

Alterations in Blood-based miRNA in Veterans Affected with Combat-related PTSD

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Individuals affected with PTSD (Post-Traumatic Stress Disorder) demonstrate changes in microRNA (miRNA) molecules associated with gene regulation. A controlled study, involving military personnel on deployment to a combat zone in Afghanistan, provided evidence for the role of blood-based miRNAs as candidate biomarkers for symptoms of PTSD. This may offer an approach towards screening for symptoms of PTSD, and holds promise for understanding other trauma-related psychiatric disorders. Limitations of the study are that this was a small pilot study, and the findings need to be validated, extended and confirmed. First results will be presented at the ECNP conference in Paris.

PTSD is a psychiatric disorder which can manifest following exposure to a traumatic event, such as combat, assault or natural disaster. Among individuals exposed to traumatic events, only a minority of individuals will develop PTSD, while others will show resiliency. Little is known of the mechanisms behind these different responses. The last few years have seen much attention given to whether the modification and expression of genes – epigenetic modifications - might be involved. But there are several practical and ethical challenges in designing a research study on humans undergoing such experiences, meaning that designing relevant study approaches is difficult.

The research group from the Netherlands, worked with just over 1,000 Dutch soldiers and the Dutch Ministry of Defense to study changes in biology in relation to changes in presentations of symptoms of PTSD in soldiers who were deployed to combat zone in Afghanistan. In a longitudinal study they collected blood samples before deployment, as well as 6 months after deployment. Most of the soldiers had been exposed to trauma, and some of the soldiers had developed symptoms of PTSD.

For this pilot study, from the initial group, subgroups were selected of in total of 24 subjects; 8 of the soldiers had developed symptoms of PTSD; 8 had endorsed traumatic experiences but had not developed symptoms of PTSD; and another 8 had not been in serious traumatic circumstances and served as a control group. Using modern sequencing techniques, several types of miRNAs of which the blood levels differed between the groups were identified.

MiRNAs (Micro RiboNucleic Acids) are small molecules with chemical building blocks similar to DNA. Unlike the more famous DNA, miRNAs are typically very short – comprising only around 20 to 25 base units (the building blocks of nucleic acids), and they do not code, in other words they do not specify the production of a protein or peptide. However, they have very important roles in biology (every miRNA regulates the expression, and thereby also the activity of several other genes), and they are known to regulate the impact of environmental factors on biology. In

addition, brain-derived miRNA can circulate throughout the human body and can be detected in the blood. Differences in miRNA levels have been associated with certain diseases, such as some cancers, kidney disease, and even alcoholism. This regulatory role makes them also a candidate for investigation in PTSD.

“We discovered that these small molecules, called miRNAs, are present in different amount in the blood of persons suffering from PTSD compared to trauma-exposed and control subjects without PTSD”, said first author Dr Laurence de Nijs (Maastricht University).

“We identified over 900 different types of these small molecules. 40 of them were regulated differently in people who developed PTSD, whereas there were differences in 27 of the miRNAs in trauma-exposed individuals who did not develop PTSD.”

“Interestingly, previous studies have found circulating miRNA levels to be not only correlated with different types of cancer, but also with certain psychiatric disorders including major depressive disorders. These preliminary results of our pilot study suggest that miRNAs might indeed be candidates as predictive blood markers (biomarker) to distinguish between persons at high and low risk of developing PTSD. However, several steps need to be performed before such results can really have an impact on the larger field and in clinical practice. In addition to working towards biomarkers, the results may also provide novel information about the biological mechanisms underlying the development of PTSD”.

Dr de Nijs explained

“Most of our stressful experiences don’t leave a long-lasting psychological scar. However, for some people who experience chronic severe stress or really terrible traumatic events, the stress does not go away. They are stuck with it and the body’s stress response is stuck in ‘on’ mode. This can lead to the development of mental illness such as PTSD.

These individuals experience symptoms including re-experiencing of the traumatic event through flashbacks or recurrent nightmares, constant avoidance of reminders of the event, negative mood, and extreme arousal. This can manifest itself through insomnia and or hyper-alertness. Individuals with PTSD are six times more at risk of committing suicide and having marital problems, and the annual loss of productivity is estimated to be approximately \$3 billion. Currently, there is no definite cure for patients with PTSD, and available treatments often are not effective”.

Commenting, Professor Josef Zohar (Ex-ECNP Chair, Tel Aviv, Israel) said:

“The relevance of a better understanding of stress related events is unfortunately becoming clearer and clearer after each terror attack. This work points to an innovative avenue regarding the potential identification of risk factors for susceptibility to developing post-traumatic stress disorder”.

Funding: Dr de Nijs was awarded a Marie Curie fellowship grant by the European Union to perform this study, within a network of other expert scientists in PTSD and epigenetics. The Dutch cohort of soldiers (PRISMO) was funded through the Dutch Ministry of Defence.

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Notes for Editors

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The European College of Neuropsychopharmacology (ECNP)

The ECNP is an independent scientific association dedicated to the science and treatment of disorders of the brain. It is the largest non-institutional supporter of applied and translational neuroscience research and education in Europe. Website: www.ecnp.eu

The 30th annual ECNP Congress takes place from 2nd to 5th September in Paris. It is Europe's premier scientific meeting for disease-oriented brain research, annually attracting between 4,000 and 6,000 neuroscientists, psychiatrists, neurologists and psychologists from around the world. Congress website: <http://2017.ecnp.eu/>

Conference abstract: P.4.a.009 Circulating microRNAs as potential biomarkers of differential susceptibility to traumatic stress

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Background: Traumatic stressors are prevalent risk factors for mental health disorders, such as post-traumatic stress disorder (PTSD). People differ strikingly in their susceptibility to develop PTSD after traumatic stress, however the exact underlying biological mechanisms of differential susceptibility are unknown. Identifying diagnostic biomarkers would enable to develop more specific preventive strategies and early interventions. Epigenetic mechanisms have been proposed to underlie the relationship between exposure to traumatic stress and the susceptibility to develop PTSD. Recent evidences suggest that microRNAs are key epigenetic players in mental health disorders. Furthermore, numerous studies demonstrated the high potential of microRNAs as promising non-invasive biomarkers for different health outcomes. We therefore aimed to identify microRNA candidates as potential biomarkers of differential susceptibility to develop PTSD after traumatic stress exposure in humans.

Methods: Next generation high-throughput sequencing was used to examine circulating microRNA profiles in 24 serum samples from a large prospective Dutch military cohort at 6 months after a 4-month deployment period. Three polarized groups were selected: susceptible subjects with PTSD after trauma exposure, unsusceptible subjects without PTSD after trauma exposure and control subject without robust exposure to stress (n=8 per group). Small RNA libraries were prepared according to Illumina v1.5 protocol and used for high-throughput sequencing on a Genome Analyser HiSeq 2000 (Illumina). Sequencing reads were processed using miRge. The expression output was analyzed using the open-source software R and Bioconductor. For the detection of differentially expressed miRNAs, the DESeq2 package was used. MicroRNAs were considered significantly differentially expressed at a p value below 0.05 (adjusted for multiple testing using Benjamini-Hochberg method). A second significance criterion, to compensate for bias introduced by very low abundant sequences, was a minimum mean of 20 read counts.

Results: The miRNA sequencing yielded an average of 9.5 million unfiltered sequencing reads. After filtering process, many reads were discarded giving on average 1.9 million high-quality reads. The number of miRNAs in each sample was normalized to the total number of reads. To identify circulating miRNAs in serum of subjects in our study, the sequencing reads were aligned to current miRBase (V21.0). A total of 919 miRNAs were detected across all serum samples. Differential expression analysis revealed 187 miRNAs differentially expressed in the serum of susceptible persons and 148 microRNAs in the serum of unsusceptible subjects compared to controls. We used the Venn tool to specify miRNAs that were uniquely expressed in each group and found 40 miRNAs specifically expressed in susceptible subjects (8 downregulated, 32 upregulated) and 27 in unsusceptible group (10 downregulated, 17 upregulated).

Conclusion: Although further experiments need to be performed with more subjects, the results of our pilot study suggest that profiles of circulating microRNAs in human serum might provide biomarker candidates and possibly mechanistic information relevant to identify differential susceptibility to develop PTSD.

How this was reviewed?

There were 1003 abstracts accepted for this conference, this work was amongst the top-scoring 170 abstracts. After initial approval from the ECNP media group, the press release was developed by the press officer and the author, with the final version being approved by the ECNP media review group. We then sought an additional view and comment from someone with expertise in the field – this is the person who comments in the press release. None of the reviewers have been involved in the work.