Report: Differential Expression Analysis of Immune Response to Influenza Vaccine

Naeela Mareta Ayudhia (BRSP Participant Number 243)

Background

Influenza (flu) is an acute respiratory infection caused by influenza viruses (types A, B, and C), with types A and B responsible for most human disease and seasonal epidemics. Severe influenza can sometimes lead to hospitalization or death. Annual vaccination is recommended to provide protective immunity and prevent serious complications. The mechanisms underlying individual responses to vaccination can be studied through transcriptomic analyses of gene expression. In this report, differentially expressed genes (DEGs) were identified between individuals who received the influenza vaccine and those who had not received it.

Methods

The dataset series GSE48018 was obtained from Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information (NCBI). The samples used for analysis consisted of whole-blood RNA collected from healthy male human volunteers who had been vaccinated. Baseline samples were obtained from the same volunteers prior to vaccination. Gene expression profiling was performed using a microarray platform, specifically the Illumina HumanHT-12 V4.0 Expression BeadChip (GPL10558). The dataset originates from a study conducted by Bucasas et al. (2011).

Transcriptomic analysis was performed in R using RStudio. The dataset was retrieved using the GEOquery package. The data then underwent preprocessing steps, including quality control assessment, log2 transformation, and inspection of normalization. Differential expression analysis was conducted using the limma package based on linear modeling. Data distribution was evaluated using boxplots, histograms, and UMAP visualization. For visualization of DEGs, volcano plots and heat maps were used to facilitate gene identification and biological interpretation. Functional enrichment analysis was performed using g:Profiler to identify Gene Ontology (GO) terms, and KEGG Mapper was used to visualize genes within KEGG pathway maps.

Result and Interpretation

The DEGs were visualized using a volcano plot to highlight genes that were significantly differentially expressed between post-vaccination samples and baseline samples. In the plot, red represents upregulated genes and blue represents downregulated genes. The visualization indicates a moderate number of upregulated genes and fewer downregulated genes. This pattern is consistent with the mechanism of vaccination, which induces

controlled transcriptional changes associated with immune activation rather than widespread transcriptomic alteration.

To identify the top upregulated and downregulated genes, the top 20 DEGs were sorted based on the highest and lowest log fold-change (logFC) values. The results are presented in Table 1.

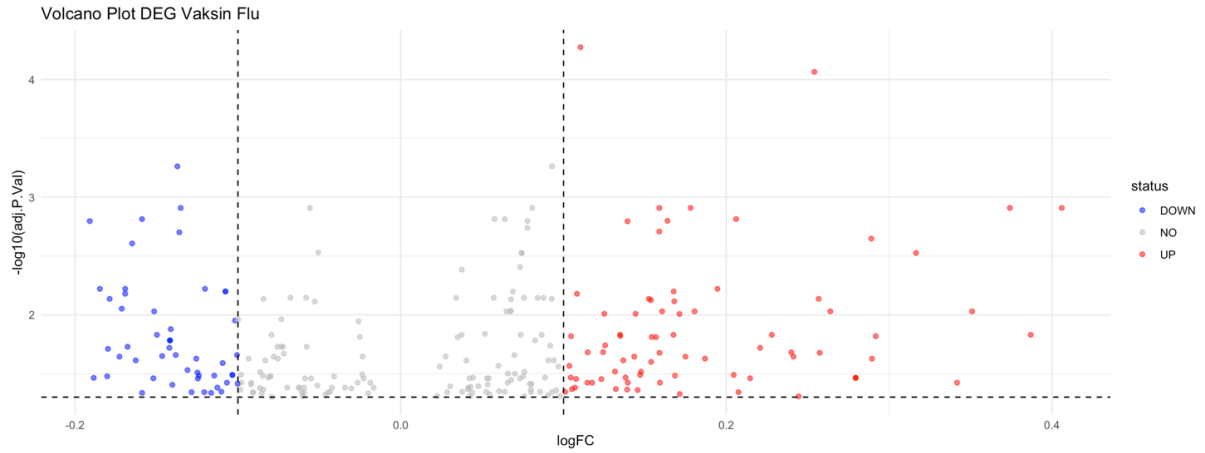


Figure 1 Volcano plot of differentially expressed genes in post-vaccination samples compared to baseline

Table 1 Top 20 upregulated and downregulated genes in post-vaccination samples compared to baseline

Upregulated genes			Downregulated genes		
logFC	**adj.P.Val**	**SYMBOL**	**logFC**	**adj.P.Val**	**SYMBOL**
0,4060	0,0012	IFIT2	-0,1910	0,0016	ERAP2
0,3870	0,0148	EPSTI1	-0,1886	0,0343	NLRP12
0,3742	0,0012	STAT1	-0,1848	0,0060	CEP19
0,3510	0,0093	GBP5	-0,1803	0,0333	NPIPB13
0,3417	0,0377	HERC5	-0,1788	0,0073	ZMAT3
0,3165	0,0030	GBP1	-0,1728	0,0226	S1PR4
0,2918	0,0152	GBP1	-0,1714	0,0089	GRIPAP1
0,2895	0,0235	UBE2L6	-0,1692	0,0060	FYN
0,2890	0,0022	GBP2	-0,1692	0,0066	ZNF394
0,2794	0,0343	FCGR1A	-0,1678	0,0187	MTMR12
0,2794	0,0343	FCGR1BP	-0,1628	0,0244	ZNF549
0,2794	0,0343	FCGR1CP	-0,1589	0,0460	ZNF69
0,2639	0,0093	STAT2	-0,1520	0,0346	XRCC2
0,2573	0,0210	DHRS9	-0,1514	0,0093	DUSP19
0,2567	0,0073	TRIM22	-0,1498	0,0148	SEMA3E
0,2540	0,0001	MAFB	-0,1465	0,0224	TBC1D10C
0,2444	0,0492	OAS2	-0,1421	0,0190	CREB1

0,2412	0,0226	PARP9	-0,1417	0,0165	STAG3L1
0,2399	0,0208	GBP4	-0,1417	0,0165	STAG3L3
0,2279	0,0148	CAVIN2	-0,1417	0,0165	STAG3L2

A heat map of the top 50 DEGs was generated to display gene expression patterns across samples. The visualization demonstrates clear gene-level expression differences with partial clustering by conditions, although the separation is not perfect. Overall, global gene expression remains largely similar between groups; however, specific immune-response genes (STAT1, GBP1, ISG20L2, TLR7, IFIT2, etc.) exhibit coordinated regulation following vaccination. This indicates pathway-level immune activation rather than broad transcriptomic remodeling.

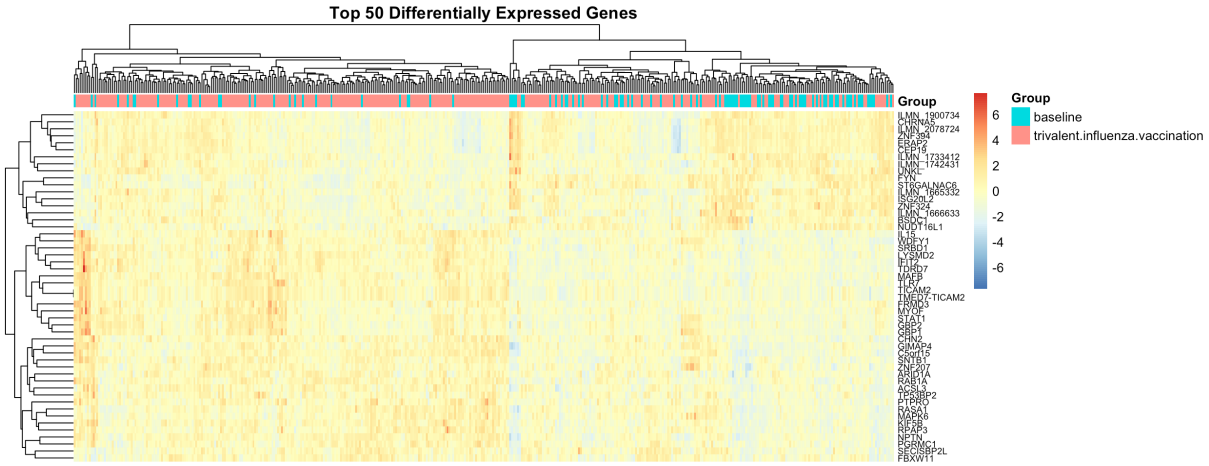
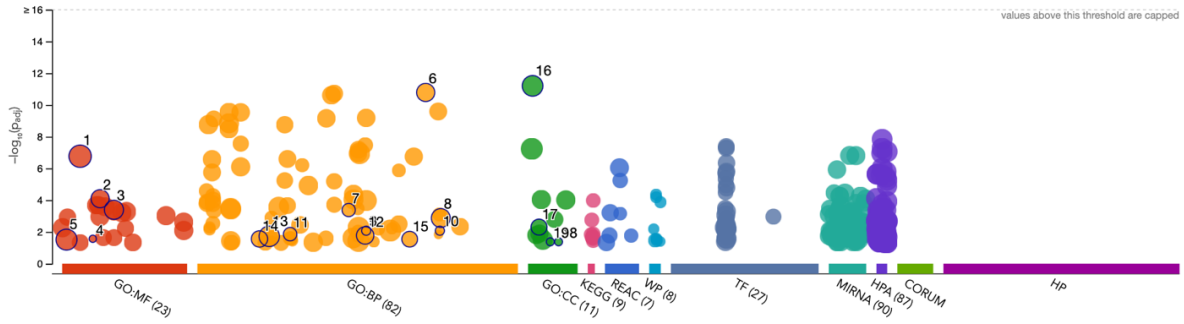


Figure 2 Heat map of the top 50 differentially expressed genes (DEGs) in post-vaccination samples compared to baseline samples

Enrichment analysis of upregulated genes was performed using g:Profiler. For Gene Ontology Biological Process (GO:BP), enriched terms included “defense response to another organism”, “antigen processing and presentation”, and “cytolysis in another organism”, indicating activation of innate antiviral sensing and adaptive immune priming. Gene Ontology Cellular Component (GO:CC) enrichment identified terms such as “cytosol,” which is associated with RIG-I-like receptor signaling, and “ISGF3 complex,” a STAT1–STAT2–IRF9 transcriptional complex downstream of type I interferon signaling. Gene Ontology Molecular Function (GO:MF) enrichment included terms such as “ribonucleotide binding” and “protein binding,” which are consistent with antiviral effector functions.

KEGG pathway mapping further supported the GO results. In the Influenza A pathway map (Figure 5), several key antiviral genes were highlighted, including TLR7, TRAF3, TBK1, STAT1, STAT2, OAS, CASP1, and Rab. These findings illustrate key mechanisms of the immune response following vaccination, including viral recognition (e.g., TLR7 and positive regulation

of type I interferon production), interferon signaling (JAK-STAT pathway leading to ISGF3 complex formation), induction of interferon-stimulated genes (e.g., OAS), inflammasome activation (e.g., CASP1), and antigen processing (e.g., TAP complex). These results indicate that influenza vaccination triggers coordinated innate immune sensing and interferon-mediated antiviral responses.



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0005515	protein binding	1.702×10^{-7}
2	GO:MF	GO:0017111	ribonucleoside triphosphate phosphatase activ...	8.074×10^{-5}
3	GO:MF	GO:0032555	purine ribonucleotide binding	4.046×10^{-4}
4	GO:MF	GO:0015433	ABC-type peptide antigen transporter activity	2.713×10^{-2}
5	GO:MF	GO:0003824	catalytic activity	3.067×10^{-2}
6	GO:BP	GO:0098542	defense response to other organism	1.638×10^{-11}
7	GO:BP	GO:0048002	antigen processing and presentation of peptid...	4.191×10^{-4}
8	GO:BP	GO:0141124	intracellular signaling cassette	1.342×10^{-3}
9	GO:BP	GO:0051715	cytolysis in another organism	8.415×10^{-3}
10	GO:BP	GO:0140970	AIM2 inflammasome complex assembly	8.415×10^{-3}
11	GO:BP	GO:0032481	positive regulation of type I interferon producti...	1.388×10^{-2}
12	GO:BP	GO:0051603	proteolysis involved in protein catabolic process	1.683×10^{-2}
13	GO:BP	GO:0019538	protein metabolic process	1.894×10^{-2}
14	GO:BP	GO:0016032	viral process	2.777×10^{-2}
15	GO:BP	GO:0072659	protein localization to plasma membrane	2.874×10^{-2}
16	GO:CC	GO:0005829	cytosol	6.290×10^{-12}
17	GO:CC	GO:0030139	endocytic vesicle	4.997×10^{-3}
18	GO:CC	GO:0070721	ISGF3 complex	4.202×10^{-2}
19	GO:CC	GO:0042825	TAP complex	4.202×10^{-2}

version	e113_eg59_p19_6be52918
date	19/02/2026, 23:11:11
organism	hsapiens

g:Profiler

Figure 3 Enrichment analysis of Gene Ontology (GO) terms for upregulated genes in post-vaccination samples

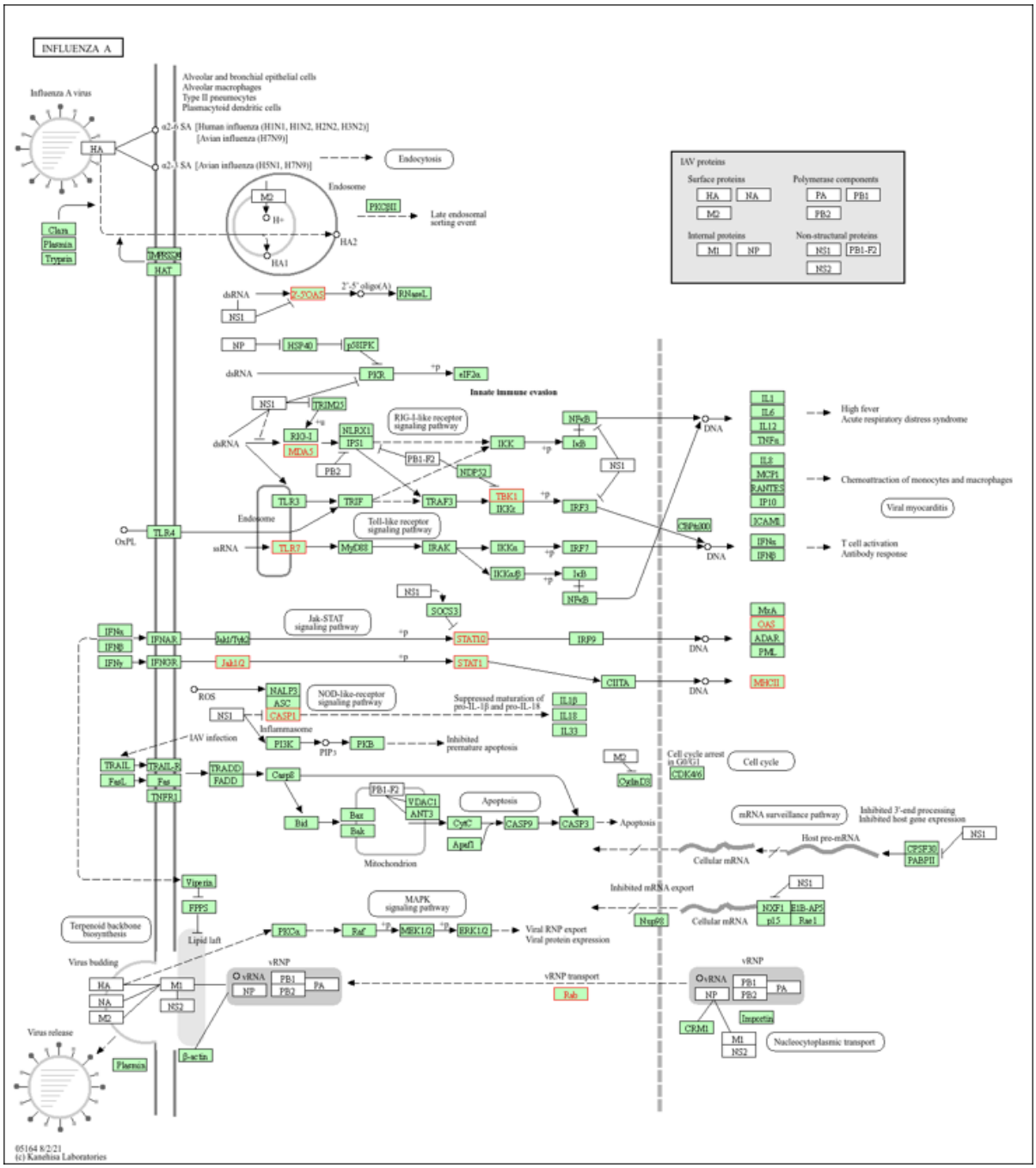


Figure 4 KEGG Influenza A pathway map with key genes highlighted in red

Conclusion

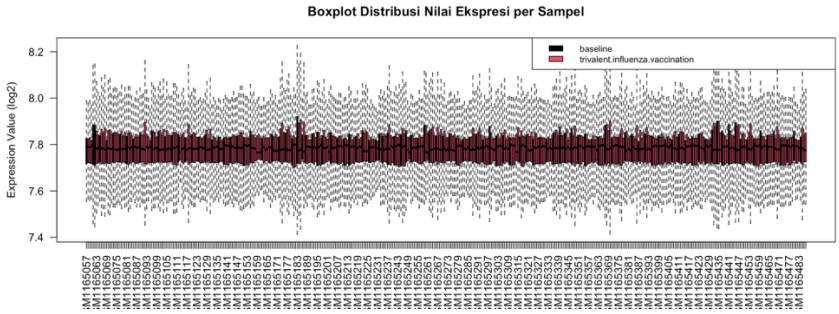
This report identified differentially expressed genes (DEGs) associated with immune responses following influenza vaccination using transcriptomic analysis of whole-blood samples. The results revealed moderate but coordinated upregulation of immune-related

genes, particularly interferon-stimulated genes such as STAT1, STAT2, IFIT2, GBP1, OAS2, and TRIM22, indicating activation of antiviral defense pathways. Functional enrichment and KEGG pathway analyses further demonstrated the involvement of innate viral sensing mechanisms, type I interferon signaling, inflammasome activation, and antigen processing and presentation pathways. Although global gene expression patterns remained largely similar between baseline and post-vaccination samples, pathway-level immune activation was clearly observed. Overall, these findings demonstrate that influenza vaccination induces a controlled yet coordinated transcriptional immune response characterized primarily by interferon-mediated antiviral mechanisms.

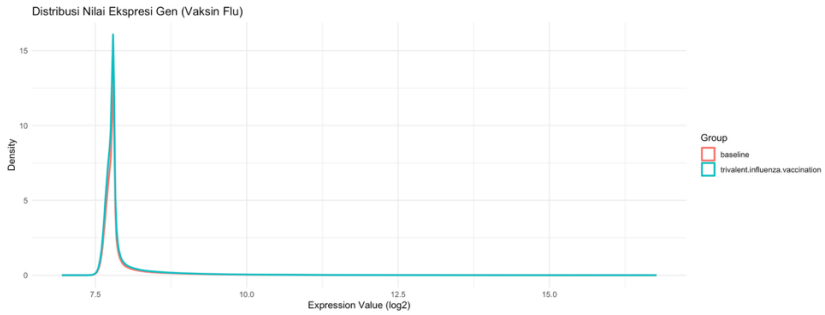
References

Bucasas, K. L., Franco, L. M., Shaw, C. A., Bray, M. S., Wells, J. M., Niño, D., Arden, N., Quarles, J. M., Couch, R. B., & Belmont, J. W. (2011). Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. The Journal of infectious diseases, 203(7), 921–929. https://doi.org/10.1093/infdis/jiq156

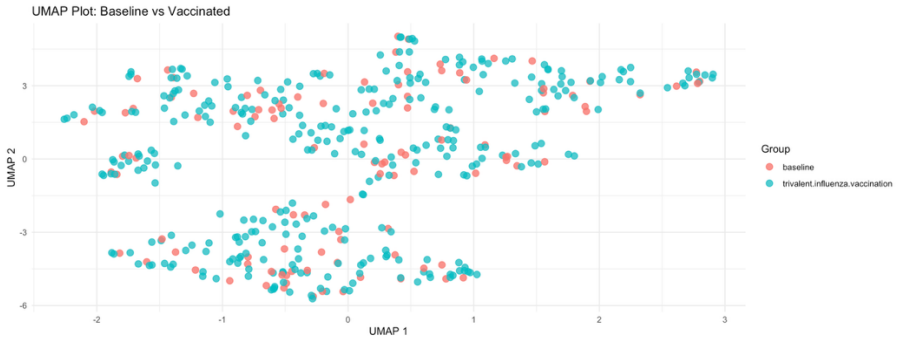
Appendix



Appendix 1 Boxplot of expression value distribution per sample



Appendix 2 Histogram of gene expression value distribution



Appendix 3 UMAP plot of baseline and post-vaccination samples