The Role of SNP Research in Identifying Biological Mechanisms Underlying Generalized Anxiety Disorder

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Generalized Anxiety Disorder (GAD) is a mental health disorder with an estimated lifetime prevalence of 5.7% among adults in the United States (Generalized anxiety disorder, November 2017). The disorder results in moderate to severe impairment in two thirds of cases in the U.S (Generalized anxiety disorder, November 2017). In such cases, the constant worry that patients experience often makes it difficult for them to fulfill their work-related responsibilities and/or maintain their personal relationships (Kessler et al., 2005). GAD negatively affects the lives of hundreds of thousands of Americans each year, yet treatments remain incomplete. The most common approaches to treating GAD are Cognitive Behavioral Therapy and pharmacological treatments (Generalized anxiety disorder, October 2017). However, approximately 50% of patients do not respond to first-line pharmacological treatments for GAD, and the side effects of each medication vary greatly between patients (Buoli et al., 2013). The development of a greater diversity of medications for GAD would allow patients the opportunity to choose a medication that provides them with maximum benefits and minimal side effects. GAD is known to be moderately heritable, but the genetic variants and biological mechanisms underlying that heritability are largely unknown (Levey et al., 2020). Investing in research dedicated to uncovering the genetic variants linked to GAD will help further the understanding of the disorder as well as the development of optimal treatments. It will also allow for the use of linkage-disequilibrium score analysis, a measure of the nonrandom co-occurrence of alleles at two or more different loci within a given population, to assess genetic correlations between GAD and other traits and conditions. Identifying genetic variants linked to GAD aids in the development of hypotheses regarding which genes influence GAD risk and the mechanisms through which they do so. If these hypotheses are substantiated, they may provide insight into ways in which modulating physiological processes with medication could reduce the burden of GAD. Studying the genetic variants linked to GAD is important for developing a comprehensive understanding of the variety of biological processes that can be involved in GAD risk, which may enable the development of a greater diversity of pharmacological treatments.

Twin studies have indicated that GAD is moderately heritable, which means there is a notable genetic component to the condition (Hettema, Neale, & Kendler, 2001). In twin studies, pairs of monozygotic twins are compared to pairs of dizygotic twins. If the correlation between outcomes for pairs of twins is higher among monozygotic twins than dizygotic twins, it is seen as evidence for a genetic component to the heritability of the condition. A crucial assumption of such studies is the Equal Environments Assumption, which asserts that differences in environment do not confound comparisons between monozygotic and dizygotic twins. This assumption has been supported by various studies, as summarized in Felson's meta-analysis "What can we learn from twin studies? A comprehensive evaluation of the equal environments assumption"(Felson, 2014). Under this assumption researchers have been able to use twin studies to show that genetics account for approximately 32% of the variation in risk for GAD (Hettema, Neale, & Kendler, 2001).

As such, twin studies indicate that genetics play at least a modest role in the heritability of anxiety disorders, with the remaining variance attributed to familial environment and individual environment. However, twin studies can only assess the overall genetic component of anxiety. They do not provide any information on genetic variations associated with anxiety disorders. This is a significant limitation because it means that twin studies can not provide much insight into the specific genes that underlie the heritability of GAD, nor the physiological processes through which they do so. Knowing that GAD is moderately heritable is very important, but that knowledge alone does provide insight into the biological mechanisms that influence GAD, nor does it open up pathways for developing treatments.

To gain insight into the various biological systems involved in susceptibility to GAD, researchers can first identify the genetic variants linked to the disorder. This is often achieved by conducting Genome Wide Association Studies (GWAS) focused on Single Nucleotide Polymorphisms (SNPs). SNPs are variations in the genome that occur as the result of a change in one nucleotide in the genome sequence. In many GWAS studies, SNPs are considered relevant if the rarer allele has a frequency >1% in a random sample of the population

(Coleman et al., 2015). When statistical analyses are performed on the results of GWAS, they tend to reveal many SNPs that are correlated with the trait of interest (Frayling, 2014).

This is both a benefit and a drawback of GWAS. Seeing so many SNPs associated with a condition indicates that it is highly polygenic, and therefore there are many different pathways to explore for treatment. Unfortunately, however, GWAS studies cannot prove causal effects, so while some of the SNPs shown to be associated with the condition may turn out to have a causal relationship, others may not (Wray et al., 2013). This is why the findings of GWAS are merely a starting place for research into the genetic causes of conditions such as GAD. Another drawback is that since many conditions are highly polygenic, any one variant in isolation is of small effect (Frayling, 2014). As a result of the small effect sizes, it can be difficult to determine whether a variant is actually linked to the trait of interest.

Despite these drawbacks, researchers have been able to use GWAS to identify many novel genetic variants linked to various psychological conditions, including GAD. For example, the largest GWAS for GAD to date, "Reproducible Genetic Risk Loci for Anxiety," identified 6 novel loci linked to GAD-2 score (Levey et al., 2020). Based on a linkage disequilibrium of genome-wide significant SNPs from the Million Veteran Program biobank, the study identified 5 genome-wide significant loci associated with GAD in European Americans and 1 genome-wide significant locus associated with GAD in African Americans. By creating a set of specific loci with a well-evidenced relationship to GAD, these findings give researchers a starting place for determining the causal mechanisms that increase vulnerability to GAD.

In this study, Levey et al. found that the SNP-based heritability for GAD estimated by linkage disequilibrium score regression based on GAD-2 score is 5.58% and the SNP-based heritability based on self-reported physician diagnosis of anxiety is 8.79%. The difference between the variability in heritability that is attributed to genetics and the variability that can be accounted for by SNPs is quite common (Eichler et al., 2010). It is known as the "missing heritability problem," and researchers have proposed many explanations. One prediction is that as more detailed genome-mapping technologies, such as whole-genome sequencing, become increasingly available, researchers will be better able to identify low-frequency and rare variants that contribute to the genetic basis of the conditions in question (Eichler et al., 2010). Working to identify the sources of this missing heritability would be one approach to cultivating a more thorough understanding of the many genes and physiological processes involved in GAD.

Continued research into the genetic variants underlying GAD is necessary for the identification of new SNPs linked to GAD as well as testing hypotheses regarding causal relationships between known SNPs and GAD. Though Levey et al.'s findings are correlational, the authors suggest a few mechanisms through which the SNPs they identified may influence susceptibility to GAD. One proposed mechanism is based on their top genome-wide significant finding among European Americans– a set of 64 genome wide significant SNPs in high linkage disequilibrium at the SATB1-AS1 locus on chromosome 3 (Levey et al., 2020). As linkage disequilibrium was measured using r^2, alleles can be considered in linkage disequilibrium when $r^2 > 0.50$ and in high linkage disequilibrium when $0.70 < r^2 < 1$. SATB1-AS1 is an antisense gene that can prevent the expression of the gene SATB1. SATB1 modulates the expression of the corticotropin releasing hormone (CRH) gene, which encodes a protein product of the same name. CRH is known to play an essential role in cognitive, behavioral, and physiological aspects of the stress response (Kalin et al., 2016; Smith & Vale, 2006). Therefore, the researchers interpret their findings as evidence that SNPs at SATB1 may influence susceptibility to GAD through their impact on the expression of CRH (Levey et al., 2020).

Convincing evidence for the relationship between overexpression of CRH and susceptibility to anxiety disorders comes from a study that showed that overexpression of CRH in the central nucleus of the amygdala (Ce) increases anxious temperament in primates (Kalin et al., 2016). Therefore, it is plausible that the SNPs identified at SATB1 may influence vulnerability to GAD by causing overexpression of CRH. However, due to the correlational nature of GWAS, it is not possible to prove that variants at SATB1 lead to GAD based on Levey et al.'s findings. Even if the relationship between SATB1, CRH, and GAD were substantiated, further research would also be required to determine whether overexpression of CRH affected susceptibility to GAD through neural mechanisms (primarily controlled by CRH expressed in the Ce), the hypothalamic pituitary adrenal axis (initiated by release of CRH from hypothalamic paraventricular nucleus neurons), or both. However,

the hypothesis that SNPs at SATB1 increase risk for GAD by causing overexpression of CRH does provide a clear and logical starting point for future research into a causal relationship between a set of genetic variants and GAD.

In addition to building a more robust understanding of the variety of systems involved in GAD risk, tracing the relationship between SNPs and the emergence of the GAD phenotype would provide opportunities to develop a greater variety of pharmacological treatments targeting specific processes within each system. If future research into the relationship between SATB1 and GAD can establish that genetic variants leading to an increased release of CRH increase vulnerability to GAD, it would suggest that creating medications designed to block expression or reception of CRH could help decrease anxiety symptoms. Fortunately, the benefits of such treatments would not be limited to GAD patients who have the SNPs at the SATB1 locus implicated in the study. Instead, understanding the relationship between CRH levels and GAD suggests that any patient with GAD may benefit from medications that block CRH reception. As researchers identify more SNPs, and thereby more biological systems, involved in vulnerability to GAD, they are likely to find more opportunities to intervene with pharmacological treatments. The development of a wide variety of pharmacological treatments for GAD, each targeting different physiological systems, would enable patients suffering from the disorder to choose a medication that provides maximal benefits and minimal drawbacks (Bandelow, Michaelis, & Wedekind, 2017). Since each individual is so unique, it is unlikely that there will ever be one medication that ensures optimal outcomes for every GAD patient. Therefore, creating a variety of medications is the best way to ensure that each patient is able to find an option that suits their needs.

The SATB1 pathway is just one of many mechanisms that may be involved in the genetic underpinnings of GAD. As researchers build upon the findings of GWAS to investigate causal relationships between SNPs and vulnerability to GAD, some of the proposed pathways will likely be substantiated while others are disproven. It is unlikely that any one variant, or even one pathway, will be identified as the primary cause of GAD. Instead, aiming to identify many SNPs of small effect that contribute to GAD risk through a variety of pathways will likely yield the most accurate and comprehensive understanding of the biological mechanisms underlying GAD.

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