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A review of systems biology research of anxiety disorders

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The development of "omic" technologies and deep phenotyping may facilitate a systems biology approach to understanding anxiety disorders. Systems biology approaches incorporate data from multiple modalities (e.g., genomic, neuroimaging) with functional analyses (e.g., animal and tissue culture models) and mathematical modeling (e.g., machine learning) to investigate pathological biophysical networks at various scales. Here we review: i) the neurobiology of anxiety disorders; ii) how systems biology approaches have advanced this work; and iii) the clinical implications and future directions of this research. Systems biology approaches have provided an improved functional understanding of candidate biomarkers and have suggested future potential for refining the diagnosis, prognosis, and treatment of anxiety disorders. The systems biology approach for anxiety disorders is, however, in its infancy and in some instances is characterized by insufficient power and replication. The studies reviewed here represent important steps to further untangling the pathophysiology of anxiety disorders.

Keywords: Anxiety disorders; systems biology; biomarkers; machine learning

Introduction

Anxiety disorders, which include generalized anxiety disorder, panic disorder, social anxiety disorder, agoraphobia and specific phobia, are the most prevalent category of psychiatric disorders.[1,2] Obsessive compulsive disorder and post-traumatic stress disorder are no longer classified as anxiety disorders[3] and will, therefore, not be discussed in this review. Anxiety disorders have a lifetime prevalence of approximately 34% and incur a substantial social burden.[4] Anxiety disorders are currently the sixth leading cause of disability worldwide, with a rate of 389.7 "disability adjusted life years" per 100,000 people.[5] Anxiety disorders are characterized by excessive fear and anticipation of threats that disrupt daily function.[3] These disorders are complex, involving environmental and polygenic contributions to their underlying pathophysiology that have independent and joint effects.[6] The clinical picture of anxiety disorders is further complicated by phenotypic heterogeneity, high rates of comorbidity, and symptom overlap with other psychiatric disorders, e.g., obsessive-compulsive disorders and addiction disorders.[1]

Our current knowledge about the pathophysiology of anxiety disorders remains incomplete and reliable biomarkers are lacking in a clinical setting.[7,8] Research on anxiety disorders has often focused on single candidate genes or specific environmental stressors. In more recent years, the scientific community has begun investigating anxiety disorders using a systems biology approach. Systems biology is a shift from traditional reductionist biology towards understanding more complex biophysical networks at various scales (from a single-cell to an organismal level)[9] for a particular outcome of interest. This more holistic approach has been given impetus by the "omics" (including genomics, proteomics, transcriptomics, metabolomics, etc.) and the era of computational biostatistics (e.g., machine learning, algorithms that automatically improve through experience).[9,10] This approach may ultimately allow fine mapping of the multiple mechanisms that contribute to these conditions.[11]

In this review, i) we provide a brief overview of current knowledge of the neurobiology of anxiety disorders, drawing on existing detailed reviews,[12-14] ii) we investigate how systems biology approaches have advanced this work, and iii) we speculate on the future clinical translation of these findings. We have selected key examples that demonstrate the capabilities of this avenue of research and provide suggestions for the way ahead.

The neurobiology of anxiety disorders

Genetics of anxiety disorders

Anxiety disorders run in families; the odds of developing this disorder are up to six-fold higher for first degree relatives of affected individuals.[15] Twin studies indicate heritability estimates between 32-67% across subtypes of anxiety disorders.[15] The genetic architecture is polygenic, with influences likely from both common and rare variations.[16] The environment is also known to play a substantial role in the etiology of these disorders through epigenetic changes and gene-environment interactions.[6] In the sections below we provide an overview of the current genetic understanding of anxiety disorders.

Candidate genes

Investigations into the genetic etiology of anxiety disorders began with linkage studies and candidate gene approaches.[17] Candidate genes were selected based on the purported biology underlying the phenotype of interest (for example, neuropeptides, monoaminergic neurotransmitter systems, and the hypothalamic-pituitary-adrenal axis) and included evidence from animal models.[18] Candidate-gene association studies for anxiety disorders have predominantly focused on polymorphisms in the genes, *SLC6A4*, *COMT*, *MAOA*, *ADORA2A*, *NPSR1*, *CRHR1*, and *RGS213*.[18] The products of these genes predominantly affect synaptic signaling by modulating neurotransmitters. Findings from candidate-gene association studies have been highly inconsistent, likely due to small effect sizes and the heterogeneous nature of anxiety phenotypes. Therefore, there is currently a focus on global hypothesis-free approaches to investigate the genetic etiology of anxiety disorders. Large-scale collaborative efforts, such as the Psychiatric Genomics Consortium (https://www.med.unc.edu/pgc/), UK Biobank (https://www.ukbiobank.ac.uk), and iPSYCH (http://ipsych.au.dk/about-ipsych) allow sufficient sample size and statistical power for such unbiased analyses. Below, we briefly review some of the omics studies that have been conducted for anxiety disorders, including genome-wide association studies (GWAS), epigenome-wide association studies (EWAS), and transcriptome-wide association studies (TWAS).

GWAS

Findings from GWASs of anxiety have not been replicated in independent cohorts or meta-analyses. Thus far, candidates that have been partially replicated include *TMEM 132D* (associated with panic disorder),[19] *GLRB* (associated with agoraphobia),[20] and *RBFOX1* (associated with generalized anxiety disorder).[21] In addition, a non-coding RNA locus on chromosomal band 3q12.3, associated with the gene *CAMKMT*, obtained genome-wide significance in a meta-analysis across various subtypes of anxiety disorders.[22] These findings highlight the potential importance of intergenic variants, which account for the majority of the associations thus far.[23] The associated genes which have been characterized suggest that altered signal transduction pathways play a key role in anxiety pathophysiology.[19,21] These GWAS findings, however, have not been unequivocally replicated and currently only account for 0.2% of the variance attributable to common variation.[24]

EWAS

Epigenetics offers an opportunity to link genetic and environmental risk factors for anxiety disorders and improve our understanding of the underlying mechanisms.[12] EWAS studies investigating methylation patterns have implicated mostly global hypomethylation associated with panic disorder,[25] hypermethylation of *HECA* in females with panic disorder,[26] and hypermethylation of *ASB1* associated with generalized anxiety disorder symptoms.[27] Unfortunately, EWAS studies are currently underpowered, even more so than GWAS work.[28]

TWAS

Analysis of transcription patterns have the potential to identify genes and pathways that either are affected by, or increase the risk of a pathology, lending insight into its pathophysiology. At this stage, TWAS among individuals with anxiety disorders are scarce, have small sample sizes, and mostly represent pilot studies. Most gene expression studies have opted for a candidate gene approach in animal models, using expression patterns to explore the functionality of findings from GWASs and EWASs (e.g., Emeny et al.[27]). TWAS tend to be more common in animal studies, with replication attempts of the identified candidates conducted in humans. For example, a TWAS of stress-exposed mice using tissue from the amygdala and prefrontal medial cortex revealed altered expression of *Ppm1f*, a protein phosphatase belonging to a family of phosphatases that negatively regulate stress response pathways.[29] Downregulated expression of *Ppm1f* was also subsequently observed in 151 human cases with anxiety symptoms compared to 165 control subjects. Few studies have investigated global expression patterns associated with anxiety. One such study investigated 336 participants (157 cases and 179 controls) and revealed differential expression of 631 genes among male participants only.[30] These genes were enriched for immune-related pathways. However, a smaller study of 102 participants with panic disorder and specific phobia was unable to identify significant differences in global expression patterns based on treatment outcomes of cognitive behavioral therapy.[31] It is evident that large-scale collaborative efforts are needed to improve the power of these analyses to identify genes and pathways with differential transcription in individuals with pathological anxiety.

Neuroimaging

Neural networks that relate to fear processing, termed the fear network, have been shown to be associated with anxiety and anxiety disorders.[14] These regions include the bed nucleus of the stria terminalis, the amygdala, and

the hippocampus, and their connections to cortical regions, such as the dorsal medial and lateral prefrontal/cingulate cortex and insula. These regions appear to be involved across the range of anxiety disorders.[14]

Slight differences can, however, be observed across anxiety disorder subtypes. A recent meta-analysis of structural and functional magnetic resonance imaging (fMRI) of generalized anxiety disorder revealed that the hippocampus, anterior cingulate cortex, and amygdala have reduced volume, and the dorsolateral prefrontal cortex and anterior cingulate cortex have reduced functional connectivity with the amygdala.[32] The sensorimotor network is also altered with greater pre- and postcentral volume, reduced supplementary motor area volume, and reduced functional connectivity in anterior and increased functional connectivity in the posterior cerebellum.[32] The neural differences in subjects with generalized anxiety disorder, compared to controls, appear to be widely distributed. Panic disorder has been associated with reduced bilateral dorsomedial prefrontal cortex, left dorsolateral prefrontal cortex, right insula, right superior temporal gyrus right middle temporal gyrus and right superior orbital frontal cortex volumes in a meta-analysis.[33] This emphasizes the role of frontal areas and an altered top-down control system in panic disorder. A structural MRI meta-analysis of social anxiety disorder indicated greater precuneus, right middle occipital gyrus, and supplementary motor area volumes, as well as lower volume in the left putamen, compared to controls.[34] This suggests that social anxiety is associated with various networks across the brain, extending beyond the fear network. A meta-analysis of fMRI revealed that subjects with specific phobia had increased activation in response to phobic stimuli in the left amygdala/globus pallidus, left insula, right thalamus, and cerebellum than controls.[35] Specific phobia is, therefore, mostly associated with alterations in the fear network.

Experimental models

Animal models are a means of studying the biological components that underlie behavior. Animal models have aided in the identification of candidate genes and molecular pathways pertinent to anxiety disorder pathophysiology.[12] For example, such models have implicated dysfunctional immune pathways in the pathophysiology of anxiety.[36,37] Animal models have also supported the role of early adversity as a risk factor for anxiety disorders, demonstrating that this affects the hypothalamic-pituitary-adrenal axis and leads to impaired brain maturation and function.[38] These effects may be associated with epigenetic modifications, which can be inherited across multiple generations.

Systems biology approaches to anxiety disorders

Systems biology research emphasizes a holistic and interdisciplinary approach to understanding biological systems related to pathophysiology.[9] In this section, we focus on key studies that not only used an omics approach to identify anxiety-related signatures, but also attempted to improve our understanding of these associations and their context in anxiety through functional analyses. The discussed studies are summarized in Table 1.

The first study we review is a GWAS of panic disorder with agoraphobia, which identified several significantly associated variants (rs78726293, rs191260602, rs17035816, and rs7688285) within or near *GLRB*, a gene encoding a transmembrane receptor.[20] These findings were further validated in two independent samples and their effects were characterized using cell cultures, post-mortem brain tissue, fMRI, and animal models.[20] Although none of the identified variants were predicted to be an expression quantitative trait locus in the GTEx database,[42] cell culture and postmortem brain tissue showed that rs7688285 was associated with increased *GLRB* expression, particularly in the midbrain. fMRI conducted on carriers of the associated variants revealed an increase in fear, sensory, and motor network activation. It was also found that these carriers had an increased startle response compared to non-carriers. Variants within *GLRB* have previously been associated with hyperekplexia, a neurological condition characterized by an exaggerated startle response and agoraphobic behavior.[43] Lastly, partial knockout of *GLRB* in mice resulted in agoraphobic behavior, demonstrated by less time spent in the center of an open field. Taken together, these findings suggest that these non-coding polymorphisms in *GLRB* increase the risk of panic disorder by, in part, altering the gene's expression and resulting in an increased startle response and agoraphobic behavior.[20]

Another GWAS of panic disorder revealed associations with variants within *TMEM132D* (rs7309727 and rs11060369), which encodes a membrane protein involved in the negative regulation of phosphatase activity.[39] This was replicated in three independent samples[39] and again in a subsequent meta-analysis that included five datasets by the same group.[19] The *TMEM132D* variants were also associated with increased severity of panic disorder.[39] mRNA expression from lymphoblastoid cell lines in the HapMap population[44] and the human postmortem cortex[45] revealed a significant correlation between anxiety and *TMEM132D* expression in the frontal cortex. Using a mouse model, associations were found between these variants, anxiety behavior, and expression of *TMEM132D* in the anterior cingulate cortex, a region involved in processing fear-related stimuli.[39]

A molecular pathway analysis, correlating phenome and transcriptomic data, is another useful example of a systems biology approach.[40] Global disruption of pathways linked to anxiety disorders were investigated by correlating broad phenotype data with publicly available transcript data from human and animal model databases across tissue types.[46] The phenotype criteria included phenotypic states of anxiety and were not restricted to an anxiety disorder diagnosis, allowing for the incorporation of larger datasets. Further, the inclusion of transcriptome data from model organisms allowed for the identification of significantly enriched pathways across experiment types. Anxiety phenotypes were significantly associated with upregulated carbohydrate metabolism, including glycolysis and the tricarboxylic acid cycle; dysregulated tight

Table 1 Summary of studies utilizing a systems biology approach to study anxiety disorders

Reference	Anxiety disorder subtype	Input	Methods	Output	Sample size
Deckert[20]	AG	1. SNPs 2. mRNA 3. Neuroimaging 4. Mice	1. GWAS 2. Cell culture and expression analysis 3. fMRI comparisons 4. Knockout study	1. Associations with GLRB SNPs (rs78726293 and rs191260602) 2. rs7688285 in GLRB affects expression in brain and cell culture 3. Increased startle reflex and fear network activation in carriers 4. GLRB knockout mice exhibit AG phenotypes	Dataset 1: 1,370 Dataset 2: 2,547 Dataset 3: 3,845 Dataset 4: 1,012
Erhardt[39]	PD	1. SNPs 2. mRNA 3. Mice	1. GWAS 2. Expression analysis in post-mortem brains 3. Animal models	1. TMEM132D SNPs (rs7309727 and rs11060369) associated with PD 2. SNPs correlate with anxiety severity 3. SNPs correlate with higher mRNA expression levels in the frontal cortex 4. TMEM132D expression in anterior cingulate cortex in mice correlated with anxiety	1,824 (909 PD, 915 HC)
Gormanns[40]	All AD-associated phenotypes	1. Transcriptomes 2. Pathway information	Phenome-transcriptome correlation analysis	1. Upregulated carbohydrate metabolism 2. Altered BBB transporter proteins	N/A
Shimada-Sugimoto[25]	PD	Genome-wide methylation	1. EWAS 2. Pathway analysis 3. Mathematical leukocyte abundance prediction	1. 40 sites are differentially methylated in PD 2. Sites enriched for positive regulation of lymphocyte activation 3. PD associated with CD4+ T-cell abundance	96 (48 PD, 48 HC)
Emeny[27]	Dimensional anxiety, PD, and AG	1. Genome-wide methylation 2. hs-CRP and IL-18 levels 3. Mice	1. EWAS 2. Correlating hs-CRP and IL-18 levels with GAD 3. Animal models	1. 48.5% increased methylation at ASB1 in severe anxiety, PD, and AG scores 2. An interaction between IL-18 and severe anxiety with methylation at ASB1 in women 3. ASB1 expression was significantly upregulated with increased stress exposure in mice	Dataset 1:1,522 Dataset 2: 131 GAD, 169 HC
Liberman[41]	Dimensional AD	Candidate genes related to diurnal preference	1. Linear regression 2. Mathematical modelling	1. rs228697 located in PER3 is associated with anxiety 2. Model satisfies available experimental knockdown conditions and existing data, and can predict circadian phenotypes associated with anxiety	546

AD = anxiety disorders; AG = agoraphobia; BBB = blood-brain barrier; EWAS = epigenome-wide association study; fMRI = functional magnetic resonance imaging; GAD = generalized anxiety disorder; GLRB = glycine receptor subunit beta gene; GWAS = genome-wide association study; HC = healthy controls; hs-CRP = high-sensitivity c-reactive protein; IL = interleukin; mRNA = messenger ribonucleic acid; N/A = not available; PD = panic disorder; SNP = single nucleotide polymorphism.

junctions and phosphatidylinositol signalling.[40] Phospho-fructokinase, the rate-limiting enzyme of glycolysis that produces lactate, was upregulated; this is notable given that panic disorder has been linked to elevated brain lactate levels, perhaps due to increased phosphofructo-kinase activity, and that panic attacks are induced by lactate infusions.[47] Speculatively, dysregulation of energy metabolism-related pathways contributes to lactate level imbalance, mediating anxiety-like phenotypes.[40] Further, variants affecting tight junctions, vital blood-brain-barrier (BBB) transporter proteins, and various other entities affecting the BBB have previously been found to influence antidepressant drug uptake and response.[48] Alterations in the BBB transport system may lead to dysregulation of metabolites and influence anxiety-like behaviors. Inositol has also previously been shown to have anxiolytic effects in animal models[49] and clinical trials in humans have been initiated.[50]

An EWAS for panic disorder identified significant differential DNA methylation at 40 CpG sites.[25] These sites were predominantly hypomethylated among panic disorder patients compared to controls. Pathway analysis revealed an enrichment of genes involved in the lymphocyte activation pathway. A comparison of the relative proportion of leukocyte subsets between panic disorder patients and controls revealed significantly increased CD4+ T cells in panic disorder patients. This suggests that the risk of panic disorder may be influenced by immune dysfunction.[25]

An EWAS of dimensional anxiety also suggested the involvement of the immune system.[27] Significant hyper-methylation of the *Asb1* promoter was associated with severe anxiety and was significantly correlated with panic severity in an independent cohort. *Asb1* appears to be a stress-responsive gene, since exposure to extreme stress is significantly associated with hypermethylation in adult mice compared to controls. Members of the *Asb* protein family have previously been shown to interact with proinflammatory cytokines,[51] and *Asb1* gene expression correlated with upregulation of the neuroimmunomodulat-ing cytokine interleukin-1 beta (IL-1β) in a mouse model.[27] This suggests that *Asb1* may be influenced by environ-mental risk factors, such as stress, leading to anxiety via neuroimmune pathways.[27]

Alterations in sleep patterns have also been linked to anxiety disorders.[52] A study of polymorphisms in *PER3*, a gene previously associated with sleep and mood dis-orders, revealed a significant association with anxiety.[41] Further, an ordinary differential equation model with other clock genes was developed that can predict circadian phenotypes in individuals with mood and sleep-related disorders. The model was trained on genetic knockout conditions previously identified in mice and various cell lines. Although this study utilizes a limiting candidate gene approach, it is an example of how mathematical modeling combined with biological associations can be used to make predictions and inform our understanding of the mechanistic underpinnings of disease. This model has the potential to guide future studies of mood disorders and their relationship with circadian rhythms.[41]

Machine learning may translate systems biology findings of anxiety disorders to clinical practice

As potential contributors to anxiety disorder pathophysiol-ogy are discovered and validated, methods to translate these findings into clinical practice are needed. Signa-tures of anxiety could improve individual predictions of diagnosis, prognosis, treatments, and treatment out-comes as we move towards a precision medicine app-roach.[53] Machine learning approaches, a discipline of computer science that utilizes mathematical and statis-tical assumptions to identify patterns from data, may be the key components to achieving this goal.[54] Given that large-scale data is becoming more widely available in the field of biology, machine learning may contribute to the systems biology approach by generating models of disease and new hypotheses.[55] Here we will present examples of studies that aim to identify robust signatures of anxiety and tools for more precise medicine models. The studies discussed here are summarized in Table 2.

Machine learning approaches to predicting anxiety dis-order diagnosis may be able to utilize a range of measures, including physiological and psychological variables.[54] Visually inferred heart-rate measurements[70] paired with the Virtual Human Distress Assessment Interview Corpus for anxiety analysis, which aims to quantify nonverbal behavior descriptors indicative of anxiety,[71] were used to predict generalized anxiety disorder using several statis-tical models.[56] From this, a Bayesian network approach was the most significant method, able to distinguish between cases and controls with an efficiency of 73%.[56] A longitudinal study of self-esteem in adolescents and young adults was used to predict adult onset of anxiety disorders.[72] An artificial neural network approach com-bined several attributes (select DSM-5 questions, age, gender, occupation, and working hours) to predict general-ized anxiety disorder diagnosis.[58] This approach had an accuracy of 96% when including sensitivity analysis, with select questions from the DSM-5 carrying the most weight in the model.[58]

Machine learning approaches have also been applied to brain imaging data. Grey matter volumes and linear support vector machine (SVM) methods have been able to distinguish between individuals who have major depression and those who have depression with comor-bid generalized anxiety disorder with an accuracy of 82%.[59] Resting-state fMRI was used in conjunction with multivariate pattern analysis to distinguish social anxiety disorder patients from controls with an accuracy of 83%.[60] This approach revealed that altered intra- and inter-network connectivity among the default mode network, visual network, sensory-motor network, affective network, and cerebellar regions were largely responsible for the classification accuracy. This finding was subsequently supported in several studies.[61,62,73] One such study found that functional analysis of the fear network alone was more accurate (72%) and that grey matter volume alter-ations across the whole brain (85%) are even more accurate.[61] This approach was also able to distinguish social anxiety disorder and panic disorder patients with an

Table 2 Summary of studies investigating signatures of anxiety disorders

Reference	Anxiety disorder subtype	Input	Methods	Output	Sample size
Chatterjee[56]	GAD	Visually inferred heart-rate measurements	1. Logistic regression 2. Naïve Bayes 3. Bayesian network	The Bayesian network was the most significant method (73% accuracy)	48 (33 GAD and 15 HC)
McGinnis[57]	Various subtypes	Motion during a fear induction task	K-nearest neighbor binary classification models	75% accuracy	63
Sribala[58]	GAD	1. DSM-5 questionnaire 2. Sociodemographic attributes	Artificial neural networks	1. Without sensitivity analysis 90% 2. With sensitivity analysis 96%	66
Chi[59]	GAD	fMRI	SVM	82%	38 (18 depressive - anxiety, 20 depression + AD)
Liu[60]	SAD	fMRI	SVM	83% accuracy	40 (20 SAD, 20 HC)
Frick[61]	SAD	fMRI and sMRI	SVM	1. Whole-brain fMRI (68%) 2. Fear-networks alone fMRI (72%) 3. Whole-brain grey matter volume (85%)	26 (14 AD, 12 HC)
Pantazatos[62]	SAD and PD	fMRI	SVM	1. SAD vs HC 88% 2. SAD vs PD 82%	51 (16 SAD, 19 HC, 16 PD)
Lueken[63]	PD	fMRI	Random undersampling tree ensemble	73% accuracy	59 (26 PD and 33 HC)
Sundermann[64]	PD/AG	1. fMRI 2. Cognitive behavioral therapy	SVM	Not able to reliably predict individual response to cognitive behavioral therapy	59 (30 responders, 29 non-responders)
Boeke[65]	Trait anxiety	fMRI	Various algorithms	Not able to reliably predict individual anxiety	Discovery: 531,307 Test: 348, 209
Hilbert[66]	GAD	1. Clinical questionnaires 2. Cortisol release 3. Grey and white matter volumes	Binary SVM	1. Combined measures improved classifications 2. Case-classification accuracy of 90% 3. Disorder-classification accuracy of 67%.	57 (19 GAD, 14 MD-GAD, 24 HC)
Whitfield-Gabrieli[67]	SAD	1. fMRI 2. DTI	Logistic regression	Combined measures achieved 84% accuracy	38
So[68]	Anxiety and depression	1. GWAS data of anxiety and depression 2. Gene sets from drugs in DSigDB	1. Gene set analysis 2. Repositioning analyses	Antipsychotic medications in clinical trials and heart medications may be useful for treating AD	N/A
Zhao & So[69]	Various combined subtypes	Transcriptome	1. Deep neural network 2. SVM	Support for psychiatric medications considered in clinical trials	N/A

AD = anxiety disorders; DSigDB = Drug SIgnatures DataBase; DTI = diffusion tensor imaging; fMRI = functional magnetic resonance imaging; GAD = generalized anxiety disorder; GWAS = genome-wide association study; HC = healthy controls; MD = major depression; N/A = not available; PD = panic disorder; SAD = social anxiety disorder; sMRI = structural magnetic resonance imaging; SVM = support vector machine.

accuracy of 82%.[62] fMRI and a random under-sampling tree ensemble in a leave-one-out cross-validation framework were also able to predict comorbidity between depression and panic disorder with agoraphobia with an accuracy of 73%.[63] Several such approaches have been unable to make successful predictions using the same neuroimaging signatures.[64,65]

Studies have also combined multiple biological measures for the validation of anxiety disorder diagnoses. Binary SVMs were used in a nested leave-one-out cross-validation framework to estimate the capacity for the joint and individual effects of various modalities (clinical questionnaires, cortisol release, grey and white matter volumes) to distinguish generalized anxiety disorder from healthy controls and subjects with major depression.[66] The clinical questionnaires were able to distinguish cases from controls most efficiently, although cortisol and neuroimaging were better suited to refining the diagnosis between generalized anxiety disorder and major depression. Combining all measures allowed for an overall improved classification (case classification accuracy of 90% and disorder classification accuracy of 67%).[66] Replication attempts and larger sample sizes are still needed to validate this promising result.

The predictive capacity of neurobiological markers to determine treatment outcomes has also been explored.[74] Such approaches have employed neuroimaging, genetic, and clinical predictors.[74] For example, resting-state fMRI and diffusion tensor imaging have been used to predict treatment response to cognitive behavioral therapy in social anxiety disorder patients with an accuracy of 84%.[67] This approach resulted in a five-fold improvement in the ability to predict treatment response compared to measures of clinical severity and single connectomic measures.[67] However, most studies of this nature remain fragmentary.[74]

Recent efforts utilizing biological variables associated with anxiety disorders and computational modeling have also begun to suggest novel targets for drug development and potential repositioning of known medications to treat these disorders. One such example compared GWAS data for anxiety and depression with gene sets from all drugs in the Drug SIGnatures DataBase.[68] This approach added support for anxiolytics already used in clinical practice and also suggested potential applications of antipsychotic medications and cardiovascular agents, e.g. fendiline, which has some evidence of antidepressant activity in animal models.[75] Another such study investigating various machine learning approaches and gene expression data provided additional evidence for the use of certain antipsychotics, antihistamines, anti-inflammatories, and histone deacetylase inhibitors to treat anxiety disorders and depression.[69] Many of the findings from this study are in agreement with evidence from animal models and current clinical trials, providing support for this approach.[69] These studies currently form the first steps in a systems biology approach that could ultimately lead to new treatments. However, they require further validation and functional evidence.

Discussion

Early work on the pathophysiology of anxiety disorders focused on specific mechanisms and particular candidate genes; this seems like an overly simplistic approach given the complex nature of these conditions. Convergent models that incorporate a range of omics-derived associations across multiple datasets (including animals and humans) and at various stages of life, offer an alternative approach.[76] Such work can potentially combine phenotype, genotype, and environome data. Systems biological approaches borrow principles from, and may contribute to the Research Domain Criteria approach, which aims to classify mental illness according to its relevant neurobiology and which focuses on continuous biological dimensions.[77] Currently, there are gaps in our knowledge and methodologies that should be refined.

Animal models have been useful in anxiety disorder research, including work using a systems biology approach (e.g., Erhardt et al.[39]). However, experimental models may have important limitations. First, many current models measure "normal"/adaptive and non-specific anxiety (e.g., exposure to predators), which may be fundamentally different from pathological/maladaptive anxiety in humans.[78] Paradigms that better model specific aspects of human anxiety disorders, such as impaired fear extinction, are therefore needed.[79] Furthermore, coordinated efforts utilizing multiple models and species could identify common pathways that mediate risk and resilience.[80]

Omics research in anxiety disorders lags behind work on other areas of psychiatry, such as schizophrenia. First, although the few GWAS, EWAS, and TWAS studies report genome-wide significant findings, they lack sufficient power and have not consistently replicated. There is a need for very large (meta-)analyses to be conducted by consortia to identify unambiguous findings. Second, a range of diagnostic tools and symptom measures are used across studies. Cross-site studies would benefit from the use of more standardized batteries. Third, leveraging of genetic covariance with other disorders, at equal power, is necessary in such a highly comorbid set of disorders. Additional well-powered comparisons across anxiety disorder subtypes may highlight unique pathophysiologies. Commonalities and disparities across anxiety disorders should be investigated using a range of methodologies. Fourth, longitudinal data are not widely available in anxiety disorder research and may provide a greater understanding of the trajectory of these conditions and their relationship with comorbid conditions.[72] And finally, since anxiety disorders are a result of both environmental risk and genetic risk, more emphasis on studies integrating both risk types may be useful. This will require deep phenotyping and adequately powered EWAS and gene-by-environment interaction studies. Genome-wide attempts of gene-by-environment studies in anxiety disorders have yet to be undertaken, and it is estimated that at least 10,000 samples are required to detect a moderately strong association.[81]

Overall, however, the field is still far from real clinical application and a personalized medicine approach, and much work has to be done to improve power, data processing, model optimization, validation, and tools that can integrate data from multiple biological variables. The machine learning models discussed above support

the importance of a holistic approach by improving predictions from systems biology data. Associations of biological variables with anxiety-related symptomatology may ultimately have the potential to refine aspects of diagnosis and treatment (Table 2). These models have indicated that the immune, endocrine, and cardiovascular systems all play a key role in underpinning anxiety disorders (e.g., Emeny et al.[27]) and data from these systems may improve specificity and power in predictive models (e.g., Camacho et al.[55]). Further, the accuracy of predictive tools may improve when multiple biological measures are combined (e.g., Boeke et al.[65]), reinforcing the complexity of anxiety disorders and the possible benefit of using multiple biological measures in future research. This is also a limitation for clinical applications, since multiple measurements are costly and time-consuming. While much hope has been put on the potential utility of a systems biology approach, time is still needed for the availability of big data and the development of new methods for its analysis.

In conclusion, current approaches to systems biology research in anxiety disorders serve as a proof-of-concept. The majority of the data collected thus far stem from research that is still exploratory, and that is underpowered and unreplicated. These findings do, however, inform potential next steps in this field. We have learned a great deal from experimental models, neurogenetics and neuroimaging about the role of processes such as fear conditioning and extinction in anxiety disorders. The development of systems biology approaches to anxiety is timely, and may help integrate different available data sources, working across different levels. This more complex approach may ultimately further our understanding of anxiety pathophysiology and development of treatments.

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