Gene Expression

Distinct transcriptomic profiles of early-onset atopic dermatitis in blood of pediatric patients

Final Project

Kavya Banerjee

Introduction & Background

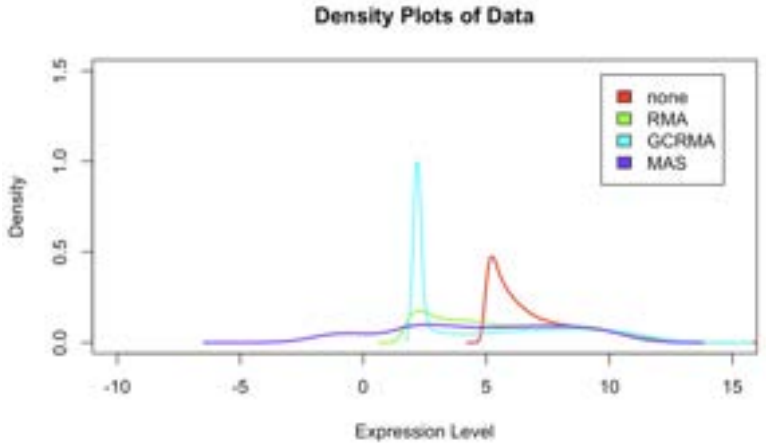
- GEO Dataset: GSE116486
- Title: Distinct transcriptomic profiles of early-onset atopic dermatitis in blood and skin of pediatric patients.
- Background
  - Atopic Dermatitis (AD): A condition predominantly affecting young children.
  - Current knowledge predominantly based on studies of adult AD using skin and blood samples from long-standing cases.
  - Recent genomic profiling of early pediatric AD biopsies revealed significant Th2 and Th17/Th22 skewing, distinct from the Th1 up-regulation seen in adults.
- Challenge: Pediatric skin biopsies are difficult to obtain, require alternative methods for understanding early AD pathogenesis.

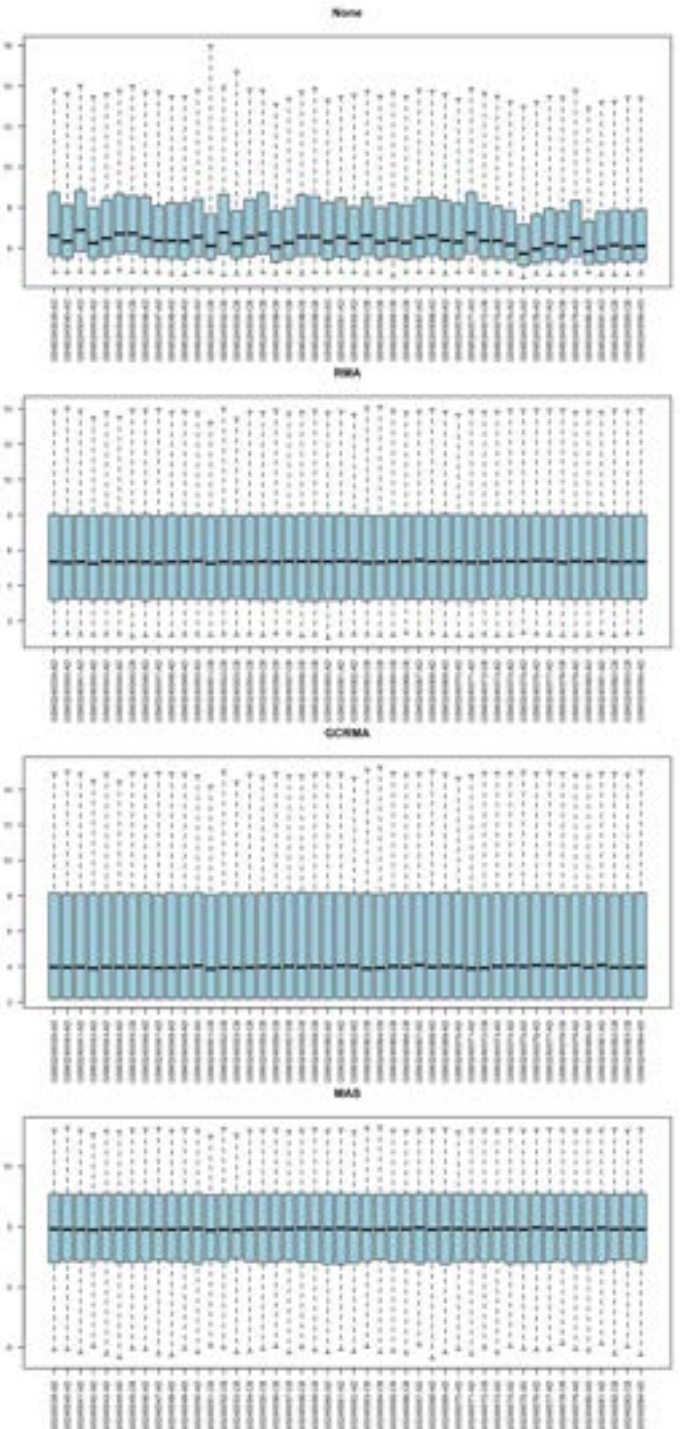
Objective & Methods

- Objective:
  - Primary Goal: To define the blood gene expression profile and identify associated biomarkers in early moderate-to-severe pediatric AD.
  - Significance: Insights into the underlying molecular mechanisms of AD in its early stages in children. Quantify systemic inflammation and contribute to unraveling new treatment targets in early AD.
- Methods:
  - Participants: Blood cells from 28 children with AD (under 5 years and within 6 months of disease onset) compared with healthy controls.
  - Sample Collection: Freshly drawn, unstimulated blood cells from the participants.
  - Approach:
    - Utilizing microarrays for gene expression analysis.
    - Identification of differentially expressed genes (DEGs) in blood (criteria: fold change >1.2, false discovery rate <0.05).
    - Microarray: [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array
    - Sample Size: n = 46 (AD = 28, CB (Healthy Control) = 18).

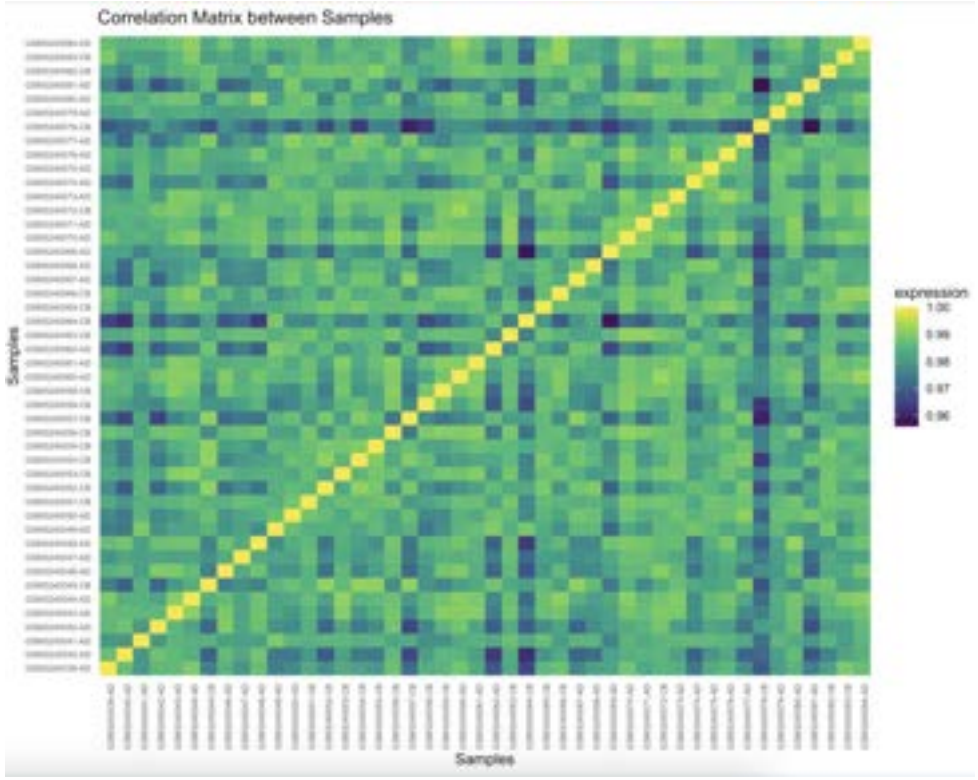
Normalization

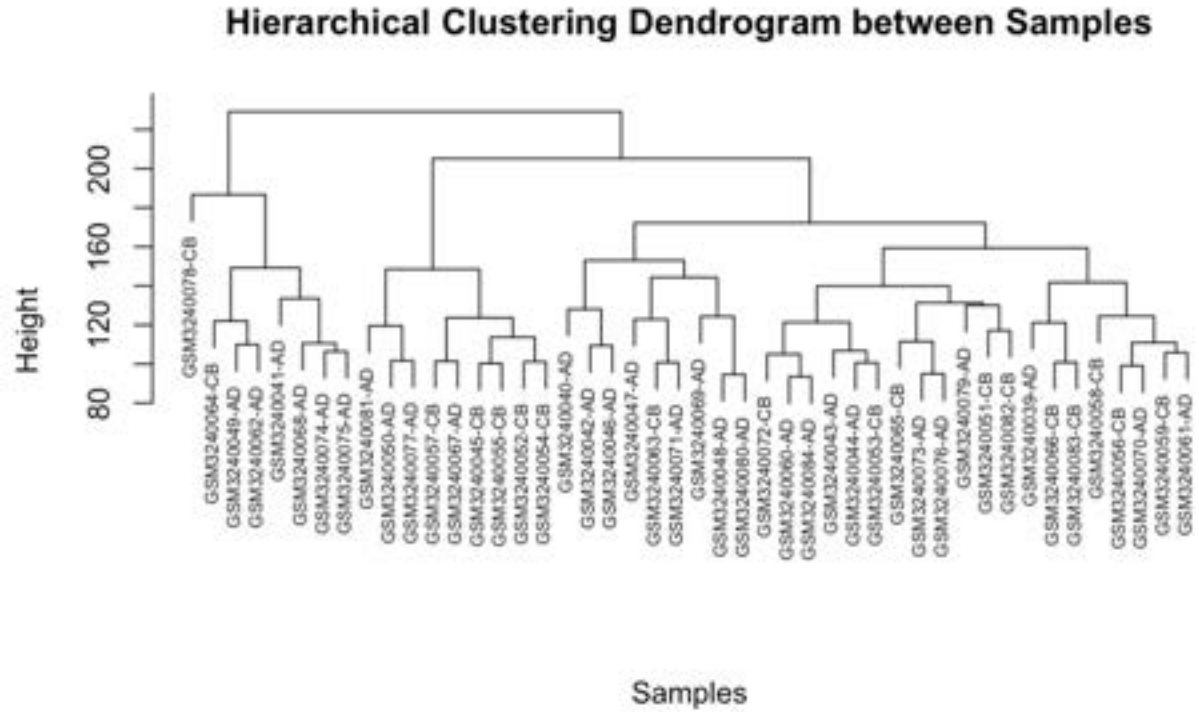
- Analysis of Methods:
  - RMA & GCRMA: Similar peak locations suggesting effective normalization, maintaining a balance in data variance.
  - MAS: Exhibits the narrowest peak, potentially signifying over-normalization, which might suppress critical biological variability.
  - RMA and GCRMA are favorable choices as they demonstrate a balanced approach to normalization, maintaining central tendency and variance.
- RMA preferred over GCRMA despite higher correspondence since boxplots for RMA show a better normalization without impacting the distribution.





Outlier Assessment

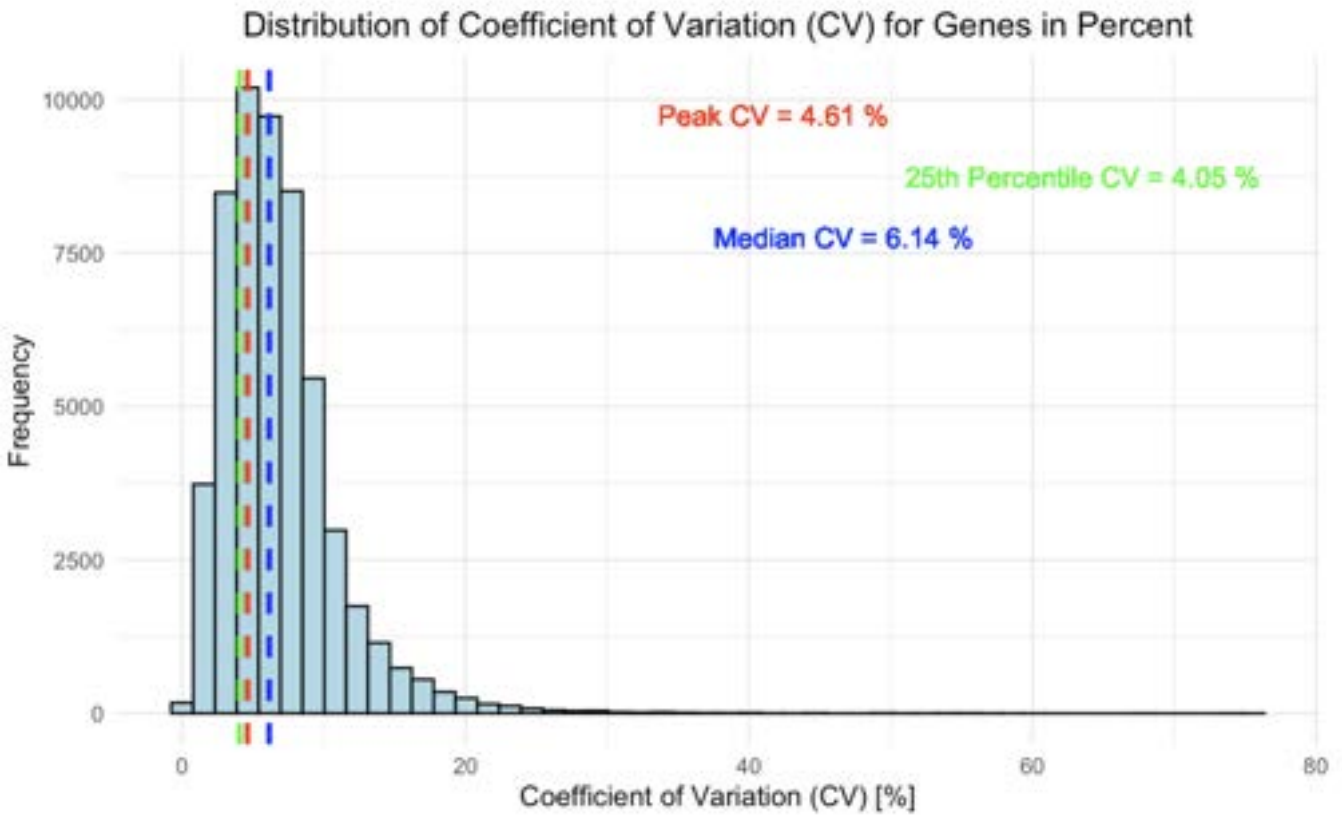




- Outlier assessment through correlation matrix and clustering of samples
- Both showed no obvious outliers; expression and clustering showed comparable grouping
- All samples considered for analysis

Filtering Low Expression Genes

- To refine gene expression data by discarding genes with minimal variation or low expression levels, which are less likely to be biologically significant.
- Reduces potential false positives, focusing on DEG.
- Method: Utilize the coefficient of variation (CV) to identify and remove genes with low variability.
- Criterion: Eliminate 25% of the low CV values



Feature Selection & Multiple Testing

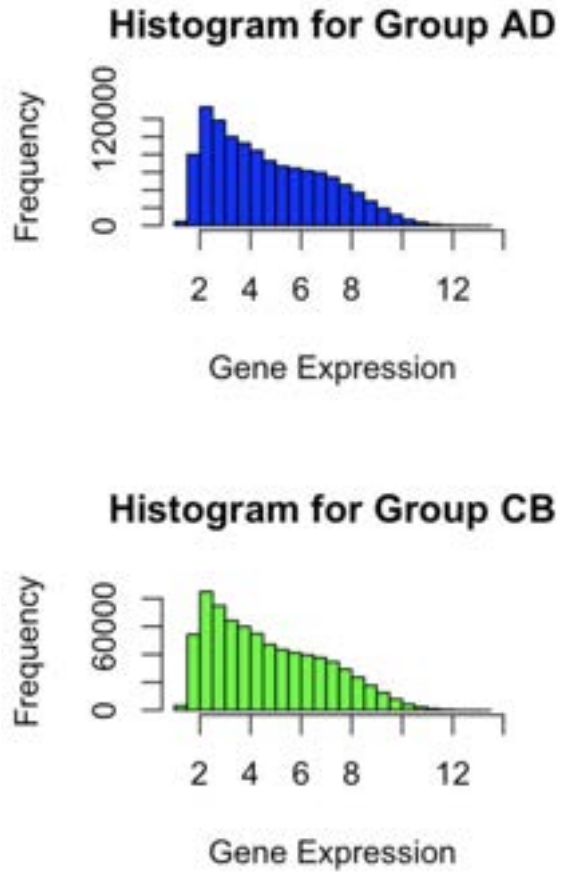


Fig 1. Non-normality of data for groups AD and CB.

- Fig 1: Data distribution follows non-normality confirmed by gene expression frequency histogram and and Anderson-Darling Test p-values for each sample (p = 3.7e-24 < 0.05). Thus, non-parametric unpaired two-sample Wilcox-test was used for finding significant genes.

- Fig 2:
  - False Discovery Rate (FDR < 0.05) correction made to p-values to reduce false positives for for multiple hypotheses testing in the analysis, conservative approach to find realistic false-positive estimate (Chen et al. 2021) .

  - Thus, the significance testing was done on FDR (=p.adjusted < 0.05). Probesets with FDR < 0.05 = 234.

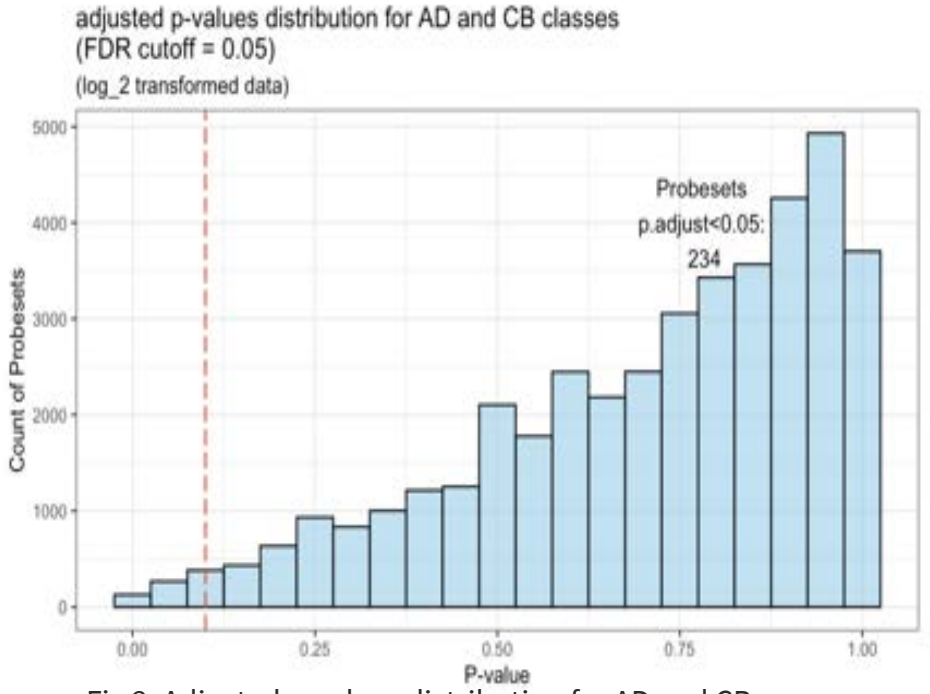


Fig 2. Adjusted p-values distribution for AD and CB groups.

Xiongzhi Chen, David G Robinson, John D Storey, The functional false discovery rate with applications to genomics, *Biostatistics*, Volume 22, Issue 1, January 2021, Pages 68–81, https://doi.org/10.1093/biostatistics/kxz010

Feature Selection & Multiple Testing – eBayes (limma)

- For exploring other significant testing methods, eBayes from limma was used. Essentially, depends on moderated t-statistic and borrow information from ensemble of genes.
- Smoothing the variance, may change the exact p-values but often preserves the rank ordering, which is critical for gene selection.
- Same FDR correction was made to the eBayes p-values and plotted against the Wilcox test p-values.
- Dual significant genes (n = 223) was obtained (eBayes p.adjust < 0.05 & Wilcox p.adjust < 0.05) were obtained.

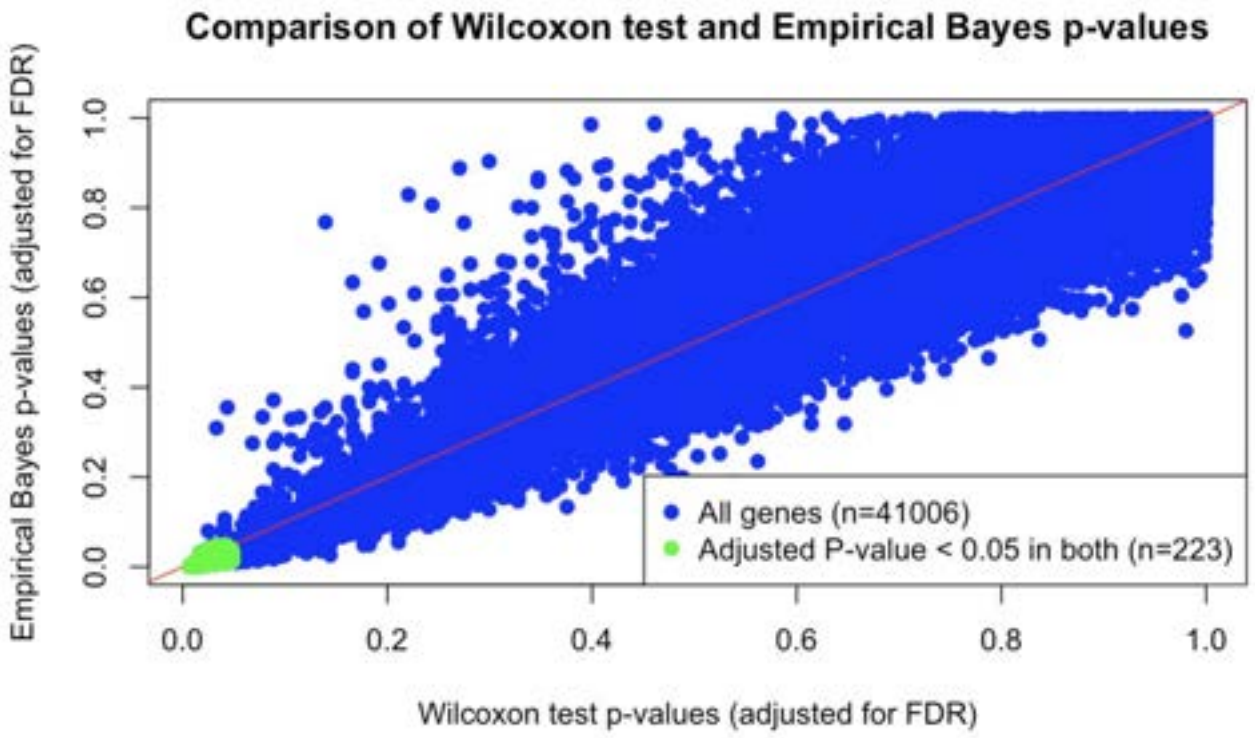


Fig 3. Adjusted p-values comparison for eBayes vs Wiclox p-values. Dual significant genes marked when p.adjust < 0.05)

Xiongzhi Chen, David G Robinson, John D Storey, The functional false discovery rate with applications to genomics, *Biostatistics*, Volume 22, Issue 1, January 2021, Pages 68–81, https://doi.org/10.1093/biostatistics/kxz010

DEG Analysis – Volcano Plot

Fig 4. Volcano plot for genes and their regulation. Regulation and significance plotted.

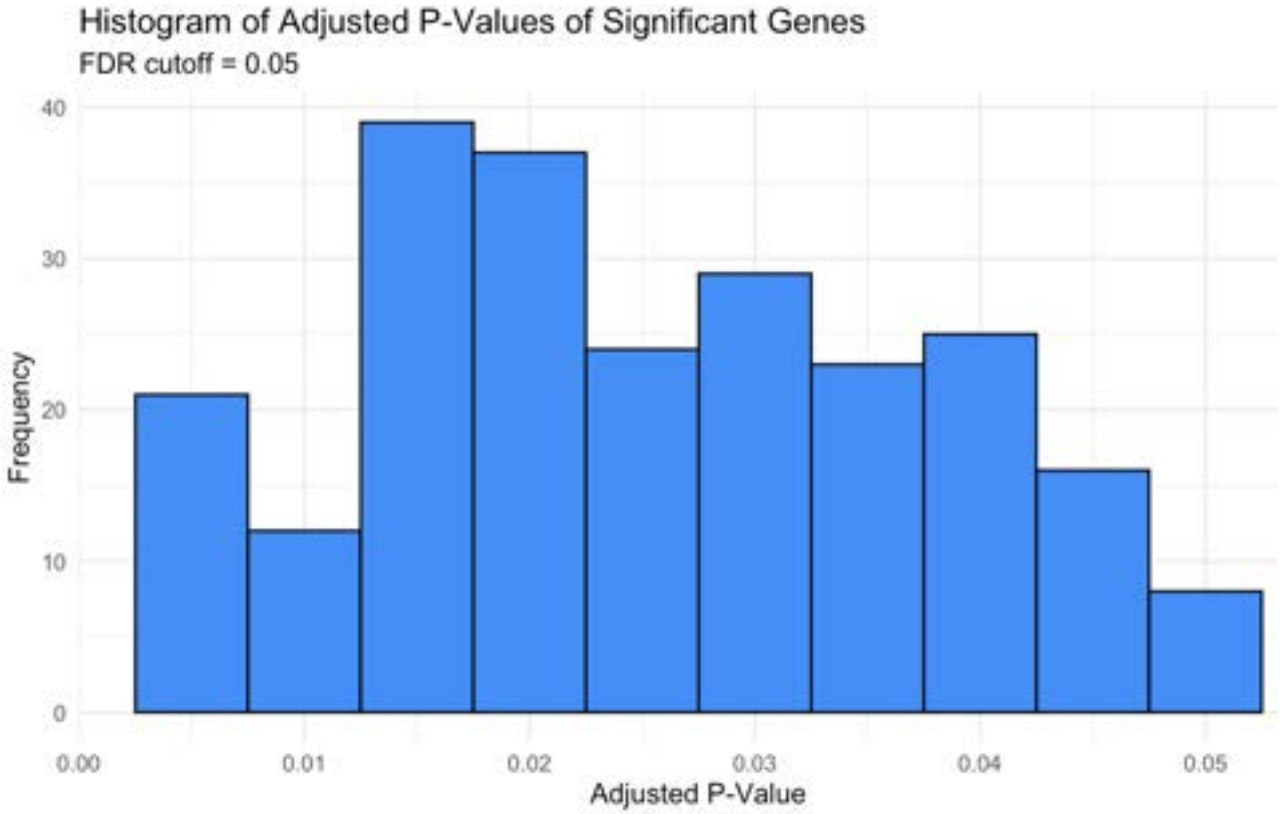


Fig 5. Histogram of significant genes with adjusted p-values.

- Volcano plot for the all genes to find DEGs. Criterion for DEGs: p.adjusted (FDR) < 0.05 and absolute Fold Change (FC) > 1.2. Total 204 DEGs were found (142 upregulated and 62 downregulated). Top 5 upregulated and downregulated genes (probesets) plotted.
- Histogram of the adjusted p-values of significant plotted majority of the genes fall below the threshold.

Dimensionality Reduction

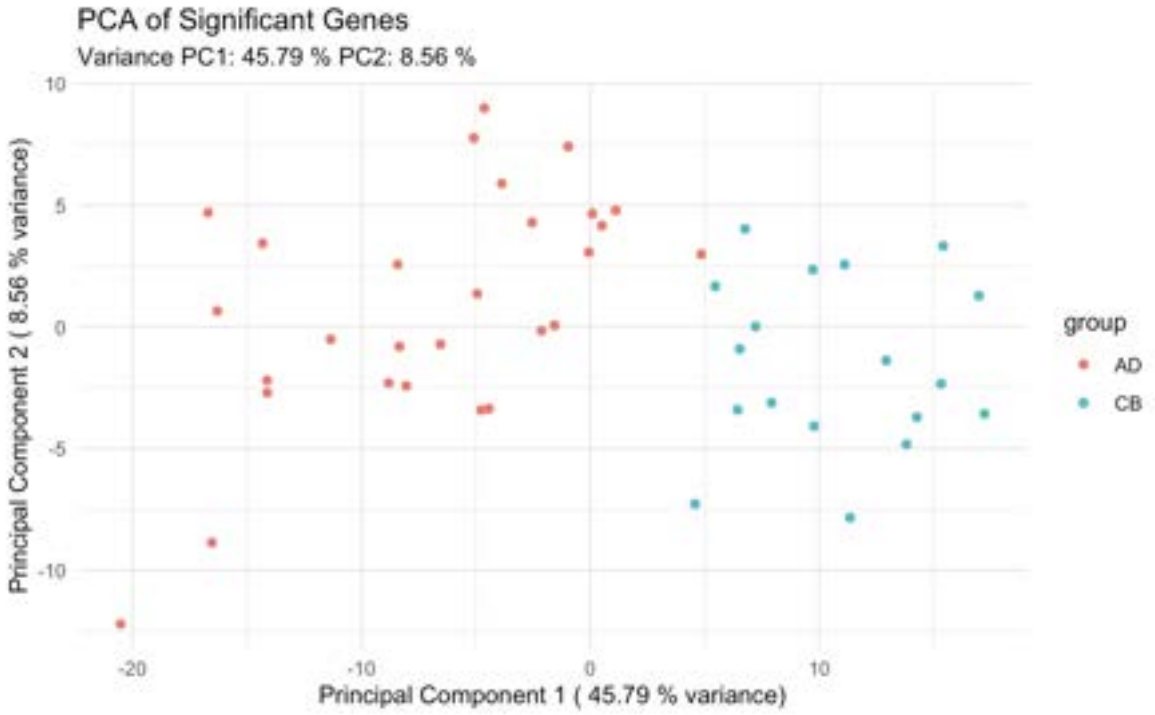


Fig 6. PCA plot showing the clustering and grouping of samples

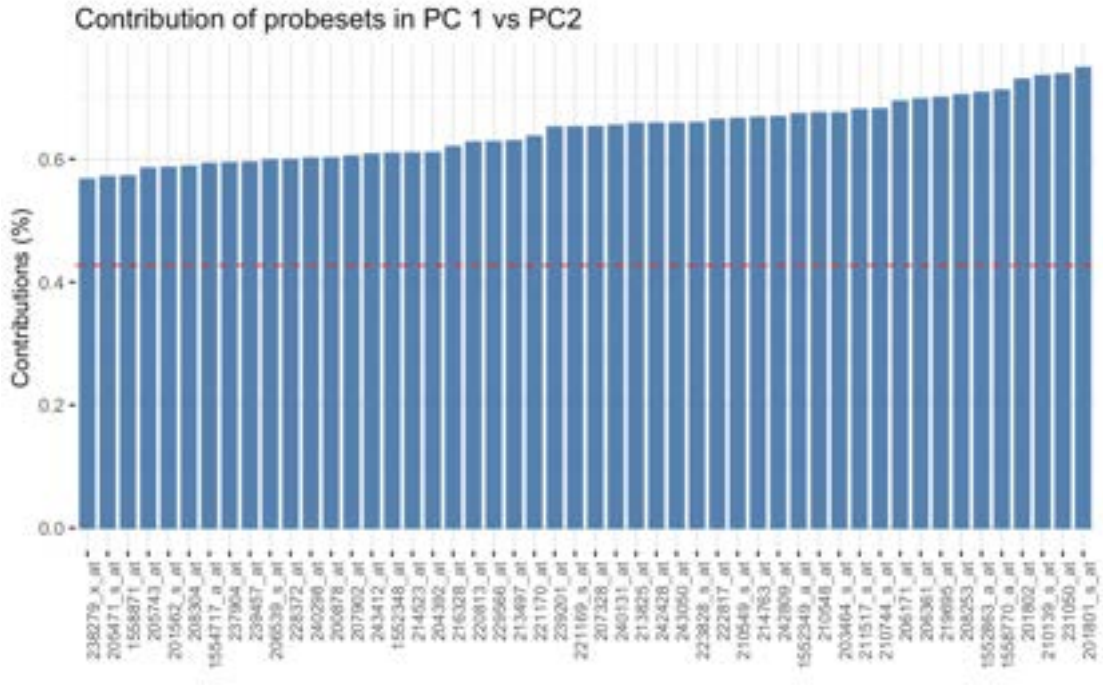


Fig 7. Contribution of probesets in PC 1 vs PC 2

- PCA is ideal for gene expression data analysis as it condenses high-dimensional data into principal components for easier visualization and interpretation, filters out noise, reveals patterns, prepares data for further statistical analysis, and efficiently handles large datasets with its scalable and computationally effective approach.

- Fig 6.PCA show some level of separation between the two groups. Visible overlap between the AD and CB samples, particularly around the center, implying significant genes differentiate to some extent, they do not create completely distinct clusters for AD and CB. Outliers in AD group from main cluster, likely due to biological validation or other underlying causes. Scree plot (not shown) shows subsequent components after PC1 explain variance lesser.

- Fig 7. Contribution of probesets in PC 1 vs PC 2. Top probesets such as 201801_s_at and 201802_at from DEG analysis show significant contribution.

Clustering

- Both CB and AD groups show similar expression in most significant genes but some show more pronounced expression such as probesets 201801_s_at, 223828_S_at and 201802_at.
- Euclidean and Complete Linkage done for its simplicity and less computational requirement.
- Doesn’t require a predefined number of clusters (suitable for exploratory analysis)
- Sensitivity to outliers helps in identifying closely related gene expressions, reflecting possible shared biological pathways or functions (Eisen et al, 1998).

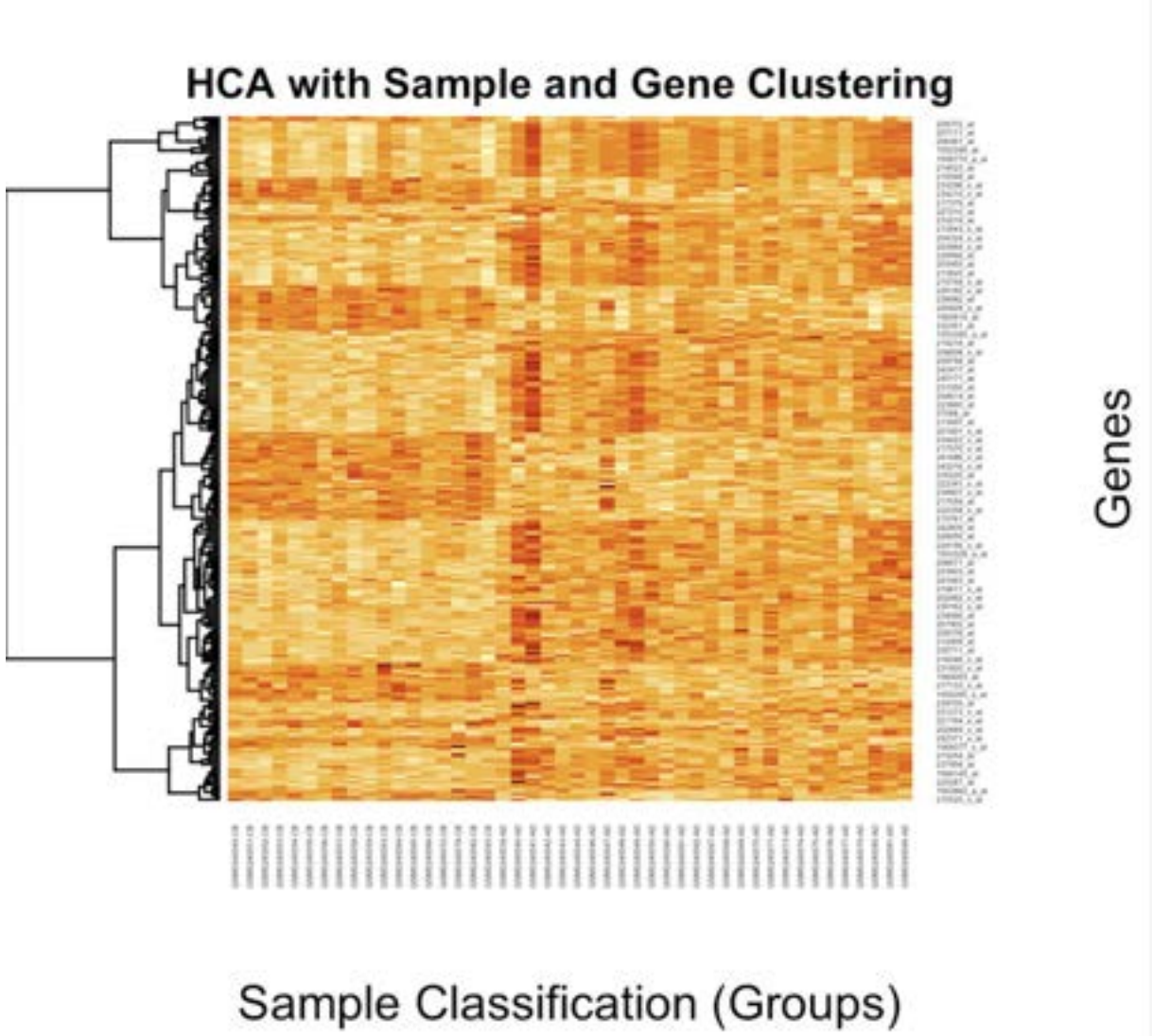


Fig 8. Clustering of Samples by groups and genes

Eisen, M. B., Spellman, P. T., Brown, P. O., & Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(25), 14863–14868.

Classification

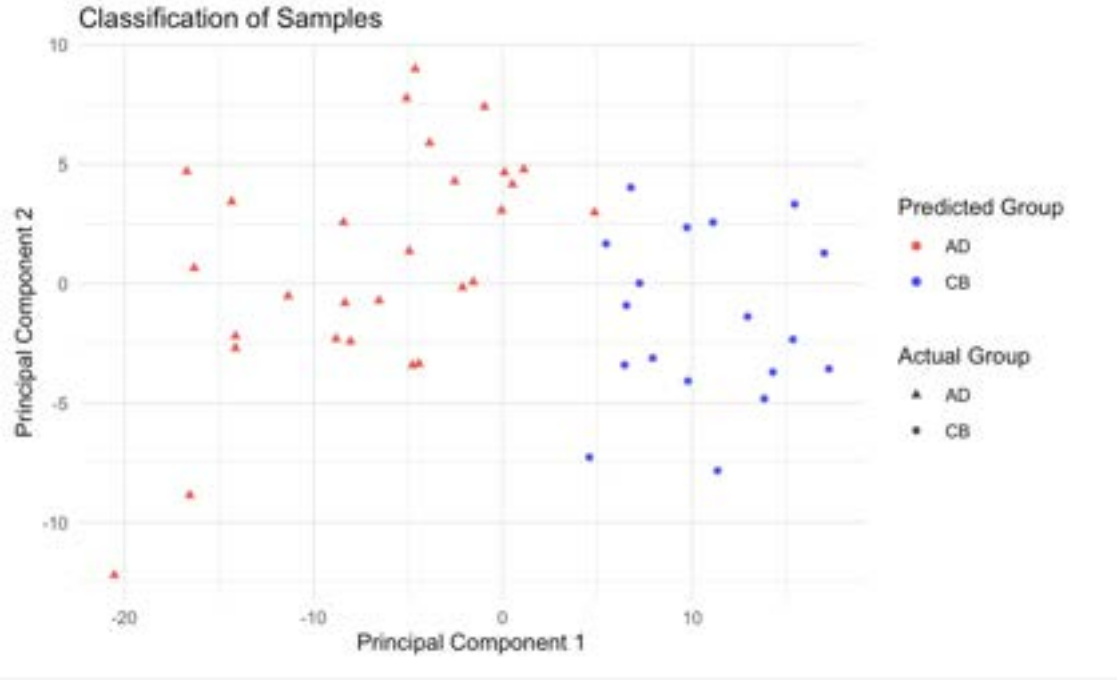


Fig 9. PCA plot of predicted and actual group classification on LRM.

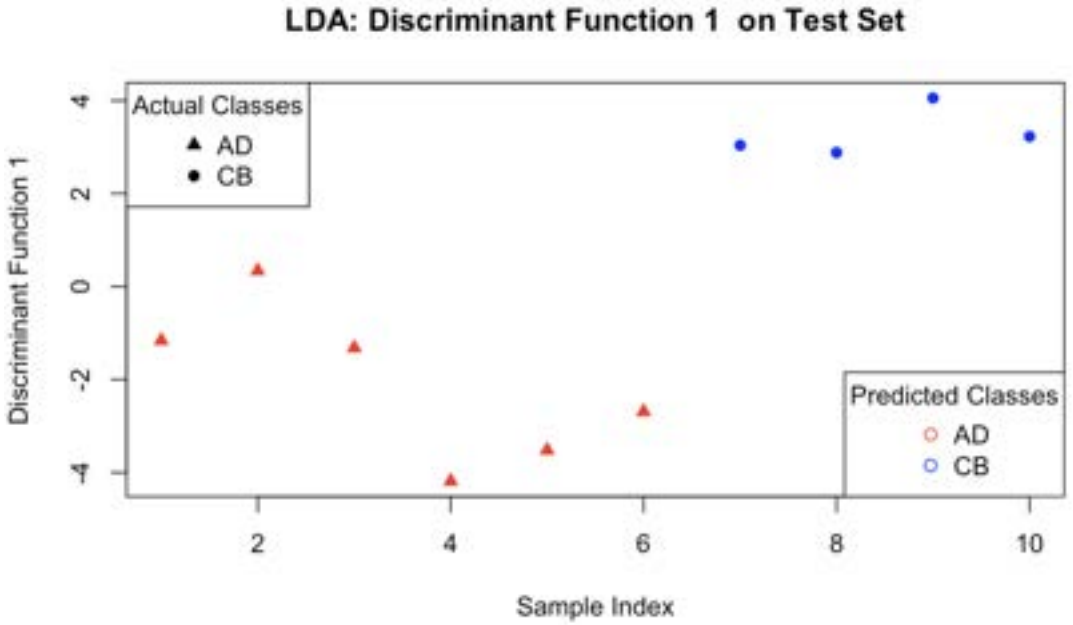


Fig 10. Plot of predicted and actual group LDA.

- Logistic Regression Model (LRM) chosen for its robustness in binary classification (Stoltzfus, 2011). However, misclassification 1 sample.

- LDA method used If the classes are well-separated (by PCA plot) since most samples are not overlapping and number of features is high relative to the number of observations. Based on 80:20 ratio, the LDA model classified all samples in test set correctly.

Stoltzfus J. C. (2011). Logistic regression: a brief primer. *Academic emergency medicine : official journal of the Society for Academic Emergency Medicine*, *18*(10), 1099–1104. https://doi.org/10.1111/j.1553-2712.2011.01185.x

Gene Enrichment Analysis

Significant probes:
1552863_a_at
1558770_a_at
201801_s_at
201802_at
216048_s_at
238279_x_at
242428_at
220828_s_at
242467_at
217559_at

NCBI DAVID Output:

216048_s_at	Rho related BTB domain containing 3(RHOBTB3)	Related Genes	Homo sapiens
GOTERM_BP_DIRECT	actin filament organization, establishment or maintenance of cell polarity, small GTPase mediated signal transduction, regulation of cell shape, male gonad development, vesicle-mediated transport, cell migration, regulation of proteolysis, cortical cytoskeleton organization, regulation of actin cytoskeleton organization, retrograde transport, endosome to Golgi, proteasome-mediated ubiquitin-dependent protein catabolic process,		
GOTERM_CC_DIRECT	cytoplasm, cytosol, cytoskeleton, plasma membrane, cell cortex, cytoplasmic vesicle, trans-Golgi network membrane, cell projection, extracellular exosome,		
GOTERM_MF_DIRECT	GTPase activity, protein binding, ATP binding, GTP binding, ATPase activity, protein kinase binding, small GTPase binding, ubiquitin protein ligase binding,		
INTERPRO	BTB/POZ-like, Small GTPase superfamily, Small GTPase superfamily, Rho type, BTB/POZ fold, P-loop containing nucleoside triphosphate hydrolase,		
SMART	BTB,		
UP_KW_BIOLOGICAL_PROCESS	Transport,		
UP_KW_CELLULAR_COMPONENT	Golgi apparatus,		
UP_KW_DOMAIN	Repeat,		
UP_KW_LIGAND	ATP-binding, Nucleotide-binding,		
UP_KW_MOLECULAR_FUNCTION	Hydrolase,		
UP_SEQ_FEATURE	DOMAIN:BTB, DOMAIN:BTB 1, DOMAIN:BTB 2, MUTAGEN:A->T: Abolishes interaction with RAB9A., MUTAGEN:D->E: Abolishes interaction with RAB9A., MUTAGEN:I->K: Abolishes interaction with RAB9A., MUTAGEN:Missing: Does not affect subcellular location, suggesting this protein is not prenylated., MUTAGEN:N->D: Abolishes ATP-binding., REGION:Disordered, REGION:Interaction with Rab9, REGION:Rho-like,		
1552863_a_at	**calcium voltage-gated channel auxiliary subunit gamma 6(CACNG6)**	**Related Genes**	**Homo sapiens**
GOTERM_BP_DIRECT	calcium ion transport, regulation of ion transmembrane transport, calcium ion transmembrane transport, regulation of calcium ion transmembrane transport via high voltage-gated calcium channel,		
GOTERM_CC_DIRECT	plasma membrane, voltage-gated calcium channel complex, integral component of membrane, L-type voltage-gated calcium channel complex,		
GOTERM_MF_DIRECT	voltage-gated ion channel activity, voltage-gated calcium channel activity, calcium channel regulator activity, calcium channel activity,		
INTERPRO	PMP-22/EMP/MP20/Claudin, Voltage-dependent calcium channel, gamma subunit, Voltage-dependent calcium channel, gamma-6 subunit,		
KEGG_PATHWAY	MAPK signaling pathway, Cardiac muscle contraction, Adrenergic signaling in cardiomyocytes, Oxytocin signaling pathway, Hypertrophic cardiomyopathy, Arrhythmogenic right ventricular cardiomyopathy, Dilated cardiomyopathy,		
UP_KW_BIOLOGICAL_PROCESS	Calcium transport, Ion transport, Transport,		
UP_KW_CELLULAR_COMPONENT	Membrane, Cell membrane,		
UP_KW_DOMAIN	Transmembrane, Transmembrane helix,		
UP_KW_LIGAND	Calcium,		
UP_KW_MOLECULAR_FUNCTION	Calcium channel, Ion channel, Voltage-gated channel,		
UP_SEQ_FEATURE	REGION:Disordered, TRANSMEM:Helical,		
1558770_a_at	**phosphoinositide-3-kinase regulatory subunit 6(PIK3R6)**	**Related Genes**	**Homo sapiens**
GOTERM_BP_DIRECT	angiogenesis, immune response, G-protein coupled receptor signaling pathway, regulation of natural killer cell mediated cytotoxicity, positive regulation of MAP kinase activity, positive regulation of T cell differentiation, positive regulation of angiogenesis,		
GOTERM_CC_DIRECT	cytosol, plasma membrane, phosphatidylinositol 3-kinase complex, phosphatidylinositol 3-kinase complex, class 1A, phosphatidylinositol 3-kinase complex, class 1B, membrane,		
GOTERM_MF_DIRECT	protein binding, kinase activity, phosphatidylinositol-4,5-bisphosphate 3-kinase activity, 1-phosphatidylinositol-3-kinase regulator activity,		
INTERPRO	Phosphoinositide 3-kinase 1B, gamma adapter, p101 subunit,		
KEGG_PATHWAY	cGMP-PKG signaling pathway, Chemokine signaling pathway, Phospholipase D signaling pathway, PI3K-Akt signaling pathway, Adrenergic signaling in cardiomyocytes, Apelin signaling pathway, Platelet activation, Cholinergic synapse, Oxytocin signaling pathway, Toxoplasmosis, Kaposi sarcoma-associated herpesvirus infection,		
UP_KW_BIOLOGICAL_PROCESS	Angiogenesis,		
UP_KW_CELLULAR_COMPONENT	Membrane, Cytoplasm, Cell membrane,		
UP_KW_MOLECULAR_FUNCTION	Kinase, Transferase,		
UP_SEQ_FEATURE	REGION:Disordered,		

Gene Enrichment Analysis

Significant probes:
1552863_a_at
1558770_a_at
201801_s_at
201802_at
216048_s_at
238279_x_at
242428_at
220828_s_at
242467_at
217559_at

NCBI DAVID Output (con't)

217559_at	ribosomal protein L10 like(RPL10L)	Related Genes	Homo sapiens
GOTERM_BP_DIRECT	ribosomal large subunit assembly, negative regulation of transcription from RNA polymerase II promoter, cytoplasmic translation, translation, regulation of translation, male meiosis I, spermatogenesis, cell differentiation, negative regulation of apoptotic process, embryonic brain development,		
GOTERM_CC_DIRECT	nucleus, endoplasmic reticulum, cytosol, ribosome, polysome, membrane, cytosolic large ribosomal subunit, cytosolic ribosome, macromolecular complex,		
GOTERM_MF_DIRECT	RNA binding, structural constituent of ribosome, translation regulator activity,		
INTERPRO	Ribosomal protein L10e, Ribosomal protein L10e/L16, Ribosomal protein L10e, conserved site,		
KEGG_PATHWAY	Ribosome, Coronavirus disease - COVID-19,		
OMIM_DISEASE	Spermatogenic failure 63,		
PIR_SUPERFAMILY	ribosomal protein L10a/L10e types,		
UP_KW_BIOLOGICAL_PROCESS	Differentiation, Meiosis, Spermatogenesis, Translation regulation,		
UP_KW_CELLULAR_COMPONENT	Cytoplasm,		
UP_KW_DISEASE	Disease variant, Intellectual disability, Autism spectrum disorder, Autism,		
UP_KW_MOLECULAR_FUNCTION	Developmental protein, Ribonucleoprotein, Ribosomal protein, Developmental protein,		
UP_KW_PTM	Citrullination, Ubl conjugation, Isopeptide bond,		
UP_SEQ_FEATURE	CROSSLNK:Glycyl lysine isopeptide (Lys-Gly) (interchain with G-Cter in SUMO2), CROSSLNK:Glycyl lysine isopeptide (Lys-Gly) (interchain with G-Cter in ubiquitin), DOMAIN:Ribosomal protein L10e/L16, REGION:Disordered,		
201801_x_at, 201802_at	solute carrier family 29 member 1 (Augustine blood group)(SLC29A1)	Related Genes	Homo sapiens
GOTERM_BP_DIRECT	neurotransmitter uptake, nucleobase-containing compound metabolic process, xenobiotic metabolic process, neurotransmitter transport, drug transmembrane transport, lactation, nucleobase transport, adenine transport, nucleoside transport, purine nucleoside transmembrane transport, cytidine transport, uridine transport, sleep, adenosine transport, inosine transport, hypoxanthine transport, thymine transport, excitatory postsynaptic potential, regulation of biological quality, cellular response to glucose stimulus, cellular response to hypoxia, pyrimidine-containing compound transmembrane transport, neurotransmitter reuptake, nucleoside transmembrane transport, guanine transmembrane transport, uracil transmembrane transport, pyrimidine nucleobase transmembrane transport, purine nucleobase transmembrane transport,		
GOTERM_CC_DIRECT	plasma membrane, integral component of plasma membrane, membrane, integral component of membrane, basolateral plasma membrane, apical plasma membrane, presynapse, postsynapse,		
GOTERM_MF_DIRECT	neurotransmitter transporter activity, nucleoside transmembrane transporter activity, adenine transmembrane transporter activity, guanine transmembrane transporter activity, uracil transmembrane transporter activity, purine nucleoside transmembrane transporter activity, cytidine transmembrane transporter activity, uridine transmembrane transporter activity, pyrimidine- and adenine-specific sodium symporter activity, AMP transmembrane transporter activity,		
INTERPRO	Equilibrative nucleoside transporter,		
KEGG_PATHWAY	Alcoholism,		
PIR_SUPERFAMILY	equilibrative nucleoside transporter,		
UP_KW_BIOLOGICAL_PROCESS	Transport,		
UP_KW_CELLULAR_COMPONENT	Membrane, Cell membrane,		
UP_KW_DOMAIN	Transmembrane, Transmembrane helix,		
UP_KW_PTM	Glycoprotein, Phosphoprotein,		
UP_SEQ_FEATURE	CARBOHYD:N-linked (GlcNAc...) asparagine, MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-193; S-213; S-222; S-297; S-333; S-378; S-414; S-416 and S-439. No change in nucleobase transport; when associated with S-193; S-213; S-222; S-297; S-333; S-378; S-416 and S-439., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-333; S-378; S-414 and S-416. No change in nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-333; S-378 and S-416., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-333; S-378; S-414 and S-439. No change in nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-333; S-378 and S-439., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-333; S-378; S-416 and S-439., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-333; S-414; S-416 and S-439. No change in nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-333; S-416 and S-439., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-378; S-414; S-416 and S-439. No change in nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-297; S-378; S-416 and S-439., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-333; S-378; S-414; S-416 and S-439. No change in nucleobase transport; when associated with S-87; S-193; S-213; S-222; S-333; S-378; S-416 and S-439., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-193; S-213; S-297; S-333; S-378; S-414; S-416 and S-439. No change in nucleobase transport; when associated with S-87; S-193; S-213; S-297; S-333; S-378; S-416 and S-439., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-193; S-222; S-297; S-333; S-378; S-414; S-416 and S-439. No change in nucleobase transport; when associated with S-87; S-193; S-222; S-297; S-333; S-378; S-416 and S-439., MUTAGEN:C->S: Loss of nucleobase transport; when associated with S-87; S-213; S-222; S-297; S-333; S-378; S-414; S-416 and S-439. No change in nucleobase transport; when associated with S-87; S-213; S-222; S-297; S-333; S-378; S-416 and S-439., MUTAGEN:Missing: No effect on subcellular localization and inosine transport., MUTAGEN:R->A: No effect on subcellular localization and inosine transport., REGION:Disordered, SITE:Essential for		

Gene Enrichment Analysis

Significant probes:
1552863_a_at
1558770_a_at
201801_s_at
201802_at
216048_s_at
238279_x_at
242428_at
220828_s_at
242467_at
217559_at

Affymetrix Output (accessed through https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL570)

ID	GB_ACC	SPOT_ID	Gene.Symbo	RefSeq.Transcript.ID	Gene.Ontology.Biological.Process	Gene.Ontology.Cellular.C	Gene.Ontology.Molecular.Function
216048_s_at	AK023621		RHOBTB3	NM_014899	0006200 // ATP catabolic process // inferre	0005794 // Golgi apparat	0000166 // nucleotide binding // inferred from
1558770_a_	AK091819		PIK3R6	NM_001010855 /// NM_00129021	0001525 // angiogenesis // inferred from el	0005737 // cytoplasm //	0005515 // protein binding // inferred from phy
238279_x_at	BF062155						
242428_at	N58513						
220828_s_at	NM_018382		FLJ11292	NM_018382			
242467_at	BF433200						
217559_at	AI001784		RPL10L	NM_080746	0006412 // translation // inferred from elec	0005622 // intracellular /	0003735 // structural constituent of ribosome /
1552863_a_	NM_145815		CACNG6	NM_031897 /// NM_145814 /// NM	0006810 // transport // inferred from electr	0005891 // voltage-gatec	0005244 // voltage-gated ion channel activity /
201802_at	NM_004955		SLC29A1	NM_001078174 /// NM_001078175	0006139 // nucleobase-containing compoun	0005886 // plasma mem	0005337 // nucleoside transmembrane transpo
201801_s_at	AF079117		SLC29A1	NM_001078174 /// NM_001078175	0006139 // nucleobase-containing compoun	0005886 // plasma mem	0005337 // nucleoside transmembrane transpo

- Majority of the enrichment show blood markers, ribosomal and transport protein markers.
- However, most show no major functional difference between AD and CB groups unlike in skin samples (Brunner et al., 2016)
- Immune Response Markers :
  - 1558770_a_at maps to phosphoinositide-3-kinase regulatory subunit 6(PIK3R6) involved in immune response. DEG significantly upregulated. Involved in MAPK signalling modulating Th2 expression. Interleukin-19 (IL-19) is implicated in the modulation of Th2 cytokine expression via MAPK signaling pathways.
  - IL-19 is a pro-inflammatory cytokine essential for the production of Th2 cytokines. Stimulated by IL-17, it serves as a bridge between the Th17 and Th2 cytokine pathways. Significant activation of both Th17 and Th2 cytokine pathways is observed in pediatric AD. In contrast, adult AD shows a dominant Th2 activation with less Th17 involvement (Esaki et al, 2016). IL-19 is potentially crucial for enhancing Th2 cytokine secretion in AD.
  - Suggested to play a similar role in other atopic diseases, such as asthma (Kannan et. al, 2012).
  - A strong Th2 signature in early-onset pediatric AD implicates IL-19 and related pathways as promising therapeutic targets.

Brunner, P. M., Israel, A., Zhang, N., … ,Estrada, Y. D., Xu, H., Krueger, J. G., Paller, A. S., & Guttman-Yassky, E. (2018). Early-onset pediatric atopic dermatitis is characterized by TH2/TH17/TH22-centered inflammation and lipid alterations. The Journal of allergy and clinical immunology, 141(6), 2094–2106.

Esaki, H., Brunner, P. M., Renert-Yuval. (2016). Early-onset pediatric atopic dermatitis is TH2 but also TH17 polarized in skin. The Journal of allergy and clinical immunology, 138(6), 1639–1651. https://doi.org/10.1016/j.jaci.2016.07.013

Kannan, Y., & Wilson, M. S. (2012). TEC and MAPK Kinase Signalling Pathways in T helper (TH) cell Development, TH2 Differentiation and Allergic Asthma. Journal of clinical & cellular immunology, Suppl 12, 11. https://doi.org/10.4172/2155-9899.S12-011

Conclusions

- A limited array of genes were identified as being dysregulated in the blood of children with AD compared to healthy controls, a contrast to the broader gene dysregulation seen in the skin.
- The gene expression patterns in blood shed light on the initial systemic immune response characteristic of early-onset pediatric AD. However, these patterns show minimal correlation with the clinical and molecular indicators of the disease observed in the skin, excluding a select number of immune markers, such as IL-19 and Th1 markers.
- This information about systemic blood inflammation could enhance our understanding of the mechanisms that contribute to the progression of the atopic march and could be instrumental in developing treatments aimed at interrupting this progression.