

MicroRNA expression profile of B lymphocytes in Multiple Sclerosis

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Introduction MicroRNAs (miRNAs) control protein synthesis by targeting mRNAs for translational repression or degradation at the post-transcriptional level. In general, miRNAs play an important role in diverse biologic processes such as development, cell proliferation and differentiation, apoptosis, oncogenesis, metabolism, angiogenesis, and inflammation. Recent studies suggest that miRNA dysregulation may contribute to the pathogenesis of multiple sclerosis (MS)¹, and further studies are needed to assess which miRNAs and which blood cell populations can be used as signature markers. MS is considered an autoimmune T-cell mediated disease, but recent evidence is mounting about a B lymphocytes role. Such evidence consists, but is not limited to **i)** oligoclonal bands IgG in cerebrospinal fluid; **ii)** the presence of B-clones expanded both in cerebral lesions and in liquor²; **iii)** the development of ectopic-follicles in intrameningeal areas³.

To further inspect single mechanism details we have analyzed microRNA expression profiles in B lymphocytes from peripheral blood of MS patients.

RESULTS Looking for differently expressed microRNAs, we found a candidate set with acceptable foldchanges but low accordance between affected twins and non-twin MS patients; for this reason we further inspected our data down to single subject profile. For all groups considered we have looked at both Principal Component Analysis (PCA) and Group-clustering (CL); such inspection highlighted the presence of an important inter-cluster overlapping, reflecting the low microRNA accordance previously observed. We thus decided to analyze separately twins and non-twin subjects. After this data partitioning both PCA and CL showed better separation between MS and HD data: we identified a cluster of 6 microRNA ($p < 0.04$ and $|\text{fold-change}| > 1.5$) differentially expressed in MS/HD groups (18 subjects), while the cluster that discriminates between affected and healthy co-twins was composed of 18 microRNA (4 subjects) with $|\text{fold-change}| > 1.5$.

Conclusions

Peripheral B cell compartment seems to be promising for the identification of microRNA signature. Future studies, including ongoing characterization of microRNAs of B cell tropic viruses, will confirm our objective.

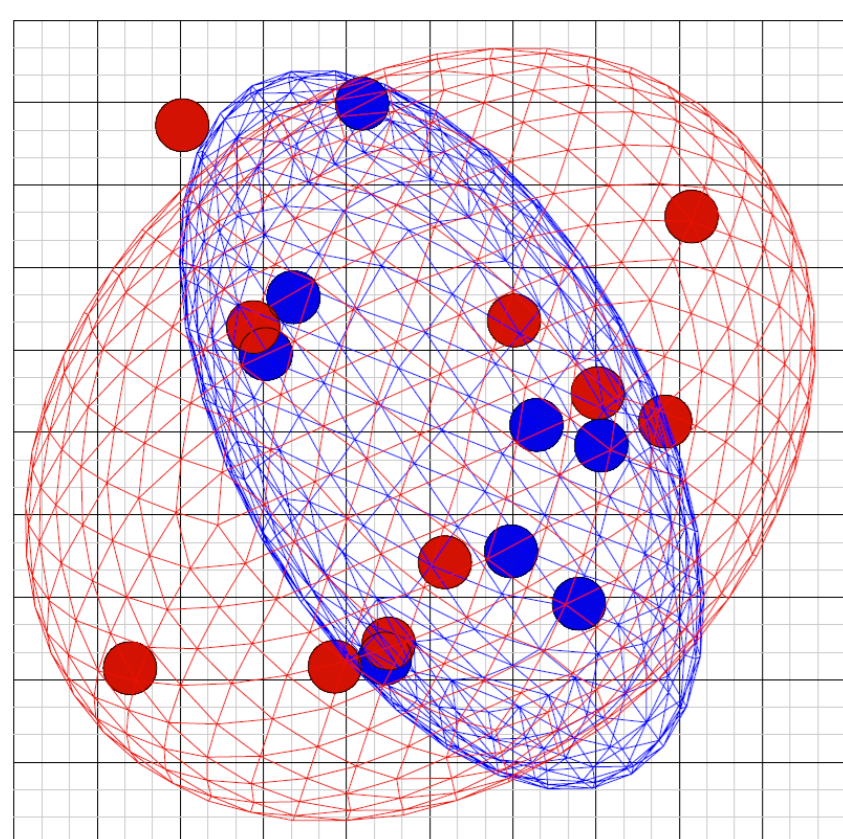


Figure 1. Principal Component Analysis of the 18 samples that we have inspected.

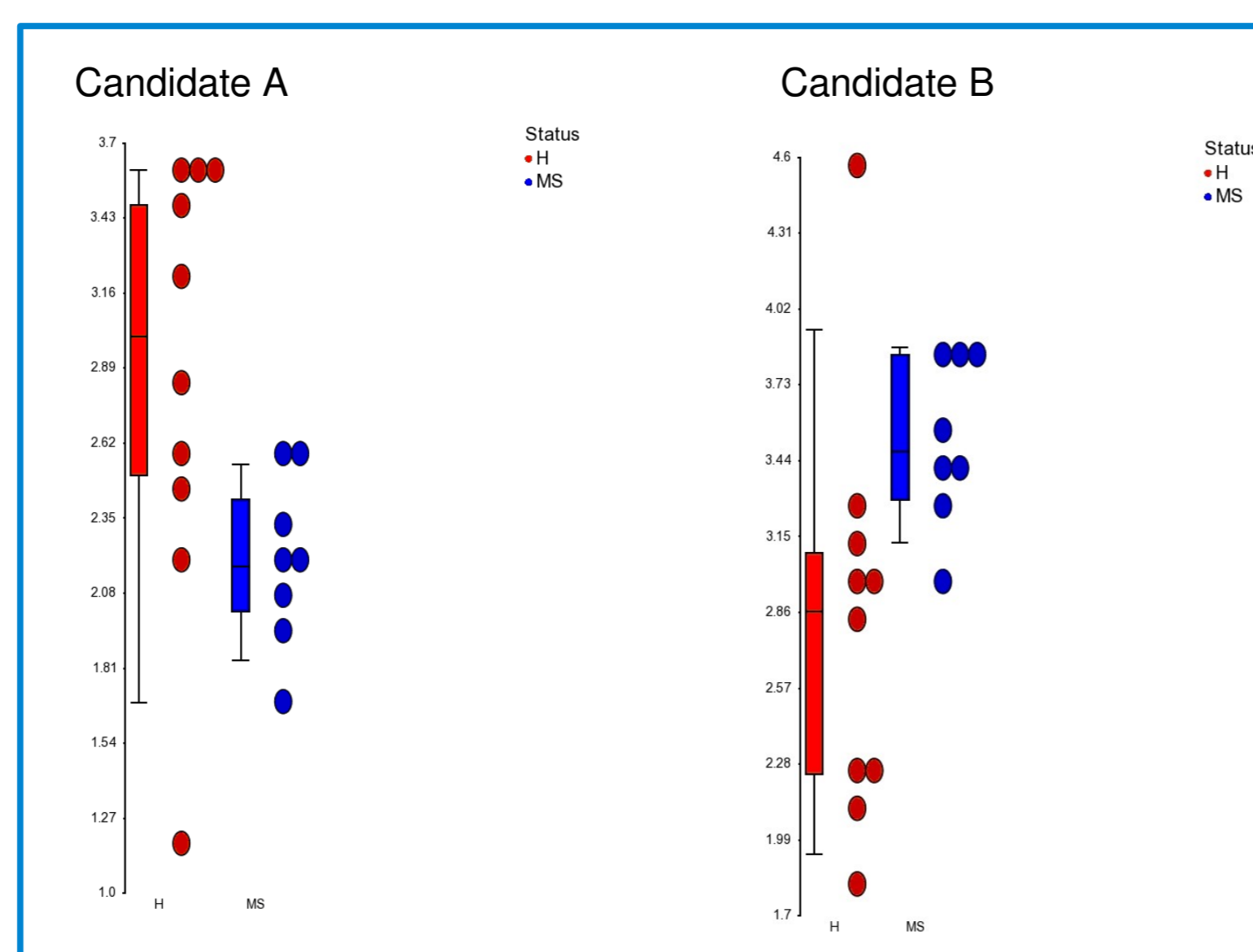


Figure 2. Non-twin MS patients. These miRNA are the best signature candidates which segregate MS/HD.

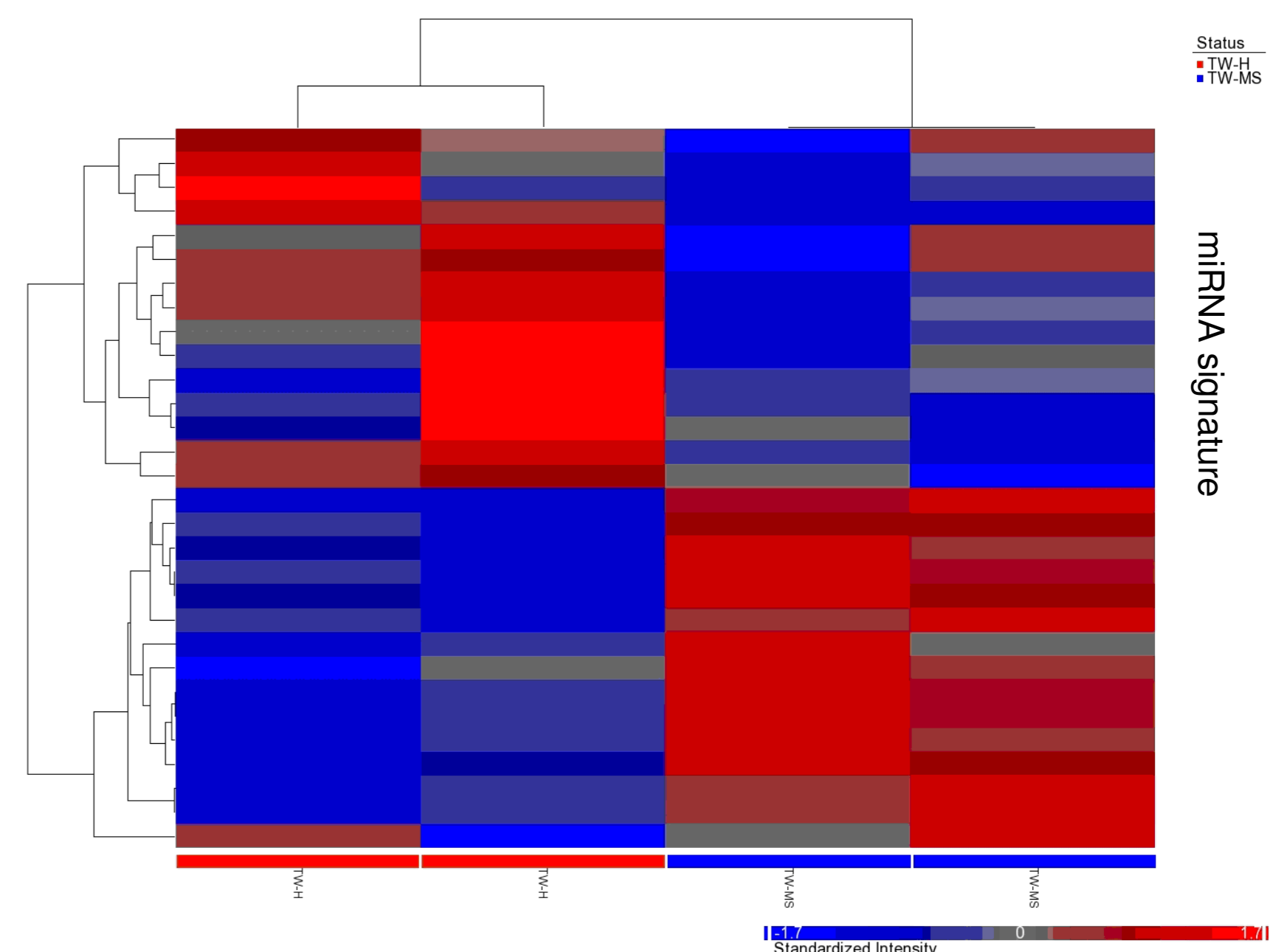


Figure 3. Dendrogram of the 30 miRNAs which exposed a $|\text{fold-change}| > 1.5$ in the MS-twins, healthy co-twins. Out of these 30, there are 18 which show a concordant trend (TW|TW or TW|TW)

Methods Using a new Affymetrix system we performed whole microRNA expression profiles in peripheral blood B cells. We studied 8 MS patients, 10 healthy donors (HD), as well as 2 female monozygotic twins discordant for MS. All affected individuals were treatment-naïve and all the investigations were performed at least three months after the last steroid therapy. A contrast-enhanced MRI was obtained from affected twins and from other MS patients within 24 hours from sampling. Peripheral blood mononuclear cells (PBMC) were obtained by density centrifugation over Ficollhpaque and highly purified CD19+ B cell subsets were sorted using MACS (Miltenyi Biotec) systems. Total RNA contains small RNA was extracted using miRNeasy Kit (Qiagen). RNA quality and purity were assessed with the use of the Agilent 2100 Bioanalyzer (Agilent). Labeled RNA from each sample was analyzed by GeneChip miRNA Array (Affymetrix). Raw data analysis have been normalized and partitioned into sub-groups using Partek. Taking Partek output as input, microRNA correlation studies between MS patient and HD have been achieved employing Ontology Reasoning Engine for Molecular Pathways (OREMP)⁴, a system that abstracts the information from different resources and combines them together into a coherent ontology. In particular we fed into OREMPdb⁴ the microRNA-Gene information augmented with the link between biochemical species and external resources such as mirBase and Gene Ontology. Twin pairs were firstly split and merged with MS patients and HD; an ANOVA analysis was evaluated on these two groups. Subsequently, four groups were considered: MS-twins, healthy co-twins, non-twin MS patients and HD.

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