# Analysis of gene expressioin of CD4+CD8+ double positive cell under granulomatiotis with polyangiitis(GPA)

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## Introduction:

T cell is considered as an essential component of adaptive immune system. Expect TCR type of T cell, mature T cells are generally considered to express either CD4+ or CD8+ co-receptor, which means T cell pool can be divided into two subsets based on the expression of CD4+ and CD8+.[1] In order to find the expansion of circulating CD4+CD8+ double positive T-cells in a disease context, we compare the mRNA expression profiles of CD4+CD8+ double positive T-cells from patients with granulomatotis with polyangiitis(GPA) to healthy controls. The detailed mechanism of CD4+ and CD8+ positive double cell is presented in **Figure 1**.[2]

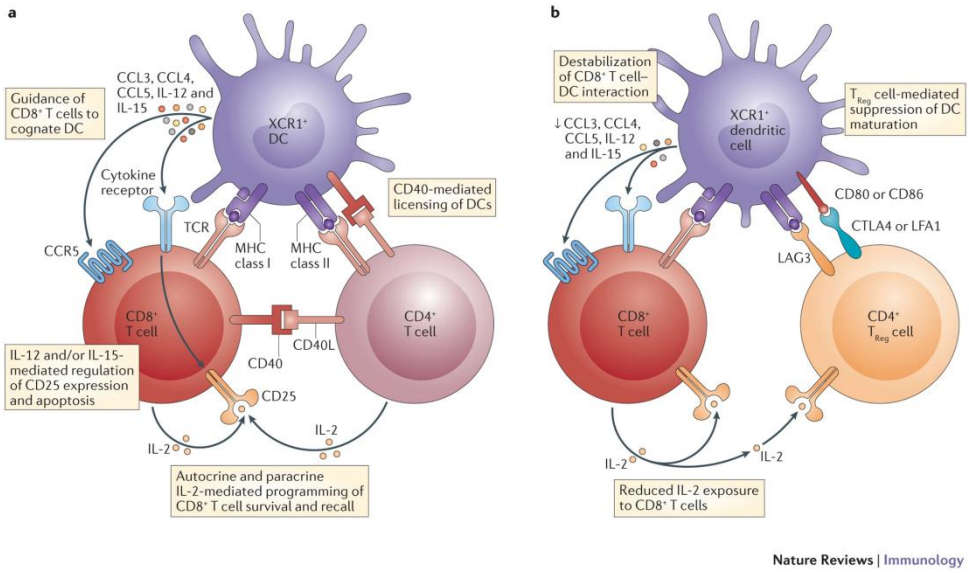


Figure 1: Regulating mechanism of CD4+ and CD8+ double positive cell

## Data analysis:

In this experiment, the data is divided into 18 sets. 9 sets are the expression of the CD4+, CD8+ and CD4+CD8+ of three patients with granulomatotis with polyangiitis(GPA). Other 9 sets are the expression of CD4+, CD8+ and CD4+CD8+ of three healthy controls. The data is extracted from GEO, the GEO accession is GSE56481. The expression data profiling is presented by mRNA micro array. To find

out the difference of the expression level of CD4+, CD8+ and CD4+CD8+ under disease, the linear model was used. The results were presented by volcano plot and heat map. Besides, the total number of the up-regulating gene and down-regulating gene were presented.

## Result:

### Regulation of gene

Initially, the linear model of contrast between GPA and healthy control analyzed. The health control was considered as the reference. Comparison with gene expression level in GPA, Figure 1 shows the total number of down-regulating gene, up-regulating gene and gene with no significant changing. Although the names of gene changing expression level were unknown, the number of up-regulating gene is higher than down-regulating gene. Based on the literature, it can be assumed that the frequency of the CD4+CD8+ double positive cell was increased with GPA.[3] The result of the linear model of contrast between GPA and healthy control is presented in **Figure 2**. The

| | GPA - health |
|---|---|
| Down | 198 |
| NotSig | 52970 |
| Up | 449 |

Figure 2: Qualitative of gene expression level changing in GPA

### Annotation

For the annotation, the hugene20sttranscriptcluster.db package in R is tried to use for the annotation of the gene name, and symbol. The annotation of up-regulating gene and down-regulating gene is showed in R script. For the result of the gene with no gene name is showed in **Figure 3**.

| | logFC | AveExpr | t | P.Value | adj.P.Val | B |
|---|---|---|---|---|---|---|
| 17112149 | -7.375445 | 7.512993 | -50.20524 | 1.249521e-22 | 6.699557e-18 | 32.26080 |
| 17116977 | 5.823362 | 5.811520 | 35.14874 | 1.512587e-19 | 4.055018e-15 | 29.36054 |
| 17117126 | 4.384196 | 5.229810 | 32.35424 | 7.798313e-19 | 1.186924e-14 | 28.50925 |
| 17116384 | 4.803789 | 5.367734 | 32.14695 | 8.854837e-19 | 1.186924e-14 | 28.44038 |
| 17118451 | 4.824495 | 5.198108 | 30.31896 | 2.812880e-18 | 2.820630e-14 | 27.79472 |
| 17116194 | 5.043164 | 5.640249 | 30.14230 | 3.156420e-18 | 2.820630e-14 | 27.72847 |

Figure 3: Head of microarray gene data

**Volcano Plot**

**Figure 4** shows the volcano plot of the analysis. Volcano plot is one of the result of the expression level. Different points in plot represent different gene respectively. The interesting gene is selected by the -log(p-value) and logFC. The p-value shows the level of difference of expression level while LogFC shows the fold change of the gene. The threshold of the p-value is 0.05 and the threshold of the logFC is 2. The red points are interesting gene with high difference expression level and high fold change. Points with logFC higher than 0 is up-regulating and vise versa. From the plot, it can be observed that the trend of gene expression level changing is up-regulating, which confirms the result from the previous part of analysis.

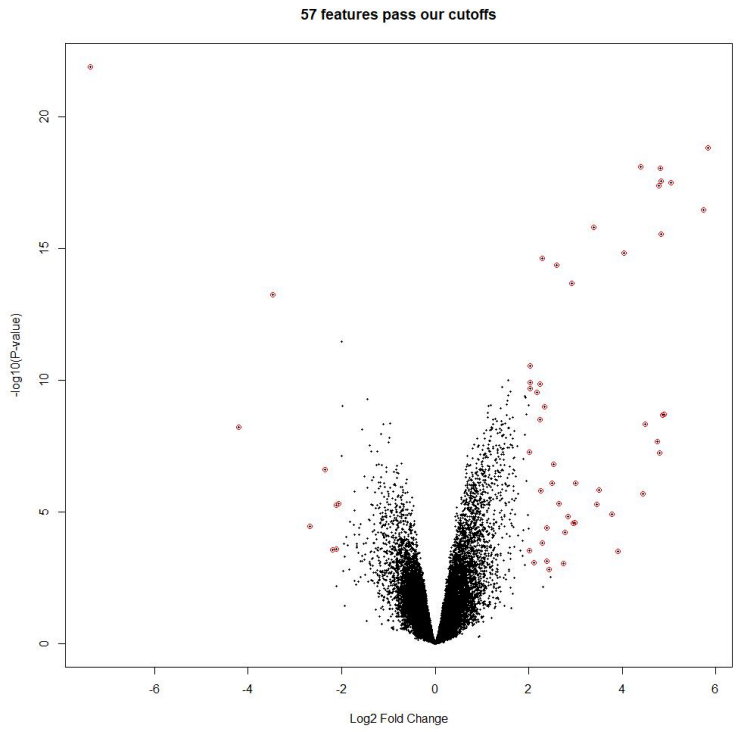


Figure 4: Volcano plot of the microarray analysis

**Heat map**

Figure 5 is the heat map of data analysis. Heat map shows the gene regulating trend in whole sample.The blue color shows the down-regulating of the gene and red color shows the up-regulating of gene. The shade of the color shows the expression level of the gene. The darker the color is, the changing of the gene expression is more obvious. From the heat map, it can be obviously observed that the most of the gene of CD4+ CD8+ and CD4+CD8+ gene is down-regulating, while gene up-regulate under the GPA, which mean CD4+CD8+ will be activated under the GPA.

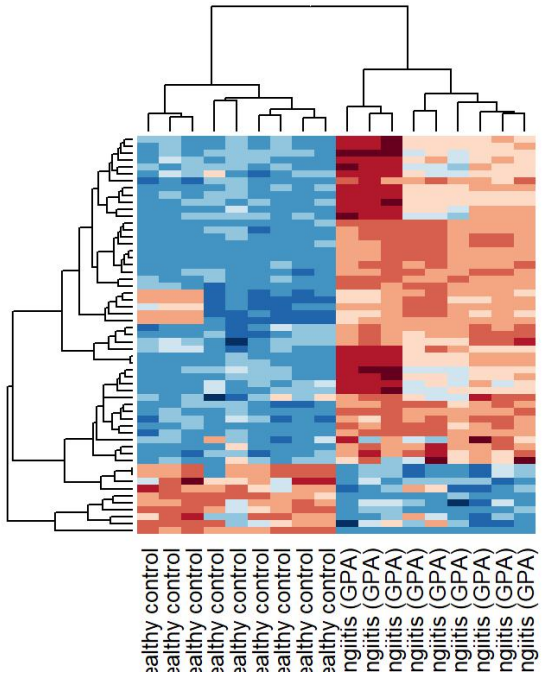


Figure 5: Heat map of the gene regulation in healthy control and GPA

**GSEA**

By using Gene Set Enrichment Analysis (GSEA), we could detect changes at the gene set or pathway level, which can contribute to phenotypic differences, even if they are low at the expression level of individual genes. Two different gene sets have been choose for our analysis (Hallmark gene sets and C7:IMMUNESIGDB) in Human MSigDB Collections. Hallmark gene sets summarize and represent specific well-defined biological states or processes and display coherent expression[4], which could reflect a general direction of gene set enrichment. In the first GSEA analysis

using the HH gene set, only one enriched gene set was obtained which is HALLMARK_INFLAMMATORY_RESPONSE. Considering that the study compared CD4+CD+8 double positive T-cell mRNA expressions from GPA patients and that GPA is a severe autoimmune disorder, we are not surprised by this result. In order to further understand the enriched gene set related to inflammatory response, we use C7 which is a immunologic signature gene sets represent cell states and perturbations within the immune system. The enrich results has shown in **Table 1.** We found that the gene sets that should be down-regulated with T-cell growth was paradoxically up-regulated in this experiment. This may suggest the activation of T cells in inflammatory diseases.

Table 1. Three gene sets with the most positive NES when using C7:IMMUNESIGDB

| ID | NES | P.adjust |
|---|---|---|
| GSE22886_NAIVE_CD8_TCELL_VS_MONOCYTE_DN | 2.56 | 1.15e-06 |
| GSE22886_NAIVE_TCELL_VS_MONOCYTE_DN | 2.51 | 2.31e-05 |
| GSE22886_NAIVE_CD4_TCELL_VS_MONOCYTE_DN | 2.45 | 2.31e-05 |

## Conclusion

The analysis showed the basic CD4+CD8+ gene expression level changing under disease. The gene expression level with disease GPA is compared to gene expression level with healthy control. The number of the regulating gene roughly shows that most of the CD4+CD8+ gene up-regulate under disease. The volcano map shows that with the threshold of p-value and fold change, the most gene still up-regulate under disease. The heat map shows the gene regulation of every sample. All healthy control in heat map shows a down-regulating while GPA sets shows the opposite.

Overall, based on the data analysis of micro array, the gene relate to CD4+CD8+ shows a high up-regulating expression when human body is under disease.

# Reference

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