THE EFFECT OF VIOLENCE EXPOSURE ON PERIPHERAL BLOOD GENE EXPRESSION

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INTRODUCTION

Peripheral blood gene expression is a very interesting recent development in biomarker research, with opportunities for both hypothesis-driven biomarker search and for hypothesis-free “omics”-based discovery (Sunde, 2010).

We know that violence exposure, and severe stress in general, affects a host of biological systems in humans, with changes in peripherally measureable biomarkers in tissues that are accessible with relatively low invasiveness, such as blood and saliva. In the blood, biomarkers can be analyzed as proteins or metabolites, in the plasma or serum, using quantitative techniques such as ELISA and high-pressure liquid chromatography. Additionally, blood cells (erythrocytes, immune cells, platelets) can be tested for functional assay, binding of ligands to receptors, or intracellular/membrane levels of proteins, using different techniques based on the research question and the cell type. Finally, we can use “gene expression”. This term usually refers to “intracellular RNA from whole blood”, as it is technically associated, in most cases, with the use of tubes for blood collection that stabilizes RNA from all cells in the blood. However, it is also possible to separate distinct blood cell populations and then extract RNA from those, as we will see in this essay.

What is RNA?

DNA sequences the codes for proteins, and each type of differentiated cell express its own pattern of proteins that confer the unique functions and features to the cell. However, DNA sequences do not directly originate the proteins, but rather they are *transcribed* into RNA sequences (or, better “messengers RNA” sequences, or mRNAs). mRNAs, in turn, interact with subcellular complexes called ribosomes in order to be *translated* into proteins through an assembly process. When we measure “RNA” we refer to measuring the entirety of the mRNAs present in the cells (also called *transcriptome*). RNA expression can be measured either through candidate-gene approach, that is, one gene at the time, or as transcriptomics approach, that is, the contemporary study of all available transcripts in the blood/cells, using array systems. More recently, plates that allow the simultaneous analysis of 30 to 100 related genes have also been developed (Sunde, 2010).

Each mRNA sequence can be specifically linked to the protein that it is destined to originate, hence we can talk about the mRNA for the glucocorticoid receptor or for the serotonin transporter. Broadly speaking, being one of the steps leading to the protein synthesis, the levels of mRNA for each specific protein are proportional to the levels of the protein itself, or to the level of activation of the cellular processes that involve the protein. Clearly this is just a broad assumption, since there are very sophisticated mechanisms of regulation of mRNA synthesis and translation that confer yet

another levels of complexity to the functional interpretation of these measurements (Proud, 2007). Theoretically, intracellular RNA could be used as a proxy measures for proteins that are then released from the cells into the blood (for example, hormones or cytokines) as well as for protein that are not released outside the cells but are functionally relevant, such as enzymes. Yet more important, we can measure peripheral blood RNA as a proxy measure for the same molecular mechanisms in less accessible tissues, such as the brain.

What do we actually measure?

Biomarker researchers have usually defined "blood gene expression" also as "leukocyte gene expression", implying that the RNA isolated from blood comes predominantly from leukocytes, or white blood cells – i.e., the cells of the immune system. This assumption has been largely based on the notion that erythrocytes (red cells), even if much more abundant than leucocytes (by a factor of approximately one thousand), do not have nucleus and as such should not have mRNA synthesis. However most recent research has suggested that indeed blood mRNA comes predominantly from erythrocytes (Sunde, 2010). However, what is really important for researchers is whether blood mRNA can be used as a proxy for mRNA expression of other tissues that are more relevant to the pathogenic processes of interest – in psychiatry and neuroscience, the brain. In this regards, peripheral blood mRNA is very promising, as studies have shown that blood cells share more than 80% of the transcriptome with other body tissues, including with brain (Liew et al., 2006). In one specific study that has compared the transcriptional profiling of 79 human tissues, including whole blood mRNA and several brain areas, whole blood was found to share significant gene expression similarities with multiple brain tissues, with an average correlation between transcripts present in both whole blood and the brain of around 0.5. The authors of this study concluded that expression of blood and brain mRNA are neither "perfectly correlated and useful nor perfectly uncorrelated and useless", and suggested that "cautious and thoughtful use of peripheral gene expression may be a useful surrogate for gene expression in the CNS" (Sullivan et al., 2006).

THE MOST IMPORTANT BIOLOGICAL PATHWAYS REGULATED BY EXPOSURE TO STRESS

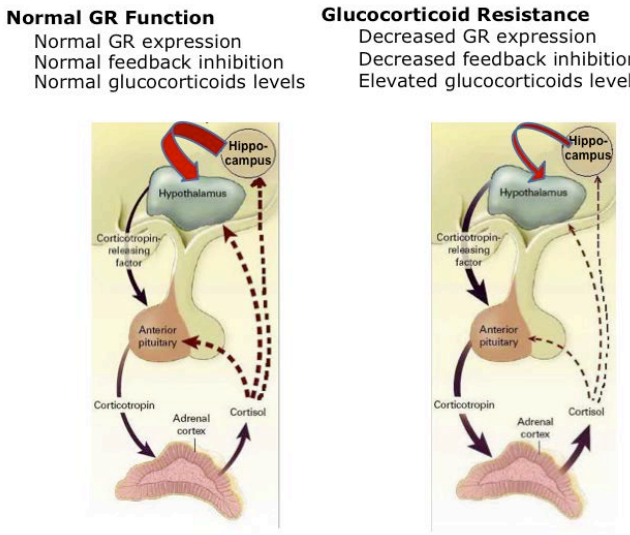
Before discussing in details the whole blood mRNA abnormalities described in individuals exposed to severe stress, especially in the context of the widely studied subjects of violence and maltreatment exposure in childhood, is important to briefly describe the biological systems involved in the stress response, and how these are abnormal following stress exposure.

Stress exposure and glucocorticoid resistance

There is clear evidence that stress leads to a persistent activation of the two main biological systems involved in the stress response, the hypothalamic-pituitary-adrenal (HPA) axis and the inflammatory system. Both these systems are also hyperactive in depression, and indeed this hyperactivity is considered part of the pathogenesis of depression (Danese et al., 2007; Heim et al.,

2008). We, and others, have extensively contributed to the understanding of the mechanism underlying HPA axis and inflammation hyperactivity in adults who experienced childhood or adults stressors, and have proposed an explanatory model centred on the glucocorticoid receptor (GR), that is, one of the most important receptors and transcription factors governing the stress response (Pariante and Lightman, 2008).

Glucocorticoid hormones, like cortisol in humans and corticosterone in rodents, are the final output of the HPA axis, and the main hormones involved in the stress response. By binding to the GR (and to the mineralocorticoid receptor, MR), cortisol effects its cellular actions, including the negative feedback regulation of the HPA axis (by which stress-induced activation of the HPA axis is followed by a rapid return to normal functioning), and the restraint of the inflammatory response (which maintains a physiological control on excessive immune processes). Although the MR is important for cortisol action, the GR is particularly relevant when the levels of glucocorticoids are high, such as during stress or depression, and thus it has traditionally been considered more relevant within this context. Exposure to stress has been shown to induce *glucocorticoid resistance*, that is, a reduction of GR function, which in turn leads to both the HPA axis hyperactivity and the increased inflammation, because of the lack of, respectively, the GR-mediated negative feedback on the HPA axis (see Figure) and the GR-mediated restraint of inflammation (Pariante and Lightman, 2008).



STRESS AND RNA EXPRESSION

Both the HPA axis and the inflammatory/immune system have been examined in a (relatively small) number of gene expression studies *in humans* that have examined peripheral blood gene expression following exposure to stressors, using either a candidate-gene approach or a transcriptomics approach. All studies have confirmed the pivotal role of the glucocorticoid receptor in mediating the biological and molecular effects of stress exposure.

Exposure to trauma and PTSD development

Studies on gene expression abnormalities in subjects with PTSD, often in the absence of a control group of individuals unexposed to stressors, are probably more relevant to understanding PTSD pathogenesis than the consequences of stress exposure. However, considering the limited evidence in this field, we felt that these are important contribution.

In two studies conducted in individuals exposed to the 9/11 events, Yehuda et al. have analysed the whole blood transcriptome of individuals who did or did not develop post-traumatic stress disorders (Yehuda et al., 2009; Sarapas et al., 2011). Among the 16 genes identified as differentially expressed (using a transcriptomics approach) (Yehuda et al., 2009), two are directly linked to GR: FKBP5 and STAT5B. Both proteins act as inhibitor of GR function, and both were *decreased* in this study. Therefore, these findings indicate *increased GR function*, rather than glucocorticoid resistance. However, the major histocompatibility complex (MHC) Class II gene was also reduced, and since expression of this gene is stimulated by glucocorticoids, this finding may indicate glucocorticoid resistance, at least for immune related genes. Of note is also that cortisol levels were not different between the two groups, and that subjects with PTSD also had an increased rate of childhood trauma. In a subsequent study that included also subjects exposed to 9/11 who had recovered from PTSD (Sarapas et al., 2011), gene expression analysis indicated that FKBP5 and MHC class II are “state” markers, as they were not present in those not currently symptomatic.

In a study from a different research group, where mRNA from isolated peripheral blood mononuclear cells, that is, lymphocytes and monocytes (as opposed to whole blood) was analysed, gene expression pattern was assessed less than an hour after exposure to a life-threatening trauma (with blood collected in hospitals) in the absence of serious medical consequences (Segman et al., 2005). The signature of genes differentially expressed in those who did develop PTSD after the trauma included an increase in the expression of transcripts involved in immune activation. Of note is the notion, discussed by these authors, that changes in blood immune cells composition may explain some of these gene expression changes – a confounder which may be less relevant when using whole blood mRNA, especially considering the most recent evidence, mentioned above, indicating a predominantly erythrocytes origin for this. Some studies using isolated immune cells do measure cells subset populations, as mentioned below.

Finally, a recent study examined gene expression in PTSD subjects following predominantly violent trauma (combat, physical or sexual abuse) and in isolated monocytes (that is, an even more distinct subcellular population) (O'Donovan et al., 2011). The transcriptome gene expression measurement was followed by bioinformatics analysis of genes whose expression is regulated by specific transcription factors, including the GR and the pro-inflammatory transcription factor, NFKB. The authors describe an upregulation of target genes for NFKB and a downregulation of target genes for GR, consistently with a pattern of glucocorticoid resistance; in addition, they identified a differential pattern in males and females for an target genes for CREB/ATF transcription factors, which convey adrenergic signals from the sympathetic nervous system, with an upregulation in men and a downregulation in women.

From social adversity to chronic stress

Of note in this context is a series of studies conducted by Miller, Cole and co-worker, using gene expression measurement in subjects exposed to various degrees of psychosocial stressors, both early in life and as adults.

In one study on socioeconomic circumstances they used a similar approach as the study mentioned above (O'Donovan et al., 2011) (bioinformatic analysis of genes that are targets of GR, NFKB and CREB) but in peripheral blood mononuclear cells (lymphocytes and monocytes) rather than monocytes only (Miller et al., 2009a). They found that a history of exposure to low socioeconomic circumstances early in life was associated, in adulthood, with an upregulation of target genes for NFKB and a downregulation of target genes for GR, consistently with the same a pattern of glucocorticoid resistance described in the study by O'Donovan above (O'Donovan et al., 2011). Moreover, they fond an upregulation of target genes for CREB/ATF, that is the same finding in males in the study by O'Donovan (O'Donovan et al., 2011), even if the sample was mixed males and females. Of note is also that there were no changes in GR expression per se. Moreover, these subjects also had functional evidence of increased inflammation, as shown by increased expression of IL-6 following stimulation of immune cells, and increased circulating cortisol levels – again, a clear pattern indicative of glucocorticoid resistance at a molecular, cellular and systemic level. Also of note is that the authors did not find any changes in the cell populations composition.

In a secondary analysis on the same samples (Chen et al., 2011), the authors also found that subjects who were exposed to high maternal warmth did not show the pattern of upregulation of NFKB-target transcripts (although, interesting, they continue to show the GR-target genes upregulation); moreover, these subjects also showed less IL-6 production following stimulation of the immune cells.

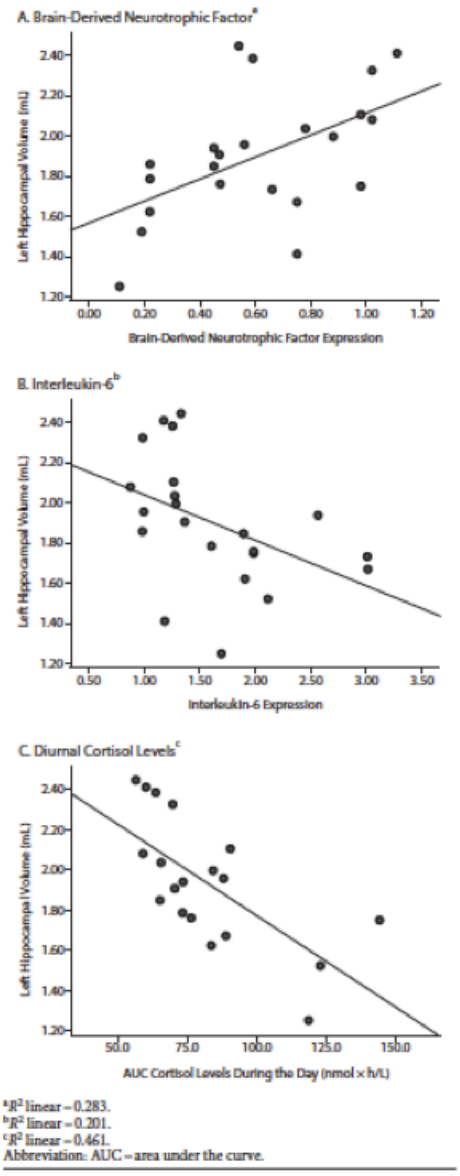
A similar pattern of upregulation of target genes for NFKB, and downregulation of target genes for GR, has been shown by these same authors in isolated monocytes of adults experiencing the chronic stress of caregiving (Miller et al., 2008), in the presence of increased cortisol levels 4 hours after awakening, increased serum markers of inflammation, and normal levels of GR mRNA.

Finally, again a similar pattern of upregulation of target genes for NFKB, and downregulation of target genes for GR, has been shown by these same author in isolated mononuclear cells (lymphocytes and monocytes) of adults who experience loneliness (Miller et al., 2009b), in the presence of increased evening cortisol levels, increased serum markers of inflammation, and normal levels of GR mRNA.

Gene expression, inflammation and brain size

We finally want to mention one study from our laboratory (Mondelli et al., 2011). We have measured gene expression in whole blood of patients with a fist-episode psychosis and in healthy controls.

Figure 1. Correlation of Left Hippocampal Volume With (A) Brain-Derived Neurotrophic Factor Gene Expression, (B) Interleukin-6 Gene Expression, and (C) Diurnal Cortisol Levels in First-Episode Psychosis Patients



[a] R^2 linear = 0.283.
[b] R^2 linear = 0.201.
[c] R^2 linear = 0.461.
Abbreviation: AUC = area under the curve.

Using direct measurement of candidate genes via qPCR, we found that patients had reduced gene expression levels of the neurotrophic molecule, BDNF, and increased gene expression levels of the two pro-inflammatory cytokines, IL-6 and TNF-alpha. Patients also had very high levels of psychosocial stressors, both in terms of exposure to childhood trauma and exposure to recent life events. Moreover, they had increased cortisol levels and a smaller left hippocampal volume, a well-known brain structural abnormality in psychosis.

Perhaps more of interest, a linear regression analysis in patients showed that a history of childhood trauma and high levels of recent stressors predicted lower BDNF expression through an inflammation-mediated pathway: that is, through the increased gene expression levels of the pro-inflammatory cytokines.

Finally, lower BDNF expression, increased IL-6 expression, and increased cortisol levels, all significantly and independently predicted a smaller left hippocampal volume (see Figure), and, together, explained a very high variance (adjusted R2 = 0.71).

This paper not only confirms the relationship between exposure to psychosocial stressors and increased inflammation-related gene expression, but also confirm the functional validity of peripheral gene expression as relevant to brain outcomes – in this case, hippocampal volume.

Conclusions

In summary, we have presented data across different samples, ranging from patients with PTSD following violent trauma to adults with early exposure to low socioeconomic status; and using different methods and different type of blood RNA, from whole blood, isolated mononuclear cells or isolated monocytes; and all studies identify a consistent pattern of stress-induced gene expression changes, indicating an increased inflammatory status and a reduced functional activity of the GR. This, as mentioned above and extensively discussed in the publications cited here, seems to represent therefore the molecular signature of exposure to stress.

In addition, we have shown evidence indicating a link between some measures of gene expressions in the blood and both functional measures of immune activation as well as brain volumetric measures, confirming that peripheral blood RNA can be used as a proxy-source for accessing mechanisms relevant to other, less available tissues.

Some of the questions that remain unanswered are: the temporal relationship of these gene expression changes with the initial exposure to stress, especially considering the frequent clustering of stressors in the same individuals; the distinction between “trait” and “state” markers, and thus whether some of these expression changes represent predisposition to encounter stress or to respond to stress with psychopathology, rather than the direct effects of the exposure; the interaction between gene expression changes and cortisol levels or HPA axis activity; and the specificity of gene expression changes within distinct blood cells subpopulations.

However, notwithstanding these unanswered questions, peripheral blood RNA is a strong and clinically relevant biomarker system and should be used for both target validation of candidate genes and target discovery of unknown mechanisms.

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