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Review

Genetic insights into the neurobiology of anxiety

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Anxiety and fear are evolutionarily conserved emotions that increase the likelihood of an organism surviving threatening situations. Anxiety and vigilance states are regulated by neural networks involving multiple brain regions. In anxiety disorders, this intricate regulatory system is disturbed, leading to excessive or prolonged anxiety or fear. Anxiety disorders have both genetic and environmental risk factors. Genetic research has the potential to identify specific genetic variants causally associated with specific phenotypes. In recent decades, genome-wide association studies (GWASs) have revealed variants predisposing to neuropsychiatric disorders, suggesting novel neurobiological pathways in the etiology of these disorders. Here, we review recent human GWASs of anxiety disorders, and genetic studies of anxiety-like behavior in rodent models. These studies are paving the way for a better understanding of the neurobiological mechanisms underlying anxiety disorders.

Highlights

Anxiety disorders are common psychiatric conditions with moderate heritability. Chronic psychosocial stress at various stages of life, or acute traumatic events, are environmental risk factors for anxiety disorders.

Genome-wide association studies in humans and gene expression studies in rodent models have revealed specific genes and pathways involved in anxiety.

Genetic associations have the potential to improve our understanding of the biological mechanisms underlying common diseases, and to help development of etiologically relevant rodent models.

Most mouse studies investigating anxiety-like behavior have been carried out with C57BL/6 substrains and male mice. Given that genetic background and sex may significantly modulate phenotypic outcomes, the use of a wider array of genetic backgrounds, as well as male and female animals, would increase the translational validity of rodent studies.

Anxiety disorders

Anxiety and fear are normal responses to potential or real threats. They may become frequent, excessive, and prolonged, impairing normal functioning, and leading to pathological anxiety [1]. Anxiety disorders, including panic disorder, social anxiety disorder, specific phobias, and generalized anxiety disorder, are the most common mental disorders, with ~14% prevalence [1,2]. They occur more frequently among women than men [1] and are treated with drugs and/or cognitive behavioral therapy. Numerous pharmacotherapeutic options are available, including anxiolytic medications, such as benzodiazepines, and antidepressants, such as selective serotonin reuptake inhibitors (SSRIs). However, because the molecular pathways leading to excessive anxiety are largely unknown, current medications are poorly targeted, and their efficacy is highly variable. They are also associated with adverse side effects and, in the case of benzodiazepines, the risk of tolerance and addiction limits their long-term use [3]. Consequently, there is a clear need to understand the molecular basis of anxiety disorders and to develop better preclinical models, prerequisites for the development of novel personalized treatments.

In this review, we describe genetic research of anxiety disorders in humans and anxiety-like behavior in rodents, and examine insights gained from these studies into the neurobiological basis of anxiety disorders. Finally, we discuss the importance of genetic background in rodent studies for better translational validity, and call for the development of more etiologically relevant rodent models of anxiety. As well as genetics, epigenetics also has important roles in anxiety disorders [4], but these and other factors contributing to the etiology of anxiety disorders are beyond the scope of the current review.

Animal models of anxiety disorders

Anxiety and fear are adaptive responses, and their necessity for survival have made them evolutionarily conserved [5]. Stress, particularly psychosocial stress, is an environmental risk factor for anxiety disorders [1]. Therefore, the most widely used animal models of anxiety disorders are based on stress exposures. Although physiological and anatomical differences exist, humans and rodents share notable similarities in their stress response and the brain circuits underlying

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threat detection and anxiety responses. Therefore, anxiety disorders are especially well suited to be modeled in rodents (Figure 1, Key figure). Cognitive capabilities are important in the human stress response, especially in coping with stress; therefore, while animal models of anxiety disorders aim to cover certain dimensions of the disorder, the full clinical symptomatology cannot be modeled in laboratory animals. Compared with human studies, a major advantage of animal models is the ability to tightly control the experimental settings, such as environmental exposures, and to have access to tissue samples at specific time points. Importantly, recent advances in transgenic, optogenetic, and chemogenetic techniques allow cell type- and circuit-specific manipulations with precise spatiotemporal control [6] (Figure 1).

Optimally, an animal model should carry etiological (cause) and construct (underlying mechanisms) validity. However, since the causes of anxiety disorders are largely unknown, current animal models of anxiety disorders are based on face (symptomatology) and predictive (treatment response) validity [7,8]. Anxiety disorders are influenced by both genetic and environmental risk factors [9,10]. The heritabilities of anxiety disorders are estimated at 30–40% [9]. In mice, both innate [11] and stress-induced anxiety-like behaviors [12,13] are strongly influenced by genetic factors. The environmental stressors used to induce anxiety-like behavior span from social to physical, from early life to adult stress, and are of varying durations (Box 1). Despite the robust genetic contribution to anxiety in humans, most mouse studies on stress and anxiety-like behavior are carried out with the C57BL/6J and related strains, which are generally more resilient to stress compared with, for example, DBA/2J or BALB/cJ inbred mouse strains, which are highly stress susceptible [12,13].

Rationale for genetically guided animal models

The development of new anxiolytic drugs requires better animal models. Identification of genetic risk factors offers a route to develop etiologically valid animal models to understand the underlying neurobiological mechanisms and to test treatment responses [14]. Once a genetic risk variant is found, it is possible to investigate which gene(s) it influences, what protein the gene encodes, and what the functions of the protein are at the molecular, cellular, and circuit levels. Alternatively, if the variant affects a noncoding RNA molecule, it is possible to determine the gene regulatory networks involved. Examples of mouse models designed on the basis of a similar approach can be found in schizophrenia. In 2014, a GWAS identified 108 independent genetic loci that predispose to schizophrenia [15]. The strongest association was located within the major histocompatibility complex region on chromosome 6, within the *C4* gene region, and it was shown that patients with schizophrenia have higher *C4A* expression levels compared with controls [16]. Transgenic mice overexpressing human *C4A* have lower cortical synapse density and increased microglial-mediated synaptic pruning accompanied by altered social behavior, working memory, and anxiety-like behavior, compared with wild-type [17]. These data suggest overactivation of the complement system as a pathogenetic mechanism of schizophrenia and offer possibilities for the development of novel treatments. Another example is provided by the NMDA glutamate receptor subunit *GRIN2A* gene, which harbors both common variants, identified through GWAS, and rare variants, identified by exome sequencing, that associate with schizophrenia [18]. These findings provide further support for the glutamate hypothesis of schizophrenia [19].

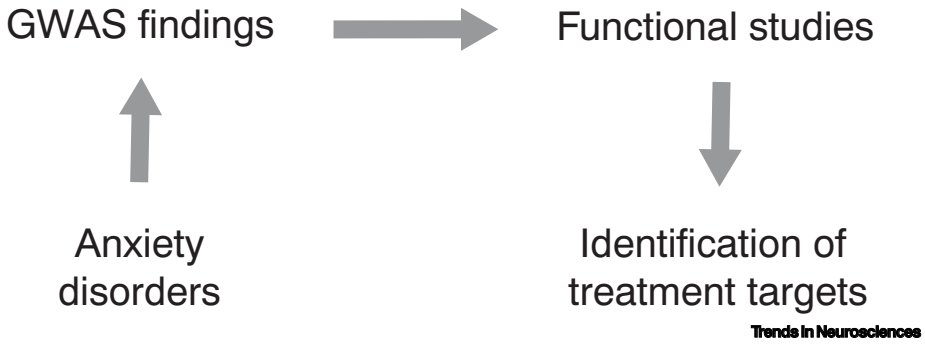
Genetic landscape of human anxiety disorders

Identification of genetic variants associating with anxiety disorders has been progressing rapidly, in part thanks to larger samples and ongoing international meta-analysis efforts. Several genetic variants predisposing to anxiety disorders have been identified in GWASs [20] (see next section). The underlying hypothesis of GWAS is that the genetic predisposition of a trait is due to a cumulative effect of many genetic variants that are common in the population [21]. Typically,

Key figure

Translational approaches to studying the etiology of anxiety disorders by combining human and rodent studies

Humans	Rodent models
Methodological advantages	
Characterization of the full symptomatology of disease	Controlled experimental environment
Ability to conduct large-scale genetic studies	Access to brain tissue at all developmental stages and at specific time points of symptom progression
	Availability of transgenic models and cell- and circuit-specific manipulations
Methodological limitations	
Genetic and phenotypic heterogeneity	Evolutionary differences in physiology and behavior
Environmental factors complex and uncontrolled	Inability to model all aspects of a psychiatric disorder
Limited access to brain tissue and confounding factors present	



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Box 1. Paradigms to test and induce anxiety-like behaviors in rodents

Tests to measure anxiety-like behavior

Commonly used tests, including the open field (OF), light–dark box (LDB), and elevated plus- and zero maze (EPM and EZM) tests, rely on the motivational conflict of the animal between exploration of a novel area and aversion of potentially dangerous open and brightly lit spaces [85]. Typically, the time spent in the open and/or brightly lit spaces is quantified. Reduced time spent in the open spaces or in the brightly lit area is thought to reflect increased anxiety-like behavior. Measures of locomotion should also be included, because changes in activity can confound the interpretation of the results. Moreover, social interaction tests in rodents are often used as a proxy of social anxiety [86]. Novelty suppressed feeding (NSF) is another conflict-based test in which a food-deprived animal faces a choice to fetch food in an open and brightly lit area or avoiding the anxiogenic environment [87]. In contrast to the OF, EPM, and EZM tests, the NSF test is not sensitive to locomotor activity.

Chronic stress-induced anxiety-like behavior

Several stress paradigms in rodents have been adopted to induce anxiety-like behavior, ranging from psychosocial to physical stress.

Stress during early life, including the postnatal period and childhood, is associated in humans with increased risk for psychiatric disorders [88]. In rodents, postnatal stress is commonly modeled using maternal separation and/or limited nesting material during the first weeks of life, which leads to anxiety-like behavior and susceptibility to stressors later in life [89]. In humans, social isolation and lack of social support are increasingly recognized as risk factors for anxiety disorders [90] and, in animal studies, social deprivation models are gaining increasing popularity. In rodents, social isolation is typically employed for several weeks starting at weaning, as this developmental time-period is crucial for social, cognitive, and emotional development [91].

In chronic mild stress, the animals undergo repeated exposures to moderate stressors, such as bright lights, noise, and limited bedding/nesting material lasting for days or weeks [92]. Repeated restraint stress, another physical stressor, results in anxiety- and depressive-like behavior in rodents [93]. *Chronic social defeat* is a model of psychosocial stress, comprising physical confrontations between an intruder and an aggressive resident animal, which results in the defeat of the intruder, and the development of anxiety- and depressive-like behavior [94–96]. Early after social defeat stress, mice show increased social avoidance toward an unfamiliar target. Not all mice develop avoidance and instead resemble nonstressed controls, mimicking individual differences in stress vulnerability in humans. Overall, these models can be used to examine neurobiological mechanisms of stress susceptibility and resilience.

500 000–1 000 000 SNPs are genotyped across the genome in cases and controls, or in a population of individuals with a continuous trait, such as symptom severity or personality traits. Taking advantage of linkage disequilibrium that occurs between nearby SNPs, genotypes from additional SNPs are then imputed using a panel of sequenced individuals from the same population, to obtain information from millions of SNPs. Linear regression analysis is then carried out for each SNP, testing for deviations in allele frequencies of the SNPs between cases and controls. Due to a large multiple testing burden, $P < 5\times10^{-08}$ has been agreed as the significance threshold for GWASs. Candidate gene studies have now been replaced by GWASs because of the significant issues of replication and small sample sizes in the former [22].

Recently, ultra-rare protein-truncating variants were identified in schizophrenia through exome sequencing of a large number of cases and controls [18]. While the odds ratios of individual common variants identified through a GWAS are very small, the carriers of the identified rare variants had a substantial risk of developing schizophrenia. The magnitude of the risk was similar to rare copy number variants (CNVs) that have been observed in patients with schizophrenia [18]. To our

Figure 1. Human studies of anxiety disorders include primarily genetic studies, neuroimaging, and studies of peripheral biomarkers. In addition to genetics, environmental factors play important roles in the etiology of anxiety disorders, and contribute to inter-individual variability. In rodent studies, environmental factors can be tightly controlled, and access to brain tissue at different time points is feasible. Rodent models offer tools to further study the functional implications of genome-wide association study (GWAS)-identified genetic variants, which ideally could lead to the identification of novel treatment targets and the development of optimized treatment practices for anxiety disorders.

knowledge, there are no exome-sequencing studies in anxiety disorders. In a study examining patients with various psychiatric disorders, a CNV within *SLC6A3* was found in one anxiety disorder case and none of the controls [23]. This finding remains to be replicated by other studies. Larger studies in anxiety disorders are needed to reveal whether the genetic landscape of these disorders extends to high risk-conferring rare variants and CNVs, which would be especially valuable for the development of etiologically relevant mouse models.

Human genetic studies of anxiety disorders

The largest GWASs of anxiety have pooled different anxiety spectrum diagnoses with the goal of identifying general anxiety- or stress-related genetic variants. In addition to clinical samples, register- and biobank-based cohorts, such as the UK Biobank [24] and the Danish iPSYCH cohort [25], have provided much-needed larger sample sizes. Table 1 summarizes some of the

Table 1. Summary of statistically significant associations of genes implicated in anxiety based on GWASs in anxiety disorders

Phenotype	Associating SNP	*P*-value	Nearest gene	Position in relation to nearest gene	Refs
Panic disorder	rs860554	4.6×10E-08	*PKP1*	Intronic	[97]
	rs7309727	5.1×10E-07	*TMEM132D*	Intronic	[26]
Anxiety and stress-related disorders	rs7528604	5.39×10E-11	*PDE4B*	Intronic	[25]
Anxiety disorders	rs1709393	1.65×10E-08	*LOC15225*	Intronic	[98]
Anxiety factor score	rs1067327	2.86×10E-09	*CAMKMT*	Intronic	
Agoraphobia Cognition Questionnaire	rs78726293	3.3×10E-08	*GLRB*	Intronic	[99]
Generalized Anxiety Disorder 2-item scale	rs4603973	6.18×10E-11	*SATB1-AS1*	Intronic	[44]
	rs6557168	1.33×10E-09	*ESR1*	Intronic	
	rs12023347	8.88×10E-09	*LINC01360*	Intronic	
	rs56226325	2.01×10E-08	*MAD1L1*	Intronic	
	rs6090040	3.28×10E-08	*TCEA2, RGS19, OPRL1*	Upstream/downstream	
	rs575403075	2.82×10E-08	*TRPV6*	Upstream	
Self-reported physician diagnosis of an anxiety disorder	rs35546597	1.88×10E-08	*AURKB*	Downstream	
	rs10534613	4.92×10E-08	*IQCE*	Intronic	
Anxiety Sensitivity Index	rs13334105	4.39×10E-08	*RBFOX1*	Intronic	[100]
Generalized anxiety disorder symptom score excluding medication users	rs78602344	4.18×10E-08	*THBS2*	Intronic	[101]
Social anxiety factor score	rs78924501	3.58×10E-08			[102]
	rs708012	1.55×10E-08	*MTCH1, FGD2*	Upstream	
Self-reported physician diagnosis of an anxiety disorder	rs10809485	1.6×10E-12	*LOC105375974*	Intronic	[24]
	rs3807866	4.8×10E-08	*TMEM106B*	Upstream	
	rs2861139	2.6×10E-09			
	rs4855559	3.7×10E-08	*MYH15*	Intronic	
	rs1187280	5.2×10E-08	*NTRK2*	Intronic	

genome-wide significant findings in anxiety disorders or anxiety-associated traits. Many risk variants identified are located within intronic regions of genes, suggesting that they regulate the function of their host genes. Here, we describe studies that have begun to reveal mechanisms by which these genes may regulate anxiety (Table 2).

One of the earliest association findings in panic disorder and anxiety severity was variants within *TMEM132D* [26–28]. Postmortem analysis revealed higher *TMEM132D* expression levels in the frontal cortex of risk variant carriers compared with nonrisk variant carriers. In addition, healthy *TMEM132D* risk variant carriers had higher trait anxiety and larger amygdala volume compared with nonrisk variant carriers [29]. Mouse studies provided further support for the involvement of *TMEM132D* in anxiety. Mice bred for high anxiety-like behavior (HAB) have higher *Tmem132d* expression levels in the medial prefrontal cortex (mPFC) compared with low-anxiety (LAB) mice [26], and this difference is possibly mediated by HAB- and LAB-specific SNPs in *Tmem132d* [30]. Concurring with the higher expression levels in the HAB mice, overexpression of *Tmem132d* in the mPFC induces increased anxiety-like behavior [30]. *Tmem132d* was also identified as a candidate gene influencing fear acquisition in a mouse quantitative trait locus (QTL) study [31]. *Tmem132d* encodes transmembrane protein 132D, which is expressed in both neurons and oligodendrocytes [32]. Although its functions are largely unknown, it has been suggested as a cell-surface marker for oligodendrocyte differentiation [33] and a regulator of cytoskeleton and morphology of dopaminergic neurons in *Caenorhabditis elegans* [34].

The Danish Register-based iPSYCH study [25] identified variants within *PDE4B* in a GWAS for a composite anxiety and stress-related phenotype. In a mouse model of chronic social defeat stress, *Pde4b* expression levels were lower in stress-susceptible mice from the C57BL/6NCrl strain compared with controls or stress-resilient mice [25]. Chronic stress did not affect *Pde4b* expression levels in the innately anxious DBA/2NCrl strain, but baseline *Pde4b* expression levels were lower in this strain than in the innately non-anxious C57BL/6NCrl mice. Phosphodiesterase 4 (PDE4) catalyzes the hydrolysis of cAMP and is highly expressed in brain regions critical for anxiety-related behavior, such as the amygdala, hypothalamus, and frontal cortex [35]. Deletion of PDE4B in mice increases anxiety-like behavior, while inhibition of its enzymatic activity has the opposite effect [36,37], supporting PDE4B as a regulator of anxiety-like behavior.

A GWAS in the UK Biobank cohort identified several SNPs associating with lifetime anxiety disorder diagnoses or current anxiety symptoms [24]. One of these hits (rs1187280) is within the intronic region of *NTRK2*, encoding tropomyosin receptor kinase B (TrkB). TrkB is a receptor for brain-derived neurotrophic factor (BDNF), a critical mediator of brain plasticity [38] and a target for antidepressants [39]. Animal studies support a broad role for TrkB in mediating anxiety-related behavior [40]. For example, overexpression of the TrkB catalytic domain has an anxiolytic effect [41], while deletion of TrkB specifically in adult-born neurons [42] or in parvalbumin-expressing interneurons [43] increases anxiety-like behavior. However, not all studies have reported changes in anxiety-like behavior in response to TrkB manipulation [40], and it remains to be shown whether and how the GWAS-identified variants affect *NTRK2* expression and TrkB function.

The largest anxiety GWAS so far, the Million Veteran Project [44], identified a significant association with self-reported anxiety and a SNP (rs4603973) near *SATB1*. This encodes Special AT-rich sequence-bindings protein 1, which acts as a chromatin organizer regulating the expression of several genes in neurons [45]. One of the targets of SATB1 is *CRH*, the gene encoding corticotropin-releasing hormone (CRH) [44], which is part of the hypothalamic–pituitary–adrenal axis, which regulates stress responses. *CRHR1*, encoding the CRH receptor, was also among the top genes associating with self-reported anxiety in a gene-based analysis [44]. Mice lacking

Table 2. Rodent studies of anxiety-associated genes identified through human GWASs[a]

Gene	Protein	Species/strain	Model	Behavioral readout	Main findings	Refs
TMEM132D	Trans-membrane protein 132D	Mouse/CD-1	HAB and LAB mice	EPM, OF	HAB mice: EPM ↑ *Tmem132d* mRNA ↑ in mPFC	[26]
		Mouse/C57BL/6J	OE of *Tmem132d* in mPFC	EPM, LDB	EPM ↑ LDB ↑	[30]
PDE4B	Phosphodiesterase 4B	Mouse/C57BL/6J	$PDE4B^{Y358C}$	EPM, OF, LDB	EPM ↓ LDB ↓ OF ↓	[37]
		Mouse/C57BL/6NCrl and DBA/2BCrl	CSDS	SA	B6-sus mice: SA ↑ *Pde4b* mRNA ↓ in mPFC and vHPC	[25]
GLRB	Glycine receptor subunit beta	Mouse/C57BL/6J	$Glrb^{+/spa}$	OF	OF ↑	[99]
		Mouse/C3H	$Glrb^{+/spa}$	EPM, OF, LDB	Not affected	[103]
RBFOX1	RNA-binding fox1 homolog-1	Mouse/C57BL/6J	*Rbfox1* KO in neurons	LDB, OF, SA	LDB ↑ OF ↑ SA ↑	[104]
TMEM106B	Trans-membrane protein 106B	Mouse/C57BL/6N	Aged $Tmem106b^{-/-}$	EPM, OF, SA	OF ↑ Locomotion ↓	[105]
ESR1	Estrogen receptor 1 alpha	Mouse/C57BL/6J	Females: CVS, OE of *Esr1* in NAc	NSF	NSF ↓	[49]
			Males: CSDS, OE of *Esr1* in NAc	SA	SA ↓	[49]
		Mouse/C57BL/6J	$Esr1^{-/-}$	EPM, OF, LDB	Not affected	[106]
		Mouse/C57BL/6J × 129/Sv	$Esr1^{-/-}$	LDB, OF	Not affected	[107]
		Rat/Wistar (ovariectomized)	Erα silenced in amygdala and hypothalamus	LDB	LDB ↓	[108]
		Mouse/C57BL/6 × CD1 ($ERα^{NesCre}$)	Erα KO in nervous system	EZM	EZM ↑	[50]
NRTK2	Tropomyosin kinase B (TrkB)	Mouse/C57BL/6N × 129Sv × CBA/J	TrkB KO in forebrain	EZM, OF	Not affected	[109]
		Mouse/C57BL/6	TrkB KO in adult-born neurons	EPM, OF	EPM ↑ OF ↑	[42]
		Mouse/C57BL/6	ELS and CUS, TrkB silenced	OF	OF ↑	[110]
		Mouse/BALB/c × DBA/2	OE of catalytic TrkB ($TrkBTK^{+}$)	EPM, OF, LDB	EPM ↓ LDB ↓	[41]
		Mouse/C57BL/6	CSDS, OE of truncated TrkB (TrkB.T1)	SA	SA ↑	[111]
		Mouse/C57BL/6 (PV^{cre})	TrkB KO in PV neurons	EPM, OF, SA	EPM ↑ OF ↑	[43]
		Mouse/C57BL/6J ($Tph2^{creERT2}$:$TrkB^{flox}$)	TrkB KO in SST neurons	EPM, OF, LDB	LDB ↑	[112]

[a]Abbreviations: CSDS, chronic social defeat stress; CVS, chronic variable stress; EPM, elevated plus maze; EZM, elevated zero maze; HAB, high anxiety-like behavior; LAB, low anxiety-like behavior; LDB, light–dark box; KO, knockout; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; NSF, novelty suppressed feeding; OE, overexcitation; OF, open field; PV, paraventricular; SA, social anxiety; SST, somatostatin.

Crhr1 show reduced anxiety-like behavior and response to stress [46], supporting the involvement of the CRH/CRHR1 system in anxiety. Other targets of SATB1 include immediate early genes (*FOSB*, *ERG*, and *ARC*), *BDNF*, and *SST* [45]. *Satb1*$^{-/-}$ mice have reduced cortical dendritic spine density during early development, suggesting potentially long-lasting effects on synapse development [45]. SATB1 also regulates the maturation of somatostatin-expressing inhibitory interneurons [47], which have recently been associated with stress responses and in the regulation of anxiety-like behavior [48].

The Million Veteran Project also found a genome-wide significant variant (rs6557168) near *ESR1*, encoding estrogen receptor alpha (ERα), which functions as a transcription factor activated by the sex hormone estrogen. A recent study identified ERα as a top upstream regulator of genes differentially expressed in stress-resilient mice after chronic social defeat stress [49]. Overexpression of ERα in the nucleus accumbens (NAc) promotes stress resilience both in female and male mice. However, the transcriptional responses to ERα overexpression were distinct between the sexes, suggesting sex specificity in downstream targets mediating proresilience. Neural ERα deletion conversely increases anxiety-like behavior only in females, suggesting that ERα-controlled regulation of anxiety-like behavior is sex specific [50].

Although these studies have identified several anxiety-associated genetic variants, the reproducibility across studies has been limited (Table 1), which is likely due to the modest sample size of most GWASs. The ongoing meta-analysis efforts of the Psychiatric Genomics Consortium and others are expected to reveal additional genetic variants that associate with composite anxiety phenotypes and specific anxiety disorder diagnoses. Another caveat of some of the current studies is that they include mostly participants of European descent, with only one ancestry-specific locus within *TRPV6* identified thus far [44].

Identification of anxiety-associated genetic variants is a starting point for understanding the underlying biology. Of note, most identified variants map onto noncoding regions, which suggests that the variants reside on regulatory elements and exert their effects by regulating gene expression. Expression QTL (eQTL) studies may provide insight into how GWAS-identified variants influence gene expression and, consequently, identify genes with expression changes that contribute to anxiety disorders [51]. Using such an approach in the Million Veteran GWAS data, combined with PsychENCODE and GTEx brain gene expression data, 26 genes the expression levels of which were significantly associated with anxiety traits were identified [52]. The associated genetic variants may mediate their effects by regulating the expression of the identified genes.

In an ideal eQTL study, the gene expression and GWAS data should derive from the same individuals. Unlike genetic mapping studies, in which an association implies potential causality (or linkage disequilibrium with a causal variant), gene expression studies have the ability to identify both causal and reactive changes, which can be distinguished using eQTL approaches [53]. So far, however, no human postmortem brain gene expression studies exist, to our knowledge, in anxiety disorders. In major depressive disorder (MDD) [54–58] and post-traumatic stress disorder (PTSD) [59,60], postmortem brain gene expression profiling has identified brain region-specific gene expression and gene network alterations and, recently, single cell RNA-sequencing further revealed cell type-specific gene expression differences between patients with MDD and controls [61], advancing the understanding of relevant disease mechanisms.

Brain gene expression profiling in rodent models of anxiety

As mentioned earlier, stress is a major risk factor for anxiety disorders, and mechanistic studies of anxiety in animal models are often based on chronic stress exposure. Brain gene expression

studies in mouse models of anxiety have revealed biological pathways involved in stress-induced anxiety. Three overall messages emerge from these studies: (i) individual behavioral differences (i.e., susceptibility or resilience to stress-induced anxiety symptoms) are associated with different gene expression responses; (ii) behavioral and transcriptomic responses to stress vary between males and females; and (iii) genetic background has an important role in regulating stress susceptibility and resilience. All studies described below were carried out with mice from the C57BL/6J strain, unless otherwise specified.

Stress susceptibility and resilience-specific gene expression responses

Although stress is a significant risk factor for anxiety disorders, especially chronic psychosocial stress and stress resulting from acute traumatic events, not everyone experiencing stress develops a clinical anxiety disorder, a situation referred to as 'stress resilience'. The chronic social defeat stress mouse model (Box 1), resulting in behavioral and physiological symptoms related to anxiety and depression, has been extensively used to investigate stress-induced gene expression changes and to characterize stress susceptibility- and resilience-specific mechanisms [13,56,62]. These studies revealed specific hub genes (elements of gene regulatory networks that regulate the expression levels of many other genes) involved in anxiety-like behaviors. Overexpression or silencing of these hub genes may result in increased susceptibility or resilience to stress, and these effects are often highly brain region specific. For example, RNA sequencing in mPFC, ventral hippocampus (vHPC), and NAc following chronic social defeat stress identified susceptibility- and resilience-specific gene regulatory networks and their hub genes [62]. It was also found that overexpression of the susceptibility-specific hub gene encoding Dickkopf-like acrosomal protein 1 (*Dkkl1)* in the vHPC, but not in the mPFC, increased stress susceptibility [62]. Other brain region-specific hub genes include, for example, those encoding sidekick cell adhesion molecule 1 (*Sdk1*) [62] and the stress resilience-promoting zinc finger protein 189 (*Zfp189*) [56]. Susceptibility- and resilience-specific changes have been found in oligodendrocyte gene expression after chronic social defeat stress in both C57BL/6NCrl and DBA/2NCrl mice [13]. These changes were accompanied with modifications in myelin thickness, suggesting myelin plasticity as a robust stress response. Overall, these studies indicate that, although chronic psychosocial stress leads to general stress-related gene expression changes, stress susceptibility and resilience are associated with distinct changes, underscoring resilience as an active process.

Sex-specific stress-induced gene expression changes

Stress-related disorders, such as anxiety disorders, occur more frequently in females, yet most of the past work on stress in animal models has been conducted on male animals. Brain gene expression studies have shown large sex differences after chronic stress, although the differentially expressed genes (DEGs) sometimes converge on the same biological pathways. In a study examining gene expression profiles after chronic variable stress, only 20–25% of the DEGs overlapped between males and females in the mPFC and NAc [55]. The gene encoding dual specificity phosphatase 6 (*Dusp6*) was identified as a female-specific stress response regulatory gene, whereas that encoding empty spiracles homeobox 1 (*Emx1*) was identified as a male-specific stress-response regulatory gene. Other genes with sex-specific effects include those encoding orthodenticle homeobox 2 (*Otx2*) in early life stress [63,64], DNA methyltransferase 3 alpha (*Dnmt3a*) [54] and slit guidance ligand 1 (*Slit1*) [58] in chronic variable stress, and cAMP-responsive element binding protein 1 (*Creb*) and hypocretin (*Hcrt*) in social isolation during adolescence [65].

The chronic social defeat stress paradigm is probably the most widely used chronic psychosocial stress model in mice. However, a caveat of this model is that its original version is only applicable to male mice, given the limited territorial aggression in females in most rodent species. The use of

rodent species in which females show aggressive behavior, for instance California mice (*Peromyscus californicus*) [66], has enabled inclusion of female subjects and identification of sex-specific effects of psychosocial stress. In studies using California mice, only females developed social avoidance after social defeat. Social stress resulted in different changes in brain gene [57] and miRNA [67] expression in males and females. For example, the gene encoding regulator of G-protein signaling 2 (*Rgs2*) was downregulated by stress in the NAc only in females [57].

Overall, brain gene expression studies in rodent models have identified novel genes and biological pathways involved in stress-induced anxiety-like behavior. However, most studies have been performed on bulk tissue involving multiple cell types. Single cell-sequencing studies, combined with spatial transcriptomics [68,69] across several brain regions and specific circuits involved in the regulation of anxiety, are expected to increase the resolution of gene expression studies and, thus, advance our understanding of the mechanisms underlying stress-induced anxiety related to different stressors and time points across the lifespan.

Genetic background matters

Inbred rodent strains vary considerably in their innate anxiety-like behavior [11,70–72], and in transgenic mouse models, anxiety-related phenotypes may differ depending on the genetic background of the mouse line. Mice from the C57BL/6 strain and its substrains, and from the FVB/NJ strain have generally low levels of innate anxiety-like behavior, whereas mice from the DBA/2J, BALB/cJ, and A/J strains have relatively high innate anxiety-like behavior [11]. Different inbred strains also vary in chronic stress-induced anxiety-like behaviors. Robust strain-dependent differences have been characterized in behavioral response to chronic social defeat stress [12,13]. Based on a social interaction test performed after a 10-day chronic social defeat stress, 31% of C57BL/6NCrl, 23% of BALB/cAnNCrl, 19% of 129S2/SvPasCrl, and 5% of DBA/2NCrl mice were identified as stress susceptible (i.e., developed social avoidance, a symptom of anxiety) after stress [13]. Moreover, inbred mouse strains differ in response to anxiolytics [73], and show divergent behavioral responses to housing conditions [74].

The large effect of genetic background on behavioral and physiological phenotypes of transgenic mice was exemplified in an analysis of F1 progeny of *Cacna1c* or *Tcf7l2*-null allele C57BL/6J carriers and wild-type female mice from 30 inbred strains [75]. These mice are heterozygous null littermates that are genetically identical to their wild-type controls at all other loci. Significant genotype–strain interactions were identified for both behavioral and physiological phenotypes, sometimes in opposite directions. For example, in the A/J strain, *Tcf7l2* haploinsufficiency increased the acoustic startle response, whereas, in DBA/2J mice, it decreased this response, illustrating the importance of modifying genes on the phenotypic outcome. The facts that mouse studies to date are frequently conducted on mice from the C57BL/6 background, and that most transgenic lines are designed based on this strain, raise the concern of generalizability across strains.

Notably, gene expression studies have also identified cases of opposite effects across different strains. To identify biological pathways involved in stress-induced anxiety-like behavior, gene expression and proteomic profiling were carried out in the bed nucleus of stria terminalis (BNST) of mice after chronic social defeat stress, and found a significant enrichment of mitochondria-related pathways [76]. Mitochondrial gene expression was upregulated in stress-susceptible C57BL/6NCrl mice compared with controls, but downregulated in DBA/2NCrl mice. Of note, in patients with panic disorder, mitochondrial-related pathways were downregulated in blood cells during a panic attack [76], similar to effects seen in DBA/2NCrl mice displaying anxiety-like phenotypes. In

another study, C57BL/6J and DBA/2J mice displayed significant differences in amygdala gene expression after chronic restraint stress [77]. For example, genes enriched in myelination and neuronal development pathways were differentially expressed in DBA/2J mice, whereas genes enriched in the synaptic plasticity pathway were differentially expressed in C57BL/6J mice.

The major strain differences in the context of modeling anxiety imply that some strains may be better suited for particular applications. When testing potential therapeutics, ceiling and floor effects of anxiogenic and anxiolytic substances may be avoided by selecting strains with appropriate baseline anxiety levels. An advantage of inbred strains is that all animals are genetically identical, thus reducing phenotypic variability, but it is important to keep in mind that the results may not generalize to other strains. The use of several inbred backgrounds, hybrid mice, or outbred strains may also be a good option depending on the research question. Outbred populations are especially well suited for systems genetics approaches that combine association mapping and gene expression profiling in relevant tissues to identify eQTLs [78,79].

Comparative studies between rodent models and humans

Comparative studies of anxiety in humans and rodent models are still limited but encouraging examples from studies in MDD exist. Several similarities were found when gene expression changes in human MDD postmortem brains were compared with mouse chronic stress models [80]. For example, chronic variable stress and social isolation largely recapitulated differentially expressed genes, and chronic social defeat stress and social isolation reproduced gene network level changes well. Moreover, several findings in mice, including key drivers of stress resilience and susceptibility, have been verified in human postmortem MDD samples [54–58], supporting the translational value of these preclinical stress models.

Concluding remarks

Despite the high prevalence and burden of anxiety disorders, current understanding of their etiology remains limited. In other psychiatric disorders, genetic research has revealed important pathogenetic mechanisms and, although small sample sizes and, therefore, low statistical power to identify significant associations, have historically hindered progress in anxiety disorders, recent larger-scale biobank or register-based studies have led to the identification of genetic variants predisposing to anxiety disorders (Table 1). Anxiety disorders have high comorbidity with each other and with other psychiatric and somatic illnesses [81] reflected also in the high genetic correlation between them [82]. Large register-based studies with information on all medical diagnoses have the potential to identify subgroups of individuals based on disease trajectories, comorbidities, and genomic information. Identification of general and subgroup-specific anxiety-associated genetic factors provides opportunities to elucidate the underlying neurobiological mechanisms (see Outstanding questions). Once the GWAS sample size required to identify the most robustly associated genes has been reached, the major question becomes how these variants influence gene function [83] and behavior. Intragenic variants provide an excellent opportunity to develop etiologically relevant disease models, which are essential for the development of specific treatments based on medication, psychosocial interventions, or brain stimulation. However, most identified variants reside outside of the coding regions of genes, making it challenging to reveal the genes they regulate. Novel high-throughput methods to identify genes the function of which is influenced by the associating SNPs, such as PASSPORT-seq, have been used, for example, in alcohol dependence [84].

Understanding of the neurobiological mechanisms underlying anxiety disorders requires a combination of studies in humans and model organisms (Figure 1). Although the overlap of human GWASs and gene expression studies in mouse models is modest so far, the gene encoding

Outstanding questions

How much of the genetic liability to anxiety is explained by common variants, rare variants, and CNVs, and how much do they increase the risk for anxiety disorders?

The genetic correlation between anxiety disorders and other psychiatric disorders is high, suggesting that the diagnostic classes do not reflect the underlying biology. What genetic and neurobiological strategies should be utilized to understand the biological basis of anxiety disorders to guide treatment practices?

What are the biological mechanisms by which anxiety-associated genetic variants influence gene function, thereby impacting vulnerability or resilience to anxiety disorders?

How could human genetic information help to develop rodent models of anxiety disorders for identification of pathogenetic mechanisms and for screening of novel anxiolytics? How can we design rodent models that incorporate the knowledge of the complex genetic background and environmental risk factors of anxiety disorders?

Anxiety disorders are more common in females than males; yet, most studies in rodent models are conducted with males. What are the anxiety-associated genetic or neurobiological mechanisms that differ between males and females? How do females and males differ in terms of susceptibility and resilience to stress-induced anxiety-like behavior?

Although it is well accepted that the genetic background modulates behavioral phenotypes, most mouse studies in neurobiology are conducted with animals from C57BL/6 substrains. How much would the translational validity of rodent anxiety models increase with inclusion of a variety of strains that differ in their susceptibility to stress-induced anxiety-like behavior?

estrogen receptor 1, for example, has been identified by both approaches [44,49]. Translational studies in which advantages of mouse models are used for unbiased 'omics screens to form hypotheses for testing in human anxiety disorders are still rare, as are 'omics experiments including rodents and humans. To reach better concurrence between rodent and human studies, the translational validity of current rodent models needs to be increased by developing etiologically valid models based on, for example, genetic information. Specific hypotheses formed based on studies in rodent models can then be tested in human clinical or epidemiological samples. The hope is that, ultimately, neurobiological insight from such studies could be translated to treatments that benefit patients with anxiety disorders.

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Declaration of interests

I.H. is listed as an inventor on a pending patent application regarding a panic disorder biomarker. M-K.K. declares no conflicts of interest.

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