma	1	D

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
CSF1R	Colony Stimulating Factor 1 Receptor	Asthma	1	D
CYSLTR1	Cysteinyl Leukotriene Receptor 1	Atopy Asthma	2	D
FCGR2B	Fc Fragment Of IgE Receptor IIb	Atopy	NA	D
HLX	H2.0 Like Homeobox	Asthma	2	D
HNMT	Histamine N-Methyltransferase	Dermatitis Asthma	NA	D
IL6R	Interleukin 6 Receptor	Dermatitis	NA	D
IL18	Interleukin 18	Asthma	1	D
INPP4A	Inositol Polyphosphate-4-Phosphatase Type I A	Asthma	1	D
IRAK3	Interleukin 1 Receptor Associated Kinase 3	Asthma	2	D
IRF2	Interferon Regulatory Factor 2	Dermatitis	1	D
MMP9	Matrix Metallopeptidase 9	Asthma Allergy	2	D
MMP12	Matrix Metallopeptidase 12	Asthma	NA	D
NLRP3	NLR Family Pyrin Domain Containing 3	Food allergy	2	D
NOD1	Nucleotide Binding Oligomerization Domain Containing 1	IgE Asthma	1	D
NOD2	Nucleotide Binding Oligomerization Domain Containing 2	Atopy Dermatitis Rhinitis	NA	D
ORAI1†	Calcium Release-Activated Calcium Modulator 1	Dermatitis	1	D
PLA2G4A	Phospholipase A2 Group IVA	Asthma	2	D
PLA2G7	Phospholipase A2 Group VII	Atopy Asthma	2	D
PTGER2	Prostaglandin E Receptor 2	Asthma	1	D
STAT6	Signal Transducer And Activator Of Transcription 6	IgE Dermatitis Asthma	3	D
TLR2	Toll Like Receptor 2	Dermatitis Asthma	1	D
TLR6	Toll Like Receptor 6	Asthma	1	D
TLR9	Toll Like Receptor 9	Dermatitis Asthma	1	D
CCL2	C-C Motif Chemokine Ligand 2	Asthma	NA	E
CCL7	C-C Motif Chemokine Ligand 7	Asthma	1	E
CTLA4†	Cytotoxic T-Lymphocyte Associated Protein 4	Asthma	1	F
EPHX1	Epoxide Hydrolase 1	Asthma	NA	F
ICOS†	Inducible T Cell Costimulator	Allergy	1	F
IL2	Interleukin 2	Allergy	2	F
IL7R†	Interleukin 7 Receptor	Dermatitis	NA	F

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
IL12RB1†	Interleukin 12 Receptor Subunit Beta 1	Dermatitis	1	F
IL17RB	Interleukin 17 Receptor B	Asthma	1	F
IL21†	Interleukin 21	Asthma	1	F
IL21R†	Interleukin 21 Receptor	IgE	1	F
ITK†	Interleukin 2 Inducible T Cell Kinase	Asthma	1	F
LTA	Lymphotoxin Alpha	Asthma	1	F
PDCD4	Programmed Cell Death 4	Asthma	1	F
PECAM1	Platelet And Endothelial Cell Adhesion Molecule 1	Asthma	NA	F
PPARGC1B	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Beta	Asthma	2	F
S1PR1	Sphingosine-1-Phosphate Receptor 1	Asthma	2	F
TBXA2R	Thromboxane A2 Receptor	IgE Dermatitis Asthma	2	F
TRAF3IP2†	TNF Receptor-Associated Factor 3 Interacting Protein 2	Dermatitis	2	F
ZPBP2	Zona Pellucida Binding Protein 2	Asthma	2	F
CAT	Catalase	Asthma	1	G
SMPD1	Sphingomyelin Phosphodiesterase 1	Allergy	1	G
CD53	Cluster of Differentiation 53 Molecule	Asthma	1	H
DOCK8†	Dedicator Of Cytokinesis 8	IgE	45	H
STK10	Serine/Threonine Kinase 10	Asthma	1	H
TGFB1	Transforming Growth Factor Beta 1	Asthma	NA	H
TYK2†	Tyrosine Kinase 2	IgE	1	H
GSTP1	Glutathione S-Transferase Pi 1	Dermatitis Asthma	NA	I
PGM3†	Phosphoglucomutase 3	Atopy IgE	6	I
CCL5	C-C Motif Chemokine Ligand 5	Dermatitis Asthma	1	J
CYSLTR2	Cysteinyl Leukotriene Receptor 2	Atopy	1	J
IFNG	Interferon Gamma	Atopy	NA	J
IL12RB2	Interleukin 12 Receptor Subunit Beta 2	Atopy	3	J
TBX21	T-Box 21	Asthma	1	J
BDNF	Brain Derived Neurotrophic Factor	Asthma	NA	K
CCL26	C-C Motif Chemokine Ligand 26	Asthma	1	K
CDHR3	Cadherin Related Family Member 3	Asthma	1	K
CFTR†	Cystic Fibrosis Transmembrane Conductance Regulator	Asthma	6	K
CHIA	Chitinase, Acidic	IgE Asthma	6	K

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
CHIT1	Chitinase 1	Asthma	NA	K
DBH	Dopamine Beta-Hydroxylase	Asthma	NA	K
FLG2	Filaggrin Family Member 2	Dermatitis	1	K
GC	GC, Vitamin D Binding Protein	Asthma	NA	K
GSDMA	Gasdermin A	Asthma	1	K
GSTO2	Glutathione S-Transferase Omega 2	Asthma	NA	K
IL9	Interleukin 9	Dermatitis	1	K
IL31	Interleukin 31	Dermatitis	1	K
KLK7	Kallikrein Related Peptidase 7	Dermatitis	1	K
NOS1	Nitric Oxide Synthase 1	Asthma	1	K
NOS2	Nitric Oxide Synthase 2	Atopy	NA	K
PTGDR	Prostaglandin D2 Receptor	Asthma Allergy	4	K
PTGDR2	Prostaglandin D2 Receptor 2	Asthma	2	K
SCGB1A1	Secretoglobin Family 1A Member 1	Asthma	1	K
SPINK5[†]	Serine Peptidase Inhibitor, Kazal Type 5	Atopy Asthma	3	K
TCHHL1	Trichohyalin Like 1	Dermatitis	1	K
TMEM79	Transmembrane Protein 79	Dermatitis	1	K
TRPV1	Transient Receptor Potential Cation Channel Subfamily V Member 1	Asthma	1	K
ADAM33	ADAM Metallopeptidase Domain 33	Asthma	NA	bin
ATG5	Autophagy Related 5	Asthma	2	bin
CASP8[†]	Caspase 8	Asthma	NA	bin
CD38	Cluster of Differentiation 38 Molecule	Asthma	1	bin
CEP68	Centrosomal Protein 68	Asthma	1	bin
CSF2	Colony Stimulating Factor 2	Dermatitis	1	bin
CSTA	Cystatin A	Dermatitis	1	bin
DEFB1	Defensin Beta 1	Dermatitis	NA	bin
F2RL1	F2R Like Trypsin Receptor 1	Atopy	1	bin
GRASP	General Receptor For Phosphoinositides 1 Associated Scaffold Protein	Asthma	1	bin
GSTM1	Glutathione S-Transferase Mu 1	Dermatitis Asthma	NA	bin
HAVCR1	Hepatitis A Virus Cellular Receptor 1	Asthma	3	bin
HMGB1	High Mobility Group Box 1	Dermatitis	3	bin
IL4R	Interleukin 4 Receptor	Atopy Dermatitis Asthma	6	bin
IL10[†]	Interleukin 10	Asthma	NA	bin

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
IL12B[†]	Interleukin 12B	Dermatitis Asthma	2	bin
IL17F[†]	Interleukin 17F	Asthma	1	bin
KAT6A	Lysine Acetyltransferase 6A	Food allergy	1	bin
LPL	Lipoprotein Lipase	Dermatitis	NA	bin
LTC4S	Leukotriene C4 Synthase	Asthma Allergy	2	bin
MAP3K1	Mitogen-Activated Protein Kinase 1	Asthma	1	bin
NAT2	N-Acetyltransferase 2	Asthma	2	bin
NFKBIA	Nuclear Factor Kappa B Inhibitor Alpha	Asthma	1	bin
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1	Asthma	NA	bin
ORMDL3	Orosomucoid Like 3	Asthma	3	bin
PAG1	Phosphoprotein Membrane Anchor With Glycosphingolipid Microdomains 1	Allergy	1	bin
PHF11	PHD Finger Protein 11	Dermatitis Asthma	2	bin
PTGS2	Prostaglandin-Endoperoxide Synthase 2	Atopy Asthma	NA	bin
RBFOX1	RNA Binding Fox-1 Homolog 1	Food allergy	2	bin
SART1	U4/U6.U5 Tri-SnRNP-Associated Protein 1	Atopy	1	bin
SCGB3A2	Secretoglobin Family 3A Member 2	Asthma	1	bin
STAT3[†]	Signal Transducer And Activator Of Transcription 3	IgE	107	bin
TLR1	Toll Like Receptor 1	Asthma	NA	bin
TNF	Tumor Necrosis Factor	Asthma	NA	bin
TNFRSF13B[†]	Tumor Necrosis Factor Receptor Superfamily Member 13B	Asthma	NA	bin
TSLP	Thymic Stromal Lymphopoietin	Asthma	1	bin
ACE	Angiotensin I Converting Enzyme	Asthma	NA	NA
CCL3L1	C-C Motif Chemokine Ligand 3 Like 1	Asthma	NA	NA
CHI3L1	Chitinase 3 Like 1	Asthma	1	NA
CYP2C19	Cytochrome P450 Family 2 Subfamily C Member 19	Asthma	NA	NA
CYP2J2	Cytochrome P450 Family 2 Subfamily J Member 2	Asthma	NA	NA
ERBIN	Erb-B2 Interacting Protein	IgE	1	NA
FCGR2A	Fc Fragment Of IgE Receptor IIa	Atopy Allergy	1	NA
FLG	Filaggrin	Dermatitis Food allergy Asthma	62	NA
FLG10.2	Fillagrin Alternative Isoform, Repeat 10.2	Dermatitis	NA	NA
FLG11	Fillagrin Alternative Isoform, Repeat 11	Dermatitis Asthma	1	NA

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
GSDMB	Gasdermin B	Asthma	4	NA
LCE3C	Late Cornified Envelope 3C	Dermatitis	NA	NA
MUC7	Mucin 7, Secreted	Asthma	1	NA
TLR10	Toll Like Receptor 10	Asthma Rhinitis	1	NA
VSTM1	V-Set And Transmembrane Domain Containing 1	Dermatitis	1	NA
ACE	Angiotensin I Converting Enzyme	Asthma	NA	NA
CCL3L1	C-C Motif Chemokine Ligand 3 Like 1	Asthma	NA	NA
CHI3L1	Chitinase 3 Like 1	Asthma	1	NA
CYP2C19	Cytochrome P450 Family 2 Subfamily C Member 19	Asthma	NA	NA
CYP2J2	Cytochrome P450 Family 2 Subfamily J Member 2	Asthma	NA	NA
ERBIN	Erb-B2 Interacting Protein	IgE	1	NA
FCGR2A	Fc Fragment Of IgE Receptor IIa	Atopy Allergy	1	NA
FLG	Filaggrin	Dermatitis Food allergy Asthma	62	NA
FLG10.2	Fillagrin Alternative Isoform, Repeat 10.2	Dermatitis	NA	NA
FLG11	Fillagrin Alternative Isoform, Repeat 11	Dermatitis Asthma	1	NA
GSDMB	Gasdermin B	Asthma	4	NA
LCE3C	Late Cornified Envelope 3C	Dermatitis	NA	NA
MUC7	Mucin 7, Secreted	Asthma	1	NA
TLR10	Toll Like Receptor 10	Asthma Rhinitis	1	NA
VSTM1	V-Set And Transmembrane Domain Containing 1	Dermatitis	1	NA

Abbreviations: NA, not available. [1]Genes were not available for clustering because they could not be identified in the mouse immune system of ImmGen. [†]Overlapping genes between atopy-related gene list and PID-related gene list (n=22).

Table S2. Ingenuity pathway analysis – top three pathways of all atopy genes (n=160)

Pathways	-log (*p*-value)[1]	Ratio[2]	Molecules
T Helper Cell Differentiation	2,95E01	3,01E-01	*IL21, STAT6, IFNG, IL4R, IL12RB1, IL10, IL21R, IL6R, IL12RB2, STAT3, TBX21, IL13, IL18, IL2, TGFB1, IL12B, NGFR, ICOS, CD86, IL17F, TNF, IL4*
Th1 and Th2 Activation Pathway	2,84E01	1,51E-01	*IL1RL1, IL12RB1, IL31, TBX21, IL2, TGFB1, IL9, IL4, STAT6, IFNG, PTGDR2, IL4R, IL10, HAVCR1, IL6R, TYK2, IL12RB2, STAT3, TLR9, IL13, TSLP, IL18, IL17RB, IL12B, LTA, ICOS, S1PR1, CD86*
Th2 Pathway	2,49E01	1,6E-01	*STAT6, IFNG, IL4R, PTGDR2, IL12RB1, IL10, HAVCR1, IL1RL1, TYK2, IL12RB2, IL31, TLR9, IL13, TBX21, TSLP, IL17RB, IL2, TGFB1, IL12B, ICOS, IL9, S1PR1, CD86, IL4*

[1]The –log (*p*-value) indicates the probability of the association of atopy-related genes with the pathway by random chance alone. [2]The ratio is calculated by the number of atopy-related genes in a given pathway that have a –log (*p*-value) equal to or greater than 1.3 (default cutoff value), divided by the total number of atopy-related genes that make up that pathway.

Table S2b. Ingenuity pathway analysis – top 3 pathways of atopy genes without 'bin' genes (n=108)

Pathways	-log (*p*-value)[1]	Ratio[2]	Molecules
Th1 and Th2 Activation Pathway	2,37E01	1,19E-01	*STAT6, IFNG, PTGDR2, IL12RB1, IL1RL1, TYK2, IL6R, IL12RB2, IL31, TBX21, IL13, TLR9, IL18, IL17RB, IL2, TGFB1, LTA, ICOS, IL9, S1PR1, CD86, IL4*
T Helper Cell Differentiation	2,18E01	2,19E-01	*IL21, IFNG, STAT6, IL12RB1, IL21R, IL6R, IL12RB2, TBX21, IL13, IL18, IL2, TGFB1, NGFR, ICOS, CD86, IL4*
Th2 Pathway	2,1E01	1,27E-01	*STAT6, IFNG, PTGDR2, IL12RB1, IL1RL1, TYK2, IL12RB2, IL31, TBX21, IL13, TLR9, IL17RB, IL2, TGFB1, ICOS, IL9, S1PR1, CD86, IL4*

[1]The –log (*p*-value) indicates the probability of the association of atopy-related genes with the pathway by random chance alone. [2]The ratio is calculated by the number of atopy-related genes in a given pathway that have a –log (*p*-value) equal to or greater than 1.3 (default cutoff value), divided by the total number of atopy-related genes that make up that pathway.