Biological markers of generalized anxiety disorder

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Introduction

Generalized anxiety disorder (GAD) is a serious psychiatric condition, affecting up to 6% of the population during their lifetime; if not appropriately treated, it has a chronic course and carries a high burden of disability and public burden. Its manifestation is complicated by the comorbidity with other psychiatric disorders, such as major depressive disorder (MDD), panic disorder, and alcohol/substance abuse, which additionally aggravate outcome and contribute to a poor treatment response. Patients with GAD are frequently users of primary care resources in Western countries, having a large impact on the health care system. As with treatment of other psychiatric disorders, the treatment of GAD involves two targets—a reduction in acute symptoms and relapse prevention in the long term. Until now, international guidelines for GAD treatment have recommended selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs), and pregabalin as first-line options, owing to their established efficacy and good safety profiles, with benzodiazepines such as diazepam as second-line options. However, delayed action, worsening of anxious symptoms in the first
Translational research

days of treatment, and troublesome side effects—such as nausea and sexual dysfunction for SSRIs and SNRIs and dizziness and sedation for pregabalin—are often reasons of treatment discontinuation and lack of desirable therapeutic outcome. In addition, a large proportion of GAD patients do not obtain a sufficient response to first-line treatment or they continue to have residual symptoms; they, therefore, are consequently at high risk of experiencing disorder chronicity and a low quality of life.

Because of these unmet needs, attempts at new pharmacological approaches for the treatment of GAD have been introduced, for example, mood stabilizers and atypical antipsychotics in monotherapy or in augmentation of standard treatment with SSRIs/SNRIs. However, no other agents—including quetiapine, which shows the most robust anxiolytic effect among antipsychotics—can be recommended, at least not as first-line options for GAD treatment. Unfortunately, recent attempts to find new targets for GAD treatment—in particular, corticotropin-releasing factor (CRF) receptors—were not successful.

GAD often remains marginalized and neglected due to traditional diagnostic conception; there is a continuous debate between researchers and clinicians about its nosological and neurobiological uniqueness. However, the one approach that is certain to distinguish GAD from other mental health disorders and to improve understanding of GAD phenomenology is the investigation of biological factors commonly referred to as biomarkers that underlie GAD pathogenesis and treatment outcomes.

Psychiatric research efforts have recently prioritized such identification of biomarkers as it may significantly improve earlier diagnosis and prevention strategies for mental disorders. GAD has recently become the focus of intensive research efforts applying neuroimaging and genetic approaches toward discovery of the pathogenetic biomarkers for GAD; however, only a few studies have specifically addressed predictors of treatment response. In this paper, we review the large amount of available data and focus in particular on evidence from neuroimaging, genetic, and neurochemical measurements in GAD in order to better understand potential biomarkers involved in its etiology and treatment.

Neuroimaging biomarkers

To date, the main evidence for biomarkers in GAD is derived from neuroimaging studies, including those using structural (magnetic resonance imaging [MRI], diffusion tensor imaging [DTI]), functional (functional MRI [fMRI], positron emission tomography [PET]/single-photon emission computed tomography [SPECT]), and biochemical (proton magnetic resonance spectroscopic imaging [MRSI]) methods. These different neuroimaging techniques have for the most part been used to study baseline neuronal characteristics or changes in correlation with symptom severity. Only a few of them have measured the effect of therapeutic interventions on brain functioning in order to explore treatment biomarkers or response predictors. It is notable that available data are not consistent and thus far, vary significantly across studies. Several factors, including sex, age, phenotype, cognitive functioning, and medication status, need to be taken into account when interpreting structural brain changes detected in GAD. Here, we briefly summarize the results of neuroimaging studies in GAD and present the key findings in Table I (a simplified summary).

Structural brain morphology studies

There is good evidence that GAD is characterized by significant anatomical changes in the brain, particularly within regions related to anxiety neurocircuitry. For example, increased gray matter (GM) volume in the amygdala has been repeatedly found in GAD patients. Notably, increased right amygdala volume in GAD patients, mostly among females, was associated with prolonged reaction times on the tracking task, indicating attentional impairment. Earlier larger volumes of the amygdala and the dorsomedial prefrontal cortex (PFC) were observed in GAD females, suggesting that these disturbances in anxiety-specific regions may be related to sex predisposition for GAD.

In contrast, GM volume in the right putamen was significantly larger in GAD patients than in healthy controls, whereas a significant sex main effect was found in the left precuneus/posterior cingulate cortex, with GM volumes larger in males than in females. However, no sex-by-diagnosis interaction effect was found in this study, suggesting that GM volume in GAD is not influenced by sex. The same group has also reported that a larger GM volume in the right putamen is positively correlated with childhood maltreatment. A study in medication-free adolescents suffering from noncomorbid GAD reported increased GM volumes in the right precuneus and right precentral gyrus and decreased GM...
| Sample   | Biomarker                                                                            | Reference          |
|----------|-------------------------------------------------------------------------------------|--------------------|
| **Structural brain morphology studies**                                                                                                                |
| 16P (14F) 31HC (13F) | Greater GMV in Amy                                                                   | Etkin et al,\(^8\) 2009 |
| 19P (17F) 21HC (18F) | Larger right Amy                                                                     | Makovac et al,\(^9\) 2015 |
| 16P (16F) 15HC (15F) | Larger Amy and DMPFC                                                                 | Schienle et al,\(^10\) 2011 |
| 26P (13F) 25HC (12F) | Larger GMV in right Put                                                              | Liao et al,\(^11\) 2013 |
| 15P (16F) 28HC (17F) | Increased GMV in right precuneus and precentral gyrus; decreased GMV in left OG and PC | Strawn et al,\(^12\) 2013 |
| 19P (16F) 24HC (17F) | Higher GMV in BG; lower WMV in DLPFC                                                 | Hilbert et al,\(^13\) 2015 |
| 20P (7F) 20HC (7F)  | Reduced GM in MB, Thal, Hip, insula, STG; reduced WMV in MB, ALIC, DLPFC, PrG       | Moon et al,\(^14\) 2017 |
| 14P (8F) 10HC (6F)  | Decreased volume in Hip                                                               | Abdallah et al,\(^15\) 2013 |
| **Functional MRI studies**                                                                                                                             |
| 17P (6F) 12HC (6F) | Greater BOLD to masked angry faces in right Amy                                       | Mink et al,\(^16\) 2008 |
| 15P (7F) 20HC (11F) | Greater BOLD to facial-threat cues in Amy, vPFC, ACC                                 | McClure et al,\(^17\) 2007 |
| 14P (12F) 12HC (10F) | Greater BOLD to both aversive and neutral pictures in bilateral dorsal Amy; higher BOLD in ACC predicts better response to treatment | Nitschke et al,\(^18\) 2009 |
| 17P (11F) 24HC (18F) | Abnormal mPFC response to emotional conflict; absent FC in PGC-Amy                    | Etkin et al,\(^19\) 2010 |
| 15P (12F) 15HC (9F) | Decreased BOLD in Amy, but increased in BNST during gambling task                    | Yassa et al,\(^20\) 2012 |
| 17P (11F) 17HC (8F) | Decreased BOLD to fearful faces in Amy, but increased to angry in MFG                 | Blair et al,\(^21\) 2008 |
| 8P (5F) 12HC (6F) | Increased BOLD to worry in ACC and PFC                                               | Paulesu et al,\(^22\) 2010 |
| 15P (15F) 16HC (16F) | Decreased BOLD to facial expressions in PFC and ACC                                  | Palm et al,\(^23\) 2011 |
| 17P (13F) 18HC (10F) | Decreased BOLD during explicit emotion regulation in dorsal ACC                       | Blair et al,\(^24\) 2012 |
| 23P (17F) 22HC (11F) | Decreased BOLD during emotion regulation in PFC                                       | Ball et al,\(^25\) 2013 |
| 17P (6F) 17HC (6F) | Increased BOLD to anxiety-inducing words in VLPFC and PrG                             | Moon et al,\(^26\) 2015 |
| 32P (32F) 25HC (25F) | Less discriminating BOLD response in vmPFC during safety versus threat                | Cha et al,\(^27\) 2014 |
| 32P (25F) 25HC (25F) | Hyperactivity in VTA during fear generalization task                                  | Cha et al,\(^28\) 2014 |
| 18P (8F) 15HC (8F) | Greater BOLD to angry faces in right VLPFC                                           | Monk et al,\(^29\) 2006 |
| 28P (14F) 28HC (14F) | Lower RSFC in PFC-Lim and Cin and higher in PFC-Hip                                  | Wang et al,\(^30\) 2016 |
| 19P (17F) 21HC (18F) | Lower RSFC between the right Amy and right SFG, right paraCin/ACC, and right SMG      | Makovac et al,\(^31\) 2016 |
| 10P (6F) 10HC (6F) | Increased BOLD in mPFC and right VLPFC                                                | Strawn et al,\(^32\) 2012 |
| 21P (7F) 22HC (8F) | Increased FC between Hip and FG                                                       | Cui et al,\(^33\) 2016 |
| 26P (16F) 20HC (11F) | Abnormal FC between Amy and dPFC, Ins and STG                                        | Liu et al,\(^34\) 2015 |
| 15P (12F) | Greater BOLD in rACC and lower in Amy predicted better response to venlafaxine (8 wk) | Whalen et al,\(^35\) 2008 |
| 14P (12F) | Higher BOLD in ACC predicted better response to venlafaxine (8 wk)                  | Nitschke et al,\(^36\) 2009 |
| 14P (7F) | Both CBT and fluoxetine increased BOLD in right VLPFC in response to angry faces     | Maslowsky et al,\(^37\) 2010 |
| 21P (16F) | CBT decreased BOLD in Amy and subGAC in response to fear/angry faces                 | Fonzo et al,\(^38\) 2014 |
| 19P (9F) | Reduced BOLD in Amy and Ins during emotional tests after acute but not 4-wk administration of alprazolam | Brown et al,\(^39\) 2015 |

Table I. Summary of neuroimaging biomarkers in generalized anxiety disorder. (see abbreviations next page)
volumes in the left orbital gyrus and posterior cingulate.\textsuperscript{13} Compared with healthy adolescents, youth with GAD exhibited increased cortical thickness in the right inferolateral and ventromedial PFC (ie, inferior frontal gyrus), the left inferior and middle temporal cortex, and the right lateral occipital cortex. No relationships were observed between cortical thickness and the severity of anxiety symptoms in the significant regions.\textsuperscript{14} Additionally, significantly higher GM volumes were found in medication-free GAD subjects, mainly in basal ganglia structures and less consistently in the superior temporal pole; however, white matter (WM) volumes were lower in the dorsolateral PFC.\textsuperscript{15} Similarly, significant reduction in the WM volumes in the dorsolateral PFC, anterior limb of the internal capsule (ALIC), and midbrain was observed in GAD patients who had working memory dysfunction.\textsuperscript{16} Notably reduced dorsolateral PFC volume was negatively correlated with clinical severity and illness duration in GAD, whereas a significantly smaller orbitofrontal cortex volume was demonstrated in female than in male patients.\textsuperscript{17}

A decrease in hippocampal volumes has also been found in GAD.\textsuperscript{18} The distinguishable brain alternatives—in particular, thinner cortices in the right medial orbitofrontal and fusiform gyri, left temporal pole, and lateral occipital regions—were found in MDD patients with comorbid GAD than in those without GAD or controls, supporting the notion that GAD is a distinct clinical entity.\textsuperscript{19} Finally, reduced frontolimbic structural connectivity was demonstrated in patients with GAD by a diffusion-tensor imaging study, suggesting a neural basis for emotion regulation deficits in GAD.\textsuperscript{20}

### Functional MRI studies

Both neuronal response to emotional stimuli and resting-state connectivity have been investigated in a number of fMRI studies in GAD, mostly not in relation to treatment effect. The several regions traditionally connected to anxiety neurocircuitry and/or emotional regulation, including the amygdala, anterior cingulate cortex (ACC), medial PFC, ventrolateral PFC, dorsolateral PFC, and some others, have shown abnormal or changed activities in GAD. In particular, greater amygdala activation was demonstrated in pediatric patients with GAD and positively correlated with anxiety sever-

| 6P (3F) | Citalopram (7 wk) reduced BOLD in PFC, Str, Ins, and paraLim regions | Hoehn-Saric et al,\textsuperscript{51} 2004 |
|---|---|---|
| **PET and SPECT studies** | | |
| 7P (3F) 7HC (3F) | Unchanged 5-HTT in MB | Maron et al,\textsuperscript{53} 2004 |
| 12P (8F) 12HC (8F) | Unchanged 5-HTT in MB, decreased DAT in Str | Lee et al,\textsuperscript{54} 2015 |
| 10P (10F) 10HC (10F) | Decreased GABA-A in left TP | Tiitonen et al,\textsuperscript{55} 1997 |
| **Metabolic MRI studies** | | |
| 15P (8F) 15HC (8F) | Higher NAA/Cr in right DLPFC | Mathew et al,\textsuperscript{56} 2004 |
| 9P (4F) 10HC (4F) | Lower bilateral NAA/Cr in Hip after 12 wk of paroxetine | Mathew et al,\textsuperscript{57} 2010 |
| 15P (9F) 8HC (5F) | Increased NAA/Cr in Hip in responders to riluzole (8 wk), but decreased in nonresponders | Mathew et al,\textsuperscript{58} 2008 |
| 15P (6F) 15HC (6F) | Lower Ch/NAA in DLPFC | Moon et al,\textsuperscript{59} 2015 |

5-HTT, serotonin transporter; ACC, anterior cingulate cortex; ALIC, anterior limb of the internal capsule; Amy, amygdala; BG, basal ganglia; BNST, bed nucleus of the stria terminalis; BOLD, blood oxygen–level dependent; CBT, cognitive behavioral therapy; Ch/NAA, choline/N-acetylaspartate; Cin, cingulate; DAT, dopamine transporter; DLPFC, dorsolateral prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; dPFC, dorsal prefrontal cortex; FC, functional connectivity; FG, fusiform gyrus; GMV, gray matter volume; Hip, hippocampus; Ins, insula; Lim, limbic; MB, midbrain; MFG, middle frontal gyrus; mPFC, medial prefrontal cortex; MRI, magnetic resonance spectroscopy; NAA/Cr, N-acetylaspartate/creatinine; OG, orbital gyrus; paraCin, paracingulate; paraLim, paralimbic; PC, posterior cingulated; PET, positron emission tomography; PGC, pregenual cingulate; PrG, precentral gyrus; Put, putamen; rACC, rostral anterior cingulate cortex; RSFC, resting-state functional connectivity; SFG, superior frontal gyrus; SMG, supramarginal gyrus; SPECT, single-photon emission computed tomography; STG, superior temporal gyrus; Str, striatum; subGAC, subgenual anterior cingulate; Thal, thalamus; TP, temporal pole; VLPFC, ventrolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex; vPFC, ventral prefrontal cortex; VTA, ventral tegmental area; WM, white matter volume; wk, week

Table I. Continued
Earlier, other pediatric GAD studies have shown hyperactivity in the amygdala in response to negative emotional faces. Also, disruptions in amygdala-based intrinsic functional connectivity networks have been reported to be similar between adult and adolescents with GAD. Similar findings were also evident in adult GAD patients. In another study, GAD patients had higher amygdala activation than healthy controls in response to neutral, but not angry, faces. However, after fear induction in a gambling task, patients with GAD demonstrated decreased activity in the amygdala and increased activity in the bed nucleus of the stria terminalis when compared with controls.

The involvement of cortical regions was evidenced by studies showing that in response to angry faces or triggered worry, GAD patients demonstrated increased blood oxygen–level dependent (BOLD) responses in a lateral region of the middle frontal gyrus and persistent activation in both ACC and PFC areas. The exaggerated early neural responses to errors, as reflected by the error-related negativity on electroencephalography (EEG), was also linked to ACC abnormalities in GAD. In contrast, hypoactivation of PFC (only in female patients) or reduced dorsal ACC BOLD activity was observed in response to fearful, sad, angry, and happy facial expressions. The BOLD hypoactivation in PFC was also demonstrated in both GAD and panic disorder during response in a reappraisal task, suggesting common neuronal pathways underlying emotion dysregulation in both ACC and PFC areas. The significantly higher neuronal activities were observed in the ventrolateral PFC and precentral gyrus BOLD response to anxiety-inducing words.

The ventromedial PFC has been shown to have a critical role in threat processing in close association with broader corticolimbic circuit abnormalities, which may synergistically contribute to GAD. Moreover, the maladaptive threat processing was observed in the ventral tegmental area and the mesocorticolimbic system in female patients with GAD, which may implicate dopaminergic pathways in clinical anxiety. In trials including an angry face, adolescents with GAD showed greater right ventrolateral PFC activation than healthy adolescents. This activation was negatively correlated with anxiety severity, suggesting that the neuronal increase in BOLD signal may serve as a compensatory response. However, functional abnormalities in ventral cingulate and the amygdala seem to be common both for major depression and GAD, perhaps because of shared genetic factors. However, those with comorbid GAD in major depression had modulated hypoactivation in response to an emotional task in middle frontal regions and the insula, as usually seen in pure depression; this gives additional support to the idea of different types of emotional information processing in anxiety and depression.

Resting-state functional connectivity was reported to be lower in prefrontal-limbic and cingulate and higher in prefrontal-hippocampus regions, and both abnormalities were correlated with clinical symptom severity. Also, the amygdala-PFC connectivity underlying worry and rumination in GAD has recently been linked to autonomic dyscontrol, suggesting overlapping neuronal substrates for cognitive and autonomic dysregulation. Furthermore, amygdala and the middle frontal gyrus activation in response to presentation of emotional faces can distinguish patients with GAD and social phobia, indicating different neural circuitry dysfunctions in these two highly prevalent anxiety disorders.

Previous research also implicates the ACC in emotion regulation through effects on the amygdala and suggests that deficits in ACC-amygdala connectivity may contribute to emotion dysregulation in patients with GAD. Different hippocampal connectivity was observed between posttraumatic stress disorder (PTSD) and GAD patients, potentially explaining the difference in fear-related memory dysregulation in two anxiety phenotypes. Increased activation of the medial PFC and right ventrolateral PFC, as well as altered connectivity between the amygdala or ventrolateral PFC and regions which subserve mentalization (eg, posterior cingulate cortex, precuneus, and medial PFC) was observed in adolescents with GAD. In addition, increased functional connectivity between hippocampus/parahippocampus and fusiform gyrus was found in GAD, whereas greater functional connectivity between somatosensory cortex and thalamus was observed in panic disorder, further suggesting these two disorders have different clinical and psychopathological processes. Finally, decreased functional connectivity was found between the left amygdala and left dorsolateral PFC and increased right amygdala functional connectivity with insula and superior temporal gyrus in adolescents with GAD, confirming that they have abnormalities in brain regions associated with the emotional processing pathways.
Only a few fMRI studies have measured changes after treatment. The greater pretreatment reactivity to fearful faces in rostral ACC (rACC) and lesser reactivity in the amygdala have predicted a better response to 8-week treatment with venlafaxine in GAD; however, no differences between patients and controls with regard to neuronal activation within these regions were detected before treatment.46 In addition, higher levels of pretreatment ACC activity in anticipation of both aversive and neutral pictures were associated with greater reductions in anxiety and worry symptoms after an 8-week treatment with venlafaxine in GAD.47 This suggests that ACC-amygdala responsiveness could prove useful as a predictor of antidepressant treatment response in GAD. A significant increase in right ventrolateral PFC activity in response to angry faces after treatment with cognitive behavioral therapy (CBT) or fluoxetine was reported in small samples of young patients with GAD.48 Greater anticipatory activity in the bilateral dorsal amygdala was shown in GAD, and a CBT course led to attenuation of amygdalar and subgenual anterior cingulate response to fearful/angry face presentation, plus heightened insular activation in response to happy faces.49 However, the treatment had no apparent effects on increased amygdala-insular connectivity, and the changes were not associated with symptoms of worry. An interesting study with the benzodiazepine alprazolam found that neuronal activation in the amygdala and insula during emotional tests was reduced after acute administration of alprazolam. However, activity returned to baseline levels at week 4 of alprazolam treatment, indicating that the neural mechanisms supporting sustained treatment effects of benzodiazepines in GAD differ from those underlying their acute effects.50 Significantly reduced BOLD responses to a pathology-specific worry in prefrontal regions, striatum, insula, and paralimbic regions were reported after 7 weeks of treatment with citalopram in a small sample of patients with GAD.51

**PET and SPECT studies**

Despite the great potential that radioisotope studies have to explore the neurochemical effects in live human brain, only very few have been conducted in GAD. The first PET report in GAD was by Wu et al.52 They found lower absolute metabolic rates in basal ganglia, but found increased rates in the left inferior gyrus, Brodmann Area (BA) 17, occipital lobe, right posterior temporal lobe, and right precentral frontal gyrus.

Unlike in MDD and other anxiety disorders, in GAD, the number of 5-HT reuptake sites available in the brain was unchanged, as measured by tracers such as [123I]nor-β-CIT and [11]ADAM.53 In contrast, dopamine-reuptake-site density measured by [Tc] TRODAT-in the striatum was significantly lower in the GAD patients than in the healthy controls.54 Additionally, significant decreases in the left temporal pole type A γ-aminobutyric acid (GABA-A) receptors were found in a PET study with GAD female patients.55

**Metabolic MRI studies**

The very first magnetic resonance spectroscopy study demonstrated a higher N-acetylaspartate/creatine (NAA/Cr) ratio in the right dorsolateral PFC in medication-free patients with GAD than in healthy participants.56 The subsequent study reported persistently lower levels of bilateral hippocampal NAA/Cr in GAD after a successful 12-week treatment with paroxetine, but excluded an association between this hippocampal neuronal marker and anxiolytic response to this medication.57 Interestingly, a significant increase in hippocampal NAA was observed in GAD responders to the glutamate-release inhibitor riluzole, whereas nonresponders had decreases over 8 weeks of treatment.58 A low level of choline/NAA in the dorsolateral PFC was observed in GAD patients in another recent study, and this negatively correlated with anxiety severity.59

**Genetic biomarkers**

Increasing efforts are being made to determine genetic factors involved in the onset and development of psychiatric disorders and also those influencing their response to therapeutic interventions. Although several approaches have been used in this search, including epidemiological (family and twin) studies and molecular (linkage and association) methods, the genetic research for GAD is still modest compared with research undertaken for other anxiety or mood disorders. For a comprehensive discussion of the genetics of GAD, see also the article by Gottschalk and Domschke in this issue. Earlier meta-analysis of twin studies has estimated the heritability of GAD to be 32%,60 but higher heritability estimates (49%) and no sex differences, in contrast to...
previous reports, were demonstrated by a recent cross-sectional twin study in Sweden.\(^61\)

So far, only a few association studies have been conducted among patients with the GAD phenotype, leaving us without a consistent or clear conclusion about GAD vulnerability genes. Specifically, genes for monoamine oxidase A (MAOA) and solute carrier family 6 member 4 (SLC6A4) have been implicated as potentially involved in the pathogenesis of GAD,\(^62,63\) and the association of GAD with a 5-hydroxytryptamine receptor 1A (5-HTRIA) gene variation has been shown to be partly mediated by comorbidity with major depression.\(^64\)

A recent study showed that the Met allele of the functional brain-derived neurotrophic factor (BDNF) Val66Met polymorphism is associated with GAD risk, along with an increase in serum BDNF levels.\(^65\) However, Val66Met variation was associated with neither GAD nor BDNF plasma levels in a Chinese Han population with GAD.\(^66\) In addition, polymorphisms both in regulator of G-protein signaling 2 (RGS2) and neuropeptide Y (NPY) genes have been shown to modify risk of post-disaster GAD under conditions of high stressor exposure among adults living in areas affected by the 2004 Florida Hurricanes\(^67,68\) and few single-nucleotide polymorphisms (SNPs) in proteasome modulator 9 (PSMD9) gene were in linkage with GAD in Italian families with type 2 diabetes.\(^69\)

Recently, the microarray study of peripheral gene expression signatures has become a powerful and promising approach in the discovery of novel biomarkers via transcriptional and microRNA analysis. For example, a microRNA (miRNA) array study performed in peripheral blood mononuclear cells (PBMCs) has revealed negative correlation between the expression level of miR-4505 and miR-663 and anxiety manifestation in GAD patients; however, the molecular mechanism of this association requires further explanation.\(^70\) Another genome-wide peripheral gene expression study in a large sample of patients with GAD found no significant differential expression in women; however, 631 genes, most of which were immune-related, were differentially expressed between anxious and control men.\(^71\)

Some other promising data have been reported by pharmacogenetic initiatives, where the intensive search for genetic treatment predictors has revealed a few genes, including the pituitary adenylate cyclase-activating peptide (PACAP), serotonin transporter (5-HTT), the serotonin 2A receptor gene (HTR2A), corticotropin-releasing hormone receptor 1 (CRHR1), dopamine receptor D3 (DRD3), nuclear receptor subfamily group C member 1 (NR3C1), and phosphodiesterase 1A (PDE1A), as potential markers predicting therapeutic response to SSRI medication in patients with GAD.\(^72-78\) In contrast, none of the investigated polymorphisms within dopamine receptor D2 (DRD2) or dopamine active transporter 1 (DAT1) genes showed an impact on venlafaxine XR treatment response in GAD.\(^79\)

**Neurochemical biomarkers**

Plasma appears to be a rational source for proteomic and metabolomic measurements because it is easily accessible and because several molecules from the brain are transported across the blood-brain barrier and reach the circulation. However, drawing inferences from the neurochemical composition of plasma on the processes in the brain is not straightforward.\(^80\) Moreover, only a few studies have been conducted on plasma-based pathogenetic and/or treatment predictors in GAD, indicating the further need to explore such potentially valuable approaches. So far, the studies measuring 5-hydroxytryptamine (5-HT, also called serotonin)-related biomarkers have found decreased platelet 5-HT-reuptake-site binding in GAD patients,\(^81\) but unchanged 5-HT binding in lymphocytes as compared with controls.\(^82\) Moreover, GAD patients showed concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in platelet-rich and platelet-poor plasma, as well as in lymphocytes, within the normal range.\(^83\)

Unlike for other anxiety disorders, particularly PTSD, it seems that GAD is not characterized by consistent evidence of possible abnormalities in the regulation of HPA-axis activity. Male patients with GAD displayed similar cortisol plasma levels after a stressful test.\(^84\) A greater cortisol awakening response has been reported only for those GAD patients who had comorbidity MDD.\(^85\) The large studies performed among 4256 Vietnam-era veterans showed similar cortisol and dehydroepiandrosterone sulfate (DHEAS) plasma levels and cortisol/DHEAS ratios between GAD sufferers and normal controls.\(^86\)

Another approach is to use challenge tests to provoke anxiety/stress. In one, administration of 7.5% carbon dioxide did not significantly change salivary cortisol levels in medication-free GAD patients.\(^86\) Moreover,
no difference in pre-sleep salivary cortisol level was found among children with GAD, despite the presence of sleep disturbances. On the other hand, both higher and lower cortisol awakening responses were observed among elderly GAD patients than with nonanxious controls, positively associated with symptomatic severity in one study and irrespective of the duration of illness in another one. Furthermore, both untreated and venlafaxine-treated GAD patients demonstrated significantly higher cortisol levels than normal controls in a clonidine-challenge study. Nevertheless, some studies reported a significant reduction in post-treatment cortisol levels after successful psychological or pharmacological treatment of GAD. For example, elevated plasma cortisol levels decreased after successful CBT and greater reductions in both peak and total salivary cortisol were shown in elderly GAD patients after escitalopram treatment than in placebo-treated patients. However, no association was reported between a positive therapeutic outcome to buspirone or alprazolam and post-treatment cortisol levels in GAD.

Although a strong link between neurotrophic factors and mood disorders is well established, it seems that this relationship in GAD is not so obvious or may have the opposite effect. No changes in BDNF levels were found in a sufficiently large sample of patients with different anxiety disorders, including GAD. However, a small study comparing patients with GAD or MDD with healthy subjects showed doubled plasma levels of BDNF and artemin, a glial-cell-line–derived neurotrophic factor family member, in GAD patients compared with normal controls, whereas depressed patients showed a reduction. The baseline plasma BDNF levels were not associated with GAD severity; however, a significantly greater mean increase in plasma BDNF level was observed in duloxetine-treated patients than in those who had received placebo. Interestingly, an increased plasma concentration of nerve growth factor was observed among GAD patients after successful CBT.

Finally, among immunological factors, C-reactive protein (CRP) was found to be elevated in some studies. A pilot study that measured peripheral levels of cytokines in small cohorts of GAD and MDD patients has demonstrated an increase in plasma concentrations of interleukin (IL)-10 and α-melanocyte-stimulating hormone (α-MSH), but no significant variations in IL-2. Earlier, a study in patients with GAD and panic disorder with agoraphobia measured cell-mediated immune functions through the lymphocyte proliferative response to phytohemagglutinin, IL-2 production, and natural killer cell activity and suggested a reduction in this function when compared with healthy controls.

**Conclusion**

GAD is still something of an orphan disorder in terms of known biomarkers, as well as in the diagnosis of anxiety disorders. To some extent, this is due to the marked and common overlap of GAD with major depression; also, because the severity of the illness’ impact on the activities of the patients is often overlooked, research funds are limited. However, as this review shows, there are meaningful differences in the biology of GAD and depression that not only help confirm that these are indeed separate diseases but that they may in some cases be polar opposites. This may help explain the well-established phenomenon that GAD often precedes depression, and the emergence of depression may represent a failure of the compensatory activities that the body mounts to protect itself against the chronic stress imposed by GAD. Such differences have significant implications for improving diagnosis and thus enhance the understanding and treatment of GAD as a different illness from its better-recognized cousins, such as panic disorder and obsessive-compulsive disorder. Also, new biological insights have major implications for developing new treatments. These are sorely needed, as there has been no major advance in the treatment of GAD for many years, and thus, patients continue to suffer. If we could better target the few treatments we have on the basis of biomarkers, then we could at least work to optimize patient response to medication.

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REFERENCES

1. Wittchen HU, Jacob F. Size and burden of mental disorders in Europe – a critical review and appraisal of 27 studies. Eur Neuropsychopharmacol. 2005;15(4):357-376.

2. Nutt D, Argyropoulos S, Hood S, Potokar J. Generalized anxiety disorder: a comorbid disease. Eur Neuropsychopharmacol. 2006;16(suppl 2):S109-S118.

3. Roberge P, Normand-Lauzière F, Raymond I, et al. Generalized anxiety disorder in primary care: mental health services use and treatment adequacy. BMC Fam Pract. 2015;16:146.

4. Buoli M, Caldironi A, Caletti E, et al. New approaches to the pharmacological management of generalized anxiety disorder. Expert Opin Pharmacother. 2014(2):175-184.

5. Bandelow B, Zohar J, Hollander E, et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders - first revision. World J Biol Psychiatry. 2008;9(4):248-312.

6. Pollack MH. Refractory generalized anxiety disorder. J Clin Psychiatry. 2009;70(suppl 2):32-38.

7. Starcevic V. Generalized anxiety disorder: psychopharmacotherapy update on a common and commonly overlooked condition. Australas Psychiatry. 2015;23(4):338-342.

8. Etkin A, Prater KE, Schatzberg AF, et al. Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. Arch Gen Psychiatry. 2009;66(12):1361-1370.

9. Makovac E, Meeten F, Watson DR, et al. Neurostructural abnormalities associated with axes of emotion dysregulation in generalized anxiety. Neuropsychiatr Dis Treat. 2015;10:172-181.

10. Schiene A, Ebner F, Schäfer A. Localized gray matter volume abnormalities in generalized anxiety disorder. Eur Arch Psychiatry Clin Neurosci. 2011;261(4):303-307.

11. Liao M, Yang F, Zhang Y, et al. Lack of gender effects on gray matter volumes in adolescent generalized anxiety disorder. J Affect Disord. 2014;155:278-282.

12. Liao M, Yang F, Zhang Y, et al. Childhood maltreatment is associated with larger left thalamic gray matter volume in adolescents with generalized anxiety disorder. PLoS One. 2013;8(8):e71898.

13. Strawn JR, Wehry AM, Chu WJ, et al. Neuroanatomic abnormalities in adolescents with generalized anxiety disorder: a voxel-based morphometry study. Depress Anxiety. 2013;30(9):842-848.

14. Strawn JR, John Wegman C, Dominick KC, et al. Cortical surface anatomy in pediatric patients with generalized anxiety disorder. J Anxiety Disord. 2014;28(7):717-723.

15. Hilbert K, Pine DS, Muehlhan M, et al. Gray and white matter volume abnormalities in generalized anxiety disorder by categorical and dimensional characterization. Psychiatry Res. 2015;234(3):314-320.

16. Moon CM, Jeong GW. Abnormalities in gray and white matter volumes associated with explicit memory dysfunction in patients with generalized anxiety disorder. Acta Radiol. 2017;58(3):353-361.

17. Moon CM, Jeong GW. Alterations in white matter volume and its correlation with clinical characteristics in patients with generalized anxiety disorder. Neuroimaging. 2015;57(11):1127-1134.

18. Abdallah CG, Coplan JD, Jackowski A, et al. A pilot study of hippocampal volume and N-acetylaspartate (NAA) as response biomarkers in riluzole-treated patients with GAD. Eur Neuropsychopharmacol. 2013;23(4):278-284.

19. Liu E, Kostic M, Agosta F, et al. Brain structural abnormalities in patients with major depression with or without generalized anxiety disorder comorbidity. J Neurol. 2015;262(5):1255-1265.

20. Tromp DP, Grupe DW, Oathes DJ, et al. Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. Arch Gen Psychiatry. 2012;69(9):925-934.

21. Monk CS, Telzer EH, Mogg K, et al. Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. Arch Gen Psychiatry. 2008;65(5):568-576.

22. McCabe EB, Adler A, Monk CS, et al. fMRI predictors of treatment outcome in pediatric anxiety disorders. Psychopharmacology (Berl). 2007;191(1):97-105.

23. Roy AK, Fudge JL, Kelly C, et al. Intrinsic functional connectivity of amygdala-based networks in adolescent generalized anxiety disorder. J Am Acad Child Adolesc Psychiatry. 2013;52(3):290-299.

24. Nitschke JB, Sarniopoulos I, Oathes DJ, et al. Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. Am J Psychiatry. 2009;166(3):302-310.

25. Etkin A, Prater KE, Hoeft F, et al. Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. Am J Psychiatry. 2010;167(5):545-554.

26. Höf meg BK, Hoge EA, Greve DN, et al. Neural mechanisms of symptom improvements in generalized anxiety disorder following mindfulness training. Neuropsychiatr Dis Treat. 2013;9:424-458.

27. Yassa MA, Hazlitt RL, Stark CE, Hoehn-Saric R. Functional MRI of the amygdala and bed nucleus of the stria terminals during conditions of uncertainty in generalized anxiety disorder. J Psychiatr Res. 2012;46(8):1045-1052.

28. Blair K, Shaywitz J, Smith BW, et al. Response to emotional expressions in generalized social phobia and generalized anxiety disorder: evidence for separate disorders. Am J Psychiatry. 2008;165(1):1193-1202.

29. Pauslous E, Sambugaro E, Torti T, et al. Neural correlates of worry in generalized anxiety disorder and in normal controls: a functional MRI study. Psychol Med. 2010;40(1):117-124.

30. Weinberg A, Olvet DM, Hajcak G. Increased error-related brain activity in generalized anxiety disorder. Biol Psychol. 2010;85(3):472-480.

31. Palm ME, Elliott R, McKie S, et al. Attenuated responses to emotional expressions in women with generalized anxiety disorder. Psychol Med. 2011;41(5):1009-1018.

32. Blair KS, Geraci M, Smith BW, et al. Reduced dorsal anterior cingulate cortical activity during emotional regulation and top-down attentional control in generalized social phobia, generalized anxiety disorder, and comorbid generalized social phobia/generalized anxiety disorder. Biol Psychiatry. 2012;72(6):476-482.

33. Ball TM, Ramsawh HJ, Campbell-Sills L, et al. Prefrontal dysfunction during emotion regulation in generalized anxiety and panic disorders. Psychol Med. 2013;43(7):1475-1486.

34. Moon CM, Yang JC, Jeong GW. Explicit verbal memory impairments associated with brain functional deficits and morphological alterations in patients with generalized anxiety disorder. J Affect Disord. 2015;166:328-336.

35. Cha J, Greenberg T, Carlson JM, et al. Circuit-wide structural and functional measures predict ventromedial prefrontal cortex fear generalization: implications for generalized anxiety disorder. J Neurosci. 2014;34(11):4043-4053.

36. Cha J, Carlson JM, Dedora DJ, et al. Hyper-reactive human ventral tegmental area and aberrant mesocorticollimbic connectivity in overgeneralization of fear in generalized anxiety disorder. J Neurosci. 2014;34(17):5855-5860.

37. Monk CS, Nelson EE, McClure EB, et al. Ventrolateral prefrontal cortical activation and attentional bias in response to angry faces in adolescents with generalized anxiety disorder. Am J Psychiatry. 2006;163(6):1091-1097.

38. Etkin A, Schatzberg AF. Common abnormalities and disorder-specific compensation during implicit regulation of emotional processing in generalized anxiety and major depressive disorders. Am J Psychiatry. 2011;168(9):968-978.

39. Schlund MW, Verduzco G, Cataldo MF, Hoehn-Saric R. Generalized anxiety modulates frontal and limbic activation in major depression. Behav Brain Funct. 2012;8:8.

40. Wang W, Hou J, Qian S, et al. Aberrant regional neural fluctuations and functional connectivity in generalized anxiety disorder revealed by resting-state functional magnetic resonance imaging. Neuroimage. 2016;624:78-84.

41. Makovec E, Meeten F, Watson DR, et al. Alterations in amygdala-prefrontal functional connectivity account for excessive worry and autonomic dysregulation in generalized anxiety disorder. Biol Psychiatry. 2016;80(10):786-795.

42. Chen AC, Etkin A. Hippocampal network connectivity and activation differentiates post-traumatic stress disorder from generalized anxiety disorder. Neuropsychopharmacology. 2013;38(10):1889-1898.
43. Strawn JR, Bitter SM, Weber WA, et al. Neurocircuitry of generalized anxiety disorder in adolescents: a pilot functional neuroimaging and functional connectivity study. Depress Anxiety. 2012;29(11):939-947.
44. Cui H, Zang J, Liu Y, et al. Differential alterations of resting-state functional connectivity in generalized anxiety disorder and panic disorder. Hum Brain Mapp. 2016;37(4):1459-1473.
45. Liu WJ, Yin DZ, Cheng WH, et al. Abnormal functional connectivity of the amygdala-based network in resting-state fMRI in adolescents with generalized anxiety disorder. Med Sci Monit. 2015;21:459-467.
46. Whalen PJ, Johnstone T, Somerville LH, et al. A functional magnetic resonance imaging predictor of treatment response to venlafaxine in generalized anxiety disorder. Biol Psychiatry. 2008;63(9):858-863.
47. Nitschke JB, Sarinopoulos I, Oathes DJ, et al. Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. Am J Psychiatry. 2009;166(3):302-310.
48. Maslowsky J, Mogg K, Bradley BP, et al. A preliminary investigation of neural correlates of treatment in adolescents with generalized anxiety disorder. J Child Adolesc Psychopharmacol. 2010;20(2):105-111.
49. Forouz G, Samsavah HJ, Flagan TM, et al. Cognitive-behavioral therapy for generalized anxiety disorder is associated with attenuation of limbic activation to threat-related facial emotions. J Affect Disord. 2014;169:76-81.
50. Brown GG, Ostrovitzki S, Stein MB, et al. Temporal profile of brain response to alprazolam in patients with generalized anxiety disorder. Psychiatry Res. 2015;233(3):394-401.
51. Hoehn-Saric R, Schiund MW, Wong SH. Effects of citalopram on worry and brain activation in patients with generalized anxiety disorder. Psychopharmacology. 2004;131(1):11-21.
52. Wu JC, Buchsbaum MS, Hershey TG, et al. PET in generalized anxiety disorder. Biol Psychiatry. 1991;29(12):1181-1199.
53. Maron E, Kuikka JT, Ulat K, et al. SPECT imaging of serotonin transporter binding in patients with generalized anxiety disorder. Eur Arch Psychiatry Clin Neurosci. 2004;254(6):392-396.
54. Lee LT, Tsai HC, Chi MH, et al. Lower availability of striatal dopamine transporter in generalized anxiety disorder: a preliminary two-ligand SPECT study. Int Clin Psychopharmacol. 2015;30(3):175-178.
55. Tiihonen J, Kuikka J, Rasinen P, et al. Cerebral benzodiazepine receptor binding and distribution in generalized anxiety disorder: a fractal analysis. Mol Psychiatry. 1997;2(6):463-471.
56. Mathew SJ, Mao X, Coplan JD, et al. Dorsolateral prefrontal cortical pathology in generalized anxiety disorder: a proton magnetic resonance spectroscopic imaging study. Am J Psychiatry. 2004;161(6):1119-1121.
57. Mathew SJ, Price RB, Shungu DC, et al. A pilot study of the effects of chronic paroxetine administration on hippocampal N-acetylaspartate in generalized anxiety disorder. J Psychopharmacol. 2010;24(8):1175-1181.
58. Mathew SJ, Price RB, Mao X, et al. Hippocampal N-acetylaspate concentration and response to rituxolz in generalized anxiety disorder. Biol Psychiatry. 2008;63(9):891-898.
59. Moon CM, Kang HK, Jeong GW. Metabolic change in the right dorsolateral prefrontal cortex and its correlation with symptom severity in patients with generalized anxiety disorder: proton magnetic resonance spectroscopy at 3 Tesla. Psychiatry Clin Neurosci. 2015;69(7):423-430.
60. Hettema JM, Prescott CA, Kendler KS. A population-based twin study of generalized anxiety disorder in men and women. J Nerv Ment Dis. 2001;189(7):413-420.
61. Mather L, Blom V, Bergstrom G, Svedberg P. An underlying common factor, influenced by genetics and unique environment, explains the covariation between major depressive disorder, generalized anxiety disorder, and burnout: a Swedish twin study. Twin Res Hum Genet. 2016;19(6):619-627.
62. Tadic A, Rujescu D, Szegedi A, et al. Association of a MAOA gene variant with generalized anxiety disorder, but not with panic disorder or major depression. Am J Med Genet B Neuropsychiatr Genet. 2003;117B(5):1-6.
63. You JS, Hu SY, Chen B, Zhang HG. Serotonin transporter and tryptophan hydroxylase gene polymorphisms in Chinese patients with generalized anxiety disorder. Psychiatr Genet. 2005;15(1):7-11.
64. Molina E, Cervilla J, Rivera M, et al. Polymorphic variation at the serotonin 1-A receptor gene is associated with comorbid depression and generalized anxiety. Psychiatr Genet. 2011;21(4):195-201.
65. Moreira FP, Fabião JD, Bittencourt G, et al. The Met allele of BDNF Val66Met polymorphism is associated with increased BDNF levels in generalized anxiety disorder. Psychiatr Genet. 2015;25(2):201-207.
66. Wang Y, Zhang H, Li Y, et al. BDNF Val66Met polymorphism and plasma levels in Chinese Han population with obsessive-compulsive disorder and generalized anxiety disorder. J Affect Disord. 2015;186:7-12.
67. Amstadter AB, Koenen KC, Ruggiero KJ, et al. NPY moderates the relation between hurricane exposure and generalized anxiety disorder in an epidemiologic sample of hurricane-exposed adults. Depress Anxiety. 2010;27(3):270-275.
68. Koenen KC, Amstadter AB, Ruggiero KJ, et al. RS52 and generalized anxiety disorder in an epidemiologic sample of hurricane-exposed adults. Depress Anxiety. 2009;26(4):309-315.
69. Gragnoni C. Proteasome modulator 9 gene SNPs, responsible for antidepressant response, are in linkage with generalized anxiety disorder. J Cell Physiol. 2014;229(11):1579-1587.
70. Chen SD, Sun XY, Niu W, et al. Correlation between the level of microRNA expression in peripheral blood mononuclear cells and symptomatology in patients with generalized anxiety disorder. Compr Psychiatry. 2016;69:216-224.
71. Wilfing AP, Gibson G. Blood gene expression profiles suggest altered immune function associated with symptoms of generalized anxiety disorder. Brain Behav Immun. 2015;43:184-191.
72. Cooper AJ, Narasimhan S, Rickels K, Lohoff FW. Genetic polymorphisms in the PACAP and PAC1 receptor genes and treatment response to venlafaxine XR in generalized anxiety disorder. Psychiatry Res. 2013;210(3):1299-1300.
73. Cooper AJ, Rickels K, Lohoff FW. Association analysis between the A118G polymorphism in the OPRM1 gene and treatment response to venlafaxine XR in generalized anxiety disorder. Hum Psychopharmacol. 2013;28(3):258-262.
74. Lohoff FW, Narasimhan S, Rickels K. Interaction between polymorphisms in serotonin transporter (SLC6A4) and serotonin receptor 2A (HTR2A) genes predict treatment response to venlafaxine XR in generalized anxiety disorder. Pharmacogenomics J. 2013;13(5):464-469.
75. Narasimhan S, Aquino TD, Multani PK, et al. Variation in the catechol-O-methyltransferase (COMT) gene and treatment response to venlafaxine XR in generalized anxiety disorder. Psychiatry Res. 2012;198(1):112-115.
76. Lohoff FW, Aquino TD, Narasimhan S, et al. Serotonin receptor 2A (HTR2A) gene polymorphism predicts treatment response to venlafaxine XR in generalized anxiety disorder. Pharmacogenomics J. 2013;13(1):21-26.
77. Narasimhan S, Aquino TD, Hodge R, et al. Association analysis between the Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene and treatment response to venlafaxine XR in generalized anxiety disorder. Neurosci Lett. 2011;503(3):200-202.
78. Perlis RH, Fjall B, Dharia S, Houston JP. Pharmacogenetic investigation of response to duloxetine treatment in generalized anxiety disorder. Pharmacogenomics J. 2013;13(3):280-285.
79. Saung WT, Narasimhan S, Lohoff FW. Lack of influence of DAT1 and DRD2 gene variants on antidepressant response in generalized anxiety disorder. Hum Psychopharmacol. 2014;29(4):316-321.
80. Bandelow B, Baldwin D, Abelli M, et al. Biological markers for anxiety disorders, OCD and PTSD: A consensus statement. Part II: Neurochemistry, neurophysiology and neurocognition. World J Biol Psychi atr. 2016;15:1-53.
81. Iny LI, Pecknold J, Suranyi-Cadotte BE, et al. Studies of a neurochemical link between depression, anxiety, and stress from [3H]imipramine and [3H]mirtazapine binding on human platelets. Biol Psychiatry. 1994;36(5):281-291.
82. Hernández E, Lastra S, Urbina M, et al. Serotonin, 5-hydroxyindole-acetic acid and serotonin transporter in blood peripheral lymphocytes of patients with generalized anxiety disorder. Int Immunopharmacol. 2002;2(7):893-900.
83. Gerra G, Zaimovic A, Zambelli U, et al. Neuroendocrine responses to psychological stress in adolescents with anxiety disorder. Neuropsychobiology. 2000;42(2):82-92.
84. Vreeburg SA, Zitman FG, van Pelt J, et al. Salivary cortisol levels in persons with and without different anxiety disorders. *Psychosom Med*. 2010;72(4):340-347.
85. Phillips AC, Batty GD, Gale CR, et al. Major depressive disorder, generalized anxiety disorder, and their comorbidity: associations with cortisol in the Vietnam Experience Study. *Psychoneuroendocrinology*. 2011;36(5):682-690.
86. Seddon K, Morris K, Bailey J, et al. Effects of 7.5% CO₂ challenge in generalized anxiety disorder. *J Psychopharmacol*. 2011;25(1):43-51.
87. Alfano CA, Reynolds K, Scott N, et al. Polysomnographic sleep patterns of non-depressed, non-medicated children with generalized anxiety disorder. *J Affect Disord*. 2013;147(1-3):379-384.
88. Mantella RC, Butters MA, Amico JA, et al. Salivary cortisol is associated with diagnosis and severity of late-life generalized anxiety disorder. *Psychoneuroendocrinology*. 2008;33(6):773-781.
89. Hek K, Direk N, Newson RS, et al. Anxiety disorders and salivary cortisol levels in older adults: a population-based study. *Psychoneuroendocrinology*. 2013;38(2):300-305.
90. Hood SD, Melicher JK, Taylor LG, et al. Noradrenergic function in generalized anxiety disorder: impact of treatment with venlafaxine on the physiological and psychological responses to clonidine challenge. *J Psychopharmacol*. 2011;25(1):78-86.
91. Tafet GE, Feder DJ, Abulafia DP, Roffman SS. Regulation of hypothalamic-pituitary-adrenal activity in response to cognitive therapy in patients with generalized anxiety disorder. *Cogn Affect Behav Neurosci*. 2005;5(1):37-40.
92. Lenze EJ, Mantella RC, Shi P, et al. Elevated cortisol in older adults with generalized anxiety disorder is reduced by treatment: a placebo-controlled evaluation of escitalopram. *Am J Geriatr Psychiatry*. 2011;19(5):482-490.
93. Cohn JB, Wilcox CS, Meltzer HY. Neuroendocrine effects of buspirone in patients with generalized anxiety disorder. *Am J Med*. 1986;80(3B):36-40.
94. Klein E, Zinder O, Colin V, et al. Clinical similarity and biological diversity in the response to alprazolam in patients with panic disorder and generalized anxiety disorder. *Acta Psychiatr Scand*. 1995;92(6):399-408.
95. Molendijk ML, Bus BA, Spinhoven P, et al. Gender specific associations of serum levels of brain-derived neurotrophic factor in anxiety. *World J Biol Psychiatry*. 2012;13(7):535-543.
96. Pallanti S, Tofani T, Zanardelli M, et al. BDNF and Artemin are increased in drug-naïve non-depressed GAD patients: preliminary data. *Int J Psychiatry Clin Pract*. 2014;18(4):255-260.
97. Ball S, Marangell LB, Lipsius S, Russell JM. Brain-derived neurotrophic factor in generalized anxiety disorder: results from a duloxetine clinical trial. *Prog Neuropsychopharmacol Biol Psychiatry*. 2013;43:217-221.
98. Jockers-Scherübl MC, Zubraegel D, Baer T, et al. Nerve growth factor serum concentrations rise after successful cognitive-behavioural therapy of generalized anxiety disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007;31(1):200-204.
99. Bankier B, Barajas J, Martinez-Rumayor A, Januzzi JL. Association between C-reactive protein and generalized anxiety disorder in stable coronary heart disease patients. *Eur Heart J*. 2008;29(18):2212-2217.
100. Copeland WE, Shanahan L, Worthman C, et al. Generalized anxiety and C-reactive protein levels: a prospective, longitudinal analysis. *Psychol Med*. 2012;42(12):2641-2650.
101. Tofani T, Manelli LD, Zanardelli M, et al. An immunologic profile study in drug-naïve generalized anxiety non depressed patients: a pilot study. *Eur Neuropsychopharmacol*. 2015;25(suppl 2):S226.
102. Koh KB, Lee BK. Reduced lymphocyte proliferation and interleukin-2 production in anxiety disorders. *Psychosom Med*. 1998;60(4):479-483.
Marcadores biológicos del trastorno de ansiedad generalizada

El trastorno de ansiedad generalizada (TAG) es una condición de salud mental prevalente y muy discapacitante; sin embargo, aún hay mucho que aprender en relación con biomarcadores específicos, como también respecto del diagnóstico, lo que se dificulta más por la sobreposición marcada y frecuente del TAG con los trastornos afectivos y de ansiedad. Recientemente, el TAG ha sido objeto de grandes esfuerzos de investigación, mediante la aplicación de neuroimágenes, estudios genéticos y exámenes sanguíneos enfocados en el descubrimiento de biomarcadores relacionados con la patogenética y el tratamiento. Este artículo revisa la gran cantidad de información disponible y se enfoca especialmente en la evidencia que proviene de los resultados de las neuroimágenes, la genética y las mediciones neuroquímicas en el TAG, con el objetivo de tener una mejor comprensión de los potenciales biomarcadores involucrados en su etiología y terapéutica. En general, la mayoría de estos estudios ha entregado resultados que constituyen hallazgos aislados, algunas veces inconsistentes y no claramente replicables. Por estas razones, estos resultados aún no se han traducido en la práctica clínica. Por consiguiente, se necesitan más esfuerzos de investigación para distinguir el TAG de otros trastornos mentales y contar con nuevos hallazgos biológicos para su patogenia y tratamiento.

Les marqueurs biologiques de l’anxiété généralisée

L’anxiété généralisée (AG) est un trouble de santé mentale prévalent et très invalidant. Il reste cependant beaucoup à apprendre sur des biomarqueurs pertinents ainsi que sur le diagnostic, rendu plus difficile par le chevauchement important et courant de l’AG avec les troubles affectifs et anxieux. Récemment, l’AG a fait l’objet d’efforts intenses de recherche, appliquant la neuro-imagerie, la génétique et les analyses sanguines à la découverte de biomarqueurs pathogènes et liés au traitement. Dans cet article, nous analysons l’important volume de données disponibles et nous nous concentrons en particulier sur des données de neuro-imagerie, de génétique et des mesures neurochimiques dans l’AG, afin de mieux comprendre les biomarqueurs potentiels impliqués dans son étiologie et son traitement. Globalement, la majorité de ces études sort des résultats isolés, parfois contradictoires et non clairement reproduits. C’est pourquoi ils n’ont toujours pas été transposés en pratique clinique. Il faut donc d’autres efforts de recherche pour différentier l’AG des autres troubles mentaux et permettre de nouvelles découvertes biologiques dans sa pathogenèse et son traitement.