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Original Article

MicroRNA expression changes in Parkinson's disease (PD) patients' leukocytes prior to and following deep brain stimulation (DBS)

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Abstract: The second most prevalent neurodegenerative disorder worldwide in the elderly is Parkinson's disease (PD). It is a major risk factor for aging. *Objectives:* Currently the involvement of miRNAs in the disease is mainly unclear. Additionally, the disease aetiology is complex and there are no available disease-modifying medications. Therefore, more evidence is required concerning its pathogenesis and developing new treatment modalities. *Methods*: Here, we studied the expression profiles of about 900 miRNAs in PD patients prior to and following deep brain stimulation (DBS) both on and following 1 hour off electrical stimulation and as compared with age and gender matched healthy control (HC) donor samples, using Affymetrix miRNA microarrays. We analysed statistically the data using Affymetrix expression console software. *Results:* We detected significantly altered miRNAs pre and post DBS treatment. *Conclusions:* Our findings indicate the involvement of miRNAs in PD. Future studies can enlarge the number of samples and use RNA sequencing platform to quantify further miRNAs in PD samples. We may also use the expression levels of miRNAs as biomarkers for PD in the blood.

Keywords: PD, RNA, DBS, microarray, blood, leukocytes

Introduction and background

Parkinson's disease (PD) is a chronic neurodegenerative disorder. The prevalence of PD increases after the age of 50 and begins to rise gradually after the age of 75. In other words, the onset of PD is age-related and would increase its prevalence as the population ages. PD is a form of neurological condition usually associated with motor symptoms arising from the gradual degeneration of the dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) [1].

The 2016 Global Burden of Disease Report found that PD prevalence and death rates have risen substantially over the last 25 years and are expected to increase more [2]. Compared to 6.1 million registered PD patients in 2016, there are currently more than 10 million PD patients worldwide and the case is expected to cross 12 million by 2050 [3].

The main molecular mechanisms that underlie PD are still largely unclear.

PD is a prevalent, multifaceted neurodegenerative disease caused by mostly unknown factors. However, there are many postulated pathophysiological hypotheses to explain it. The DA population of SNpc is affected by PD. By the time of disease diagnosis, the majority of brain dopaminergic cells have already died. As the nigrostriatal dopaminergic system has its roots in this unique brain area, progressive depletion of DA neurons in SNpc triggers the resulting loss of dopamine feedback in the brain region of striatum, which primarily encourages volunteer movements [4, 5]. Another pathology in PD is the intracellular accumulation of alpha synuclein (SNCA) proteins or the development of Lewy bodies. It is said that in the olfactory bulb, and in other brain regions as the condition grows, from the olfactory bulb and medulla oblongata [6].

There is actually no medication available for PD currently that can entirely heal the disease. Dopamine replacement therapy is actually symptomatic and not therapeutic after nearly 60 years since the 1960s'. Patients usually take medications such as levodopa, dopamine and monoamine oxidase inhibitors to restore dopamine level and neurotransmission to their brain and enhance their motor functions. DBS is offered only to patients above certain cognitive score following estimation of UPDRS disease motor score. The dopamine-agonist drugs recover the concentration of dopamine but do not delay the development of PD and create many inevitable side effects concurrently. Chronic levodopa treatment may result in problems of motor response due to wear and tear. Mid to later stage PD patients need higher and more common dosage of levodopa that can contribute to neuropsychiatric adverse events such as sensory disturbances and delusions. DBS is an approved therapy for PD patients who cause motor reaction problems. It is an approved treatment. Electric stimulation is provided to the subthalamic nucleus (STN) or globus pallidus (GP) by implantable pulse generator in order to enhance the irregular firing of the basal ganglia (BG) system. While DBS surgery is effective in ameliorating motor response complications and motor features of PD, it has no effect on the progressive neurodegenerative process.

MicroRNAs (miRNAs) are small (~21 nucleotides long) RNAs that regulate up to hundreds of target genes. Our current study aimed at investigating the role of miRNAs in PD patients following surgical DBS treatment response with the examination of the expression profiling of miRNAs in peripheral blood leukocyte cells of PD patients pre- and post-DBS both on electrical stimulation and following a short one-hour electrical stimulation cessation compared to healthy control volunteers (which brings back the disease motor symptoms). MicroRNAs may also be involved in neuroinflammation, and in the cholinergic system control [7]. They rapidly emerge as global controllers of gene expression yet the scope of their role in PD is yet largely unknown.

Methods

Patients recruitment

3 PD patients and were recruited from Hadassah neurosurgery department (Jerusalem, Israel) following signature on ethics approval and short interview, and 3 samples from age- and gender-matched healthy control professors were collected from subjects recruited at the Edmond Safra campus (Jerusalem, Israel Givat Ram) following signatures on ethics approval.

Inclusions criteria

For PD patients: below the age of 75, cognitive score positive.

Exclusion criteria

For patients: no adverse side effects to the therapy, no other diseases (e.g cancer diabetes etc.). for healthy controls: age and gender matched, no medication taken.

RNA sample collection

Total RNA was extracted from the blood leukocytes of 3 PD patients in three states: pre-DBS, post-DBS on electrical stimulation and following one hour off electrical stimulation, and of 3 healthy control (HC) volunteers. miRNA expression was examined (a total of 12 samples).

Data analysis

The data was analysed using Affymetrix expression console software and the results were compared to results of analysis of small RNA sequencing from same patients prior to, and following stimulation using SOLID technology. PCR can be applied to validate selected gene targets.

Ethics committee approval statement

The Hadassah ethics committee approved the study under Helsinki committee approval (approval number 6-07.09.07 code 2507), and each recruited patients was signed on informed consent form. There was no registered clinical trial.

Results

We applied Affymetrix expression console software on the microarray data to analysed the miRNA expression profiles. About 900 miRNA genes were analysed. The following miRNAs were detected as changed in both platforms between PD to HC: mir-21 and mir-92b. Additionally, we mapped miRNA probes from exon microarrays from the same patients to the

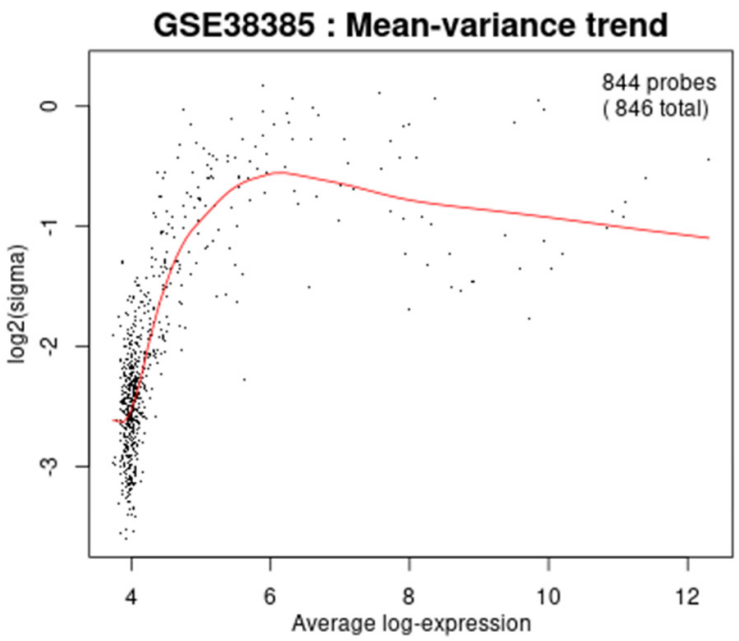

Figure 1. Legend: mean variance change (average log expression), 844 probes (846 total).

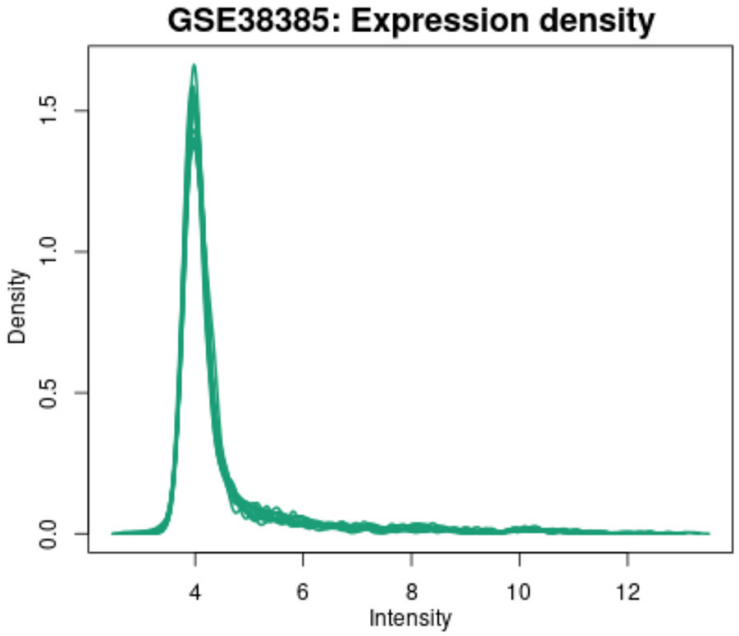

Figure 2. Legend: expression intensity.

genome and compared the expression levels to the miRNA arrays. We found the following miRNAs as significantly altered post compared to pre DBS in both platforms: Mir-424*, mir-20a, mir-18b*, mir-210, mir-143 and mir-1308. Additionally, the following miRNAs were found as changed post DBS off stim compared to on stim in both the Affymetrix arrays and the sequencing data: mir-486 and mir-15a*. We generated both PCA and box plots based on the data. **Figure 1** shows mean variance trend of the data. **Figure 2** shows expression density of the quantified miRNAs. **Figure 3** shows box plots, **Figure 4** UMAP (PCA) of the samples and **Figure 5** the general experimental design. **Figure 5** the general experimental design.

Our results show that several miRNAs were significantly altered in the patient samples. This is in agreement with our findings from small RNA sequencing experiment (SOLiD technology) on the same samples.

The current results are in line with one of our previous studies, in which we found alternative splicing changes by analysis of junction arrays and constructed miRNA-target networks for miRNAs found as changes by analysis of the sequenced samples [8]. Overall, we found reversible expression changes in several miRNAs (in terms of gene expression fold change). Altogether, our findings exhibit the importance of microRNAs in the aetiology of PD.

Future prospects: the relatively new technology of single cell sequencing technology [9] allows a detailed inspection into expression changes of miRNAs, long non coding RNAs (lncRNAs) and coding genes in a more precise resolution. Additionally, gene networks can be analyzed [10]. Further comparisons to publicly available networks may enable further insights into the disease underlying molecular mechanisms. Targeting of detected gene targets by

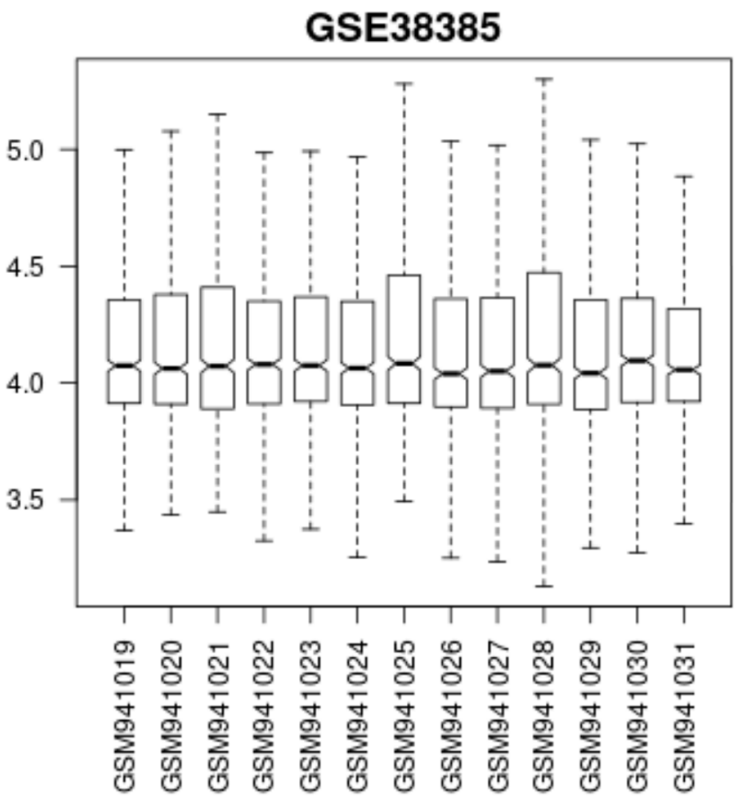

Figure 3. Legend: Box plots of the changed miRNAs.

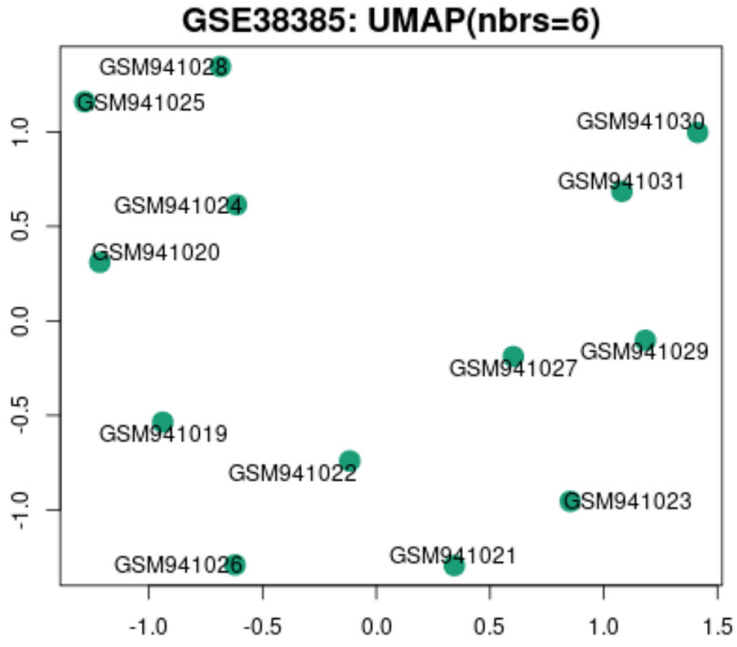

Figure 4. Legend: PCA of the 12 microarray samples.

Cas9/CRISPR guides may enable new treatment methods [11]. Additionally, DNA copy number alterations can be quantified. Drug screens may be enabled through RNAi. Further comparisons to mice PD models expression data may enable further insights into disease underlying molecular mechanisms and involved genes [12-14]. Staining of post mortem brain samples to different cell types followed by machine learning quantification. Advanced computational image analyses may also enable quantification of oligodendrocytes (OLGs), neurons and further cell types in PD brains compared to control brain samples (e.g astrocytes-AC, microglia-MG and epithelial cells) [6].

Based on the aforementioned results, we will be able to better understand the molecular mechanisms that underlie PD that will enable development of both early diagnostic methods using blood test as well as potential genomic based therapeutics. Additionally, comparison of the results to results based on analyses of PD models mice brain samples (e.g., MPTP exposed) can reveal further insights into the underlying mechanisms. Future studies using sequencing technologies will enable generation of additional data from patient's blood leukocytes followed by computational analyses. Additionally, the data can be compared to immune inflammatory diseases [9, 15].

Discussion

A better understanding of the molecular mechanisms that underlie PD will enable development of both early diagnostic methods using blood test as well as potential genomic based therapeutics. Future studies using sequencing technologies will enable generation of additional data from patient's blood leukocytes followed by compu-

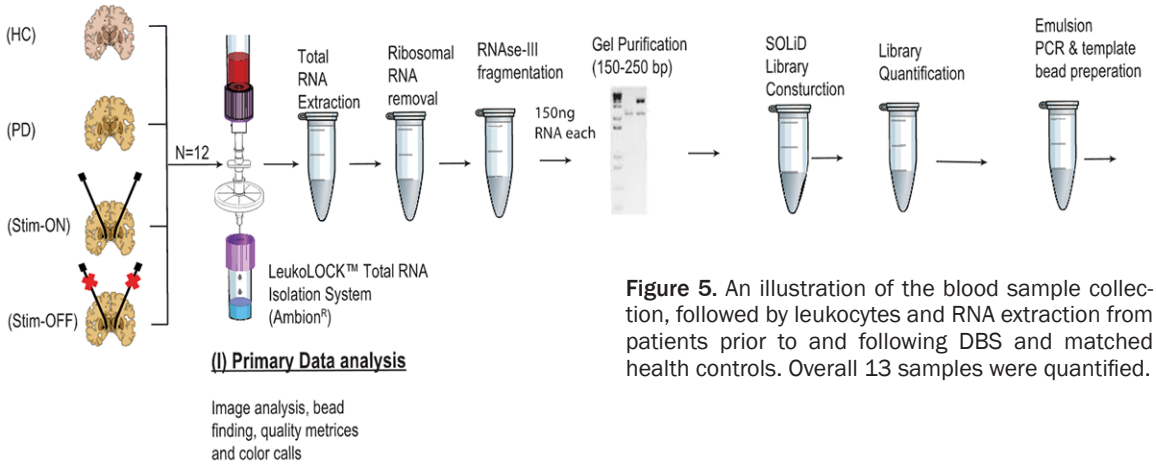

Figure 5. An illustration of the blood sample collection, followed by leukocytes and RNA extraction from patients prior to and following DBS and matched health controls. Overall 13 samples were quantified.

tational analyses. Additionally, the data may be compared to immune diseases. The advance of single cell sequencing may allow gaining further insights into the disease underlying molecular mechanisms.

Conclusions and future direction

We found overlaps between miRNAs that changed in the arrays and in small RNA sequencing experiment. Future direction will be to increase the number of tested samples (both patients and controls) and to reanalyse the data following interrogation by RNA-Seq. Additionally, the findings can be compared to findings based on analyses of Alzheimer's disease (AD) patients' blood samples.

Limitations

The data set is currently a little bit small in terms of number of samples. In future studies the number of samples will be increased (to 10 in each group for patients, pre and post DBS and post DBS OFF STIM), and potentially another sequencing experiment will be performed on miRNAs extracted from the patient's and control samples and the data will be analysed and compared.

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Disclosure of conflict of interest

None.

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