# INSIGHT IN MIRNOME OF SEVERE MULTIPLE SCLEROSIS: PILOT STUDY OF DISTINCTIVE RELAPSE-ONSET MS PHENOTYPES

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## INTRODUCTION

Molecular background and biomarkers of highly heterogonous and hardly predictable disease progression among relapse-onset MS patients are of high research interest.

In the current pilot study, we aimed to employ next-generation sequencing to investigate the expression of whole small non-coding microRNAs (miRNome) in two groups of MS patients with highly distinctive progression phenotype: one with fast progressing, severely disabling course vs. mild course of MS, longitudinally followed >10 years.

## METHODS

**Sample:** Mild phenotype MS (n=4 patients), progressive phenotype MS (n=5 patients)

**miRNome-seq:** PBMC total RNA was processed using TakaraBio SMARTer smRNA-Seq Kit and sequenced on Illumina iSeq100 instrument.

**Differentially expressed miRNA (DEmiRNA) analysis:** Pre-processing of raw sequencing data, quality control miRNA mapping and annotation and differential expression analysis was performed using sRNAtoolbox pipeline. Results of DEseq2 algorithm are presented.

**Functional interpretation of DEmiRNA target genes:** DIANA-miRPathv3.0 based on Tarbase v7.0 as a resource of miRNA:gene interactions was employed.

## RESULTS

**DEmiRNA analysis**

Achieved read depth was approximately **1 million raw reads/sample**

After genome alignment and miRbase v22 annotation up to **92 mature miRNAs/sample** were detected.

Differential expression analysis identified the significant **upregulation of hsa-miR-23c (log2FC=4.29, Padj= 0.03) in progressive phenotype.**

**Top significantly enriched KEGG pathways in hsa-miR-23c targets**

| KEGG pathway | p-value | #genes |
|---|---|---|
| Antigen processing and presentation | 4.85E-06 | 3 |
| Phagosome | 2.44E-05 | 10 |
| Allograft rejection | 0.000131 | 3 |
| Viral carcinogenesis | 0.00073 | 10 |
| Protein processing in endoplasmic reticulum | 0.002962 | 10 |
| Graft-versus-host disease | 0.011572 | 3 |
| Glycosaminoglycan biosynthesis - keratan sulfate | 0.015224 | 1 |
| Epstein-Barr virus infection | 0.043198 | 8 |

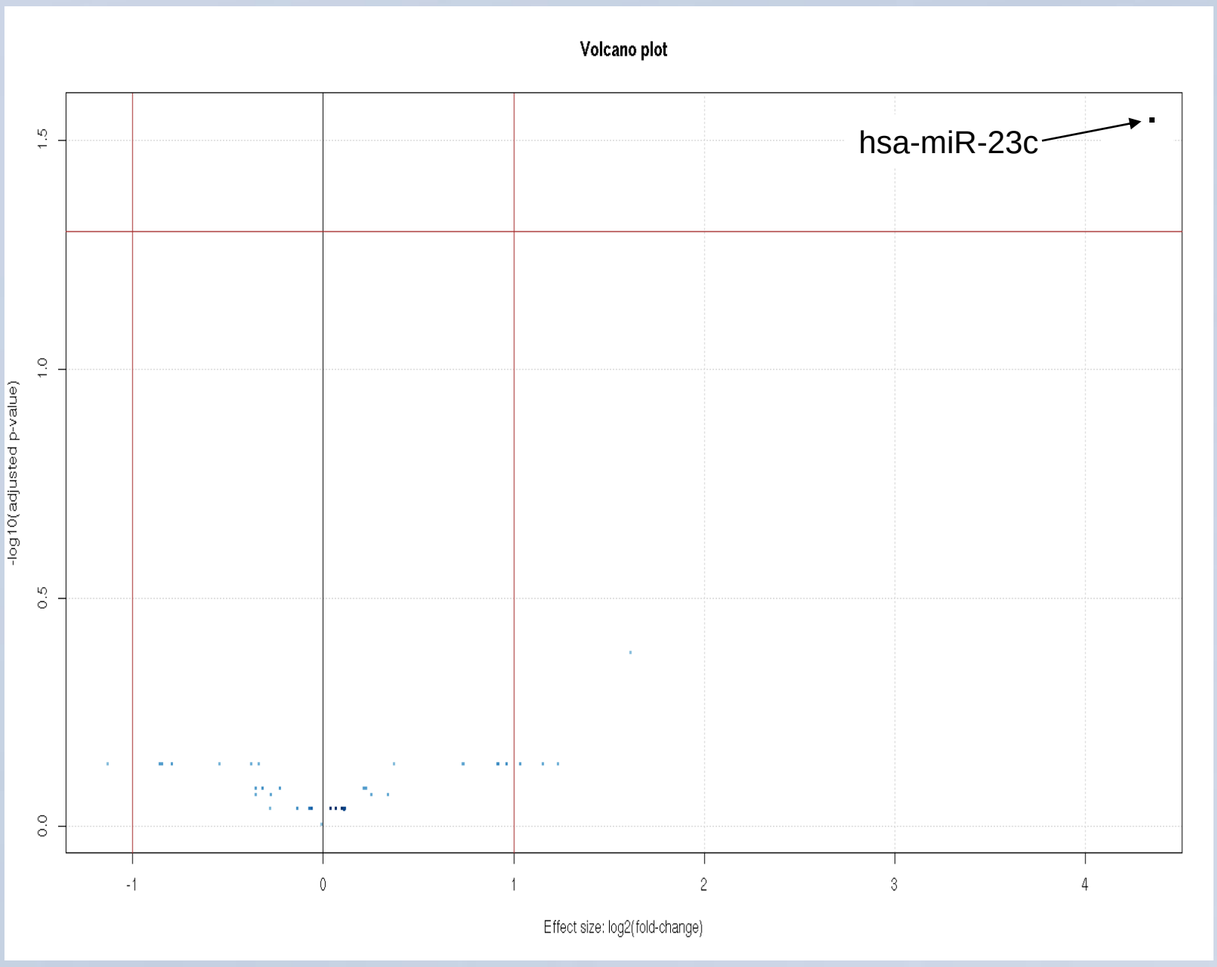


Fig 1. Volcano plot of DEmiRNAs

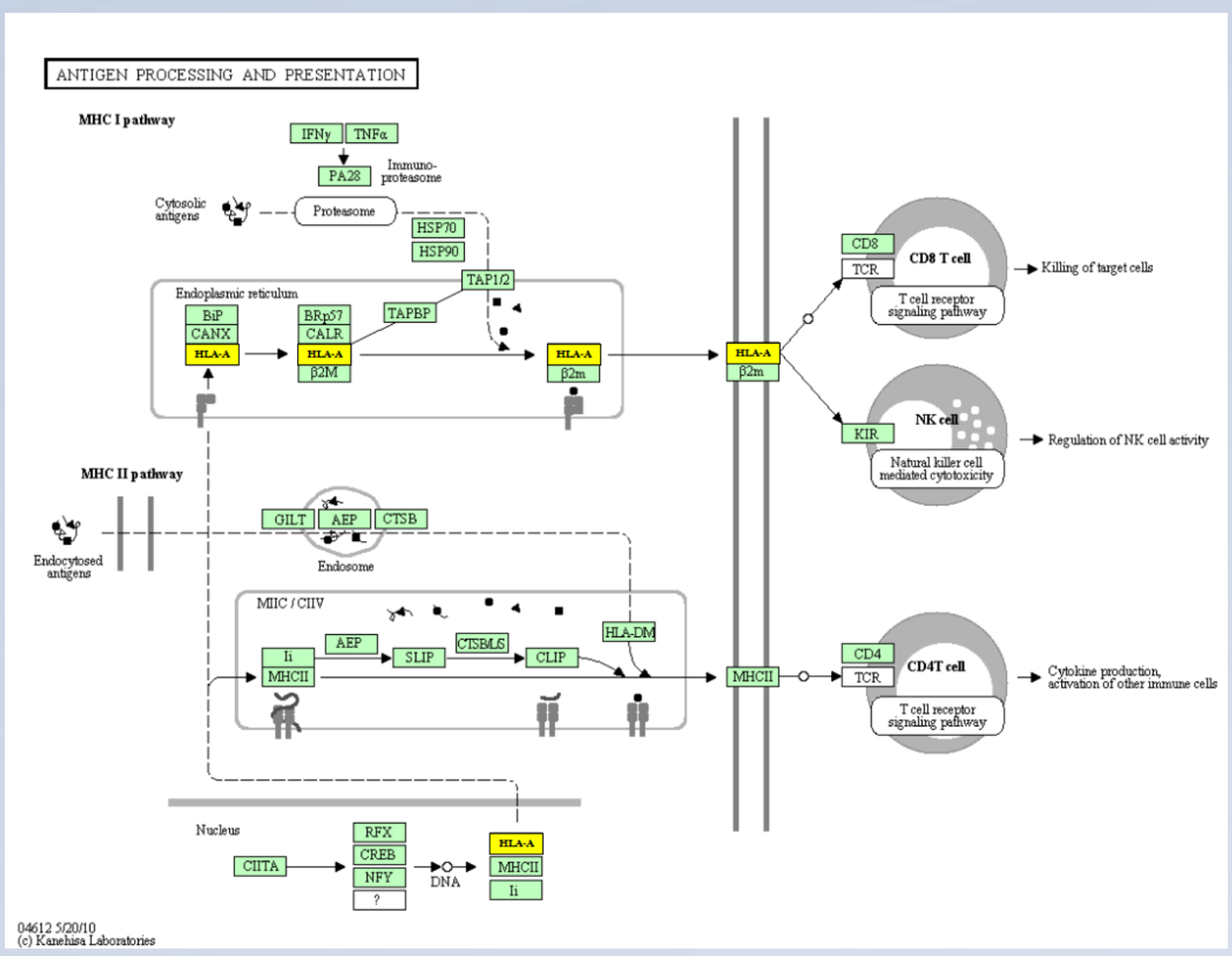


Fig 2. The KEGG pathway enriched in hsa-miR-23c target genes with the lowest enrichment p-value

## CONCLUSIONS

In conclusion, this pilot study indicates phenotype-related differences in expression of miRNAs, molecules with high regulatory and biomarker properties.

Although detected in PBMC, hsa-miR-23c is highly expressed in the brain and target MS relevant genes such as, HLA (A, B, C), transferrin receptor, Nrf2, recently proposed to play important role in neurodegeneration.

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