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Common Polymorphisms in the Age of RDoC: Integration and Translation

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Abstract

The value of common polymorphisms in guiding clinical psychiatry is limited by the complex polygenic architecture of psychiatric disorders. Common polymorphisms have too small an effect on risk for psychiatric disorders as defined by clinical phenomenology to guide clinical practice. To identify polymorphic effects that are large and reliable enough to serve as biomarkers requires detailed analysis of a polymorphism's biology across levels of complexity from molecule to cell to circuit and behavior. Emphasis on behavioral domains rather than clinical diagnosis as proposed in the Research Domain Criteria (RDoC) framework facilitates the use of mouse models that recapitulate human polymorphisms because effects on equivalent phenotypes can be translated across species and integrated across levels of analysis. A knock-in mouse model of a common polymorphism in the brain-derived neurotrophic factor gene (*BDNF*) provides examples of how such a vertically integrated translational approach can identify robust genotype-phenotype relationships that have relevance to psychiatric practice.

Keywords

Common polymorphism; Behavioral dimension; Genetic biomarker; BDNF Val66Met; Fear learning; Anxiety

One of the major rationales for the Research Domain Criteria (RDoC) framework is that “diagnostic categories based on clinical consensus fail to align with findings emerging from clinical neuroscience and genetics (1).” This point is emphasized by how complex the

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genetic architecture of the major clinical psychiatric disorders have proven to be (2–4). Initially, genome wide association studies (GWAS) of neuropsychiatric disorders were driven by a model in which common human disorders were the result of a moderate number of common genetic risk factors with genotype relative risk effect sizes in the range of 1.5 to 4.0 (5–7). This common disease/common variant (CD/CV) model was attractive because it suggested that, with the newly available large-scale genomic technologies, genetic risk factors could be readily identified and then used as biomarkers that would have adequate predictive power to guide clinical practice and would be applicable to a substantial portion of the population (8).

As genome-wide approaches have been applied to very large samples it has become clear that the genetic risk in human populations for clinical disorders such as autism, schizophrenia, depression, the anxiety disorders, and others is comprised of a complex mix of many distinct and often *de novo* rare mutations of moderate effect size and thousands of common polymorphisms of very small effect size (3, 9–11). The complex polygenic architecture of psychiatric disorders demonstrates that categorical psychiatric diagnoses, defined by clinical phenomenology, capture an extremely wide array of biological complexity, which prevents the practical application of genetic biomarkers to clinical psychiatry.

It may be that the diffuse genetic architecture of psychiatric disorders is due to the inevitable complexity of behavior. However, as posited in the RDoC framework, it may be due instead to the poor mapping of the clinical phenomenology of psychiatric disorders onto the biological pathways that subserve behavior (12). If this is the case it may be possible to identify effects of common polymorphisms on core aspects of neurobiology and behavior that are of adequate effect size and reliability to guide clinical practice. The challenge then becomes how to identify robust genotype-phenotype relationships and apply them to clinical practice short of diagnosis *per se*.

The goal of the RDoC framework is to relate the neurobiology underlying different levels of analysis to one another ultimately with implications for behavior and its disorders. That being the case, polymorphisms should be treated as the biological entities they are and studied in detail for their effects on biological function at different levels of analysis. To accomplish this, polymorphisms must be taken out of their human genomic context and introduced into experimental systems which: minimize the inevitable off-target genetic and behavioral complexity of human subjects, allow controlled and invasive manipulations across levels of analysis, and facilitate systematic studies on interactions of these polymorphic effects with important biological phenomena such as sex, development, and environmental exposures. In this review we discuss a *vertically integrated translational approach* to identifying the effects of common genetic variation on behavior. In this approach human variants are introduced into the genomes of inbred mouse strains allowing for controlled experiments to understand the phenotypic effects of that variation at different levels of complexity and relate them to one another. That information is then used to develop constrained *a priori* hypotheses for association testing in humans (Figure 1A). The vertically integrated translational approach is reminiscent of and integrates well with the RDoC framework in that it emphasizes relationships between the biology of different levels

of analysis applied to behavioral domains that are relevant to human disorders but can be studied in parallel in humans and non-human species (Figure 1B).

Genetic knock-out mouse models have been extremely powerful in elucidating the contributions of individual genes to neurobiological function and behavior (13) but their translational value for developing clinical biomarkers is limited because they do not recapitulate the detailed biology of naturally occurring human variants. Genetic knock-in technologies allow the targeted introduction of single nucleotide changes into the mouse genome providing an exact molecular recapitulation of a human variant on an otherwise homogeneous genetic background which provides construct validity in translation of mouse findings to humans (14). An additional benefit of a mouse model system is that controlled breeding can produce as many animals of each genotype as needed regardless of the prevalence of the variant in human populations facilitating analysis of allele-dose effects and factors such as sex and development that may modify the polymorphism's effects. Finally, environmental exposures can be controlled in mouse models minimizing the confounding effects of diverse experiences in human populations and facilitating controlled studies of how specific exposures interact with the polymorphism.

We have implemented this approach targeting a common single nucleotide polymorphism (SNP) in the human gene coding for brain-derived neurotrophic factor (*BDNF*), a neurotrophin involved in neuronal growth and survival as well as experience-dependent learning (15–17). The *BDNF* Val66Met SNP (dbSNP ID:rs6265) codes for the replacement of an evolutionarily conserved valine with a methionine at position 66 in the *BDNF* protein. The *BDNF* Val66Met polymorphism is common in most human populations with the minor allele frequency ranging from 0.48 in Asian population to 0.01 in African populations with European populations in between at 0.2 (18). *In vitro* analysis of *BDNF* Val66Met has demonstrated that the variant *BDNF* Met protein is less efficiently targeted to the regulated secretory pathway than the *BDNF* Val protein, which leads to its decreased activity-dependent secretion (19–21). The *BDNF* SNP has been associated in humans with hippocampal volume, cognitive performance, and psychiatric disorders including schizophrenia, bipolar disorder, major depression, and anxiety disorders however none of these associations has been consistently replicated limiting their value in refining understanding of *BDNF* SNP effects and clinical use (22).

BDNF Val66Met knock-in mouse

The prodomain of the *BDNF* peptide in which the Val66Met polymorphism occurs is highly conserved from mouse to human and implies strong structure-function constraints in this region of the *BDNF* peptide and because wild-type inbred mouse strains naturally express the ancestral allele (*BDNF*^{Val}), the same variant nucleotide knocked into the mouse genome causes the same valine to methionine substitution (*BDNF*^{Met}). The *BDNF*^{Met} allele displayed a dose-dependent effect on regulated *BDNF* secretion in cultured hippocampal neurons from the variant *BDNF* mice (i.e., *BDNF*^{Val/Val} > *BDNF*^{Val/Met} > *BDNF*^{Met/Met}) despite equal levels of total *BDNF* protein in the brains of *BDNF*^{Val/Val} and *BDNF*^{Met/Met} mice (20). This phenotype recapitulates the cellular phenotype of *BDNF* Val66Met identified *in vitro* (19, 21) and distinguishes the polymorphic mouse model from *BDNF*

knock-out mice, which express their phenotypic effects through reduced BDNF expression. The BDNF^{Met} allele also caused decreased dendritic complexity in hippocampal neurons and decreased total hippocampal volume, the latter providing an external source of validation for one of the relatively well replicated anatomical phenotypes of BDNF Val66Met in human subjects (20, 23). Because BDNF is a key modulator of experience-dependent learning, particularly in the hippocampus, conditioned fear learning presented an ideal initial behavioral domain to test for higher-level effects of the BDNF Val66Met polymorphism. Conditioned fear learning is a form of Pavlovian learning in which a neutral conditioned stimulus (CS) such as an acoustic tone, when paired with an intrinsically aversive unconditioned stimulus (US) rapidly takes on the properties of the US and can then elicit a fear response when presented alone (24, 25). In addition to the CS, the context in which the US occurs can also take on aversive properties and this contextual fear learning requires input from the hippocampus, which expresses the highest levels of BDNF in the brain (26). In a standard fear learning paradigm the BDNF^{Met} allele displayed a dose dependent effect reducing the formation efficiency of contextual fear learning (20).

Vertically integrated translational approach to the role of BDNF Val66Met in cued fear extinction learning

Additional aspects of conditioned fear responses are also attractive intermediate phenotypes for understanding the effects of BDNF Val66Met in mice and humans on a behavioral domain relevant to human psychopathology. Translation is also facilitated by rich literatures that relate altered fear learning to human anxiety disorders and their treatment, define differential developmental trajectories of components of fear learning, and have identified the effects of certain environmental exposures on fear learning (27–30). Having identified a contextual fear-specific effect of the BDNF SNP on the formation of fear associations, we tested for its effects on cued fear extinction in parallel mouse and human studies. In both mice and humans the BDNF Met allele was associated with reduced fear extinction learning (31). In mice we were able to identify a dosage effect of the Met allele on fear extinction learning but, as is often the case with human population samples, there were too few Met allele homozygotes to allow a meaningful statistical analysis and they were pooled with BDNF Val66Met heterozygotes in human analyses (31).

We then sought convergent evidence across levels of analysis for BDNF Val66Met effects on the neural substrates of fear extinction using species-specific analyses. We assessed activation of amygdala and ventromedial prefrontal cortex (vmPFC) during fear extinction learning in humans as a function of BDNF Val66Met genotype using functional magnetic resonance imaging (fMRI). Consistent with the behavioral results, human Met allele carriers displayed elevated activation in the amygdala and decreased activation in vmPFC during fear extinction learning (31) suggesting that the behavioral effects of BDNF Val66Met on extinction learning are due to reduced extinction-activated plasticity in the vmPFC, which impairs its ability to regulate amygdala responses (32, 33). Detailed *in vitro* electrophysiological testing in the BDNF Val66Met mice facilitated testing this hypothesis through activity-dependent plasticity in the mouse analogue of vmPFC, infralimbic medial prefrontal cortex. In the absence of stimulation, excitatory postsynaptic potentials (EPSPs)

were similar in BDNF^{Val} and BDNF^{Met} mice however spike timing-dependent plasticity, a neural correlate of experience dependent learning and memory was reduced in BDNF^{Met} mice (34). The same experiments performed using selective pharmacologic agents allowed electrophysiological dissection of these effects and demonstrated that BDNF^{Met} mice have reduced postsynaptic responses to both NMDA receptor and GABA receptor related plasticity (35). Thus, the availability of a behavioral construct, acute fear responses, that has well defined and similar cross-species behavioral attributes and neural underpinnings provided an opportunity to validate the association between BDNF Val66Met and a complex behavior with clinical relevance through integration across levels of analysis and across species.

Exploratory analyses of gene interactions in polymorphic mice

The BDNF Val66Met knock-in mouse provides an efficient and reliable method of testing exploratory hypotheses on how the polymorphism interacts with other factors that would be extremely difficult in human samples. This is because adequately powered samples can be easily generated and assessed to test specific *a priori* hypotheses whereas collecting a large human sample to test a single hypothesis requires a great deal of prior probability to justify the effort. We have used the polymorphic mouse model to dissociate the effects of the BDNF Val66Met polymorphism on sub-constructs of conditioned fear learning and identify interactions with development and stress that can be used to guide the design of prospective human genetic studies and also provide examples of why polymorphic effects do not map cleanly onto diagnostic categories.

Gene x Development Interactions

Although a genetic variant is encoded in an individual's genome at the time of fertilization, its effects on complex phenotypes are not necessarily fixed across the lifespan. Behavioral domains including fear learning display complex and distinct developmental trajectories that may modify the effects of a polymorphism on neural function and behavior (36). The development of cued fear extinction learning displays a similar non-linear trajectory in mice and humans (37, 38). Adolescence is a unique period during which cued fear extinction learning is dramatically less efficient than childhood or adulthood, which appears related to imbalances in the functional maturity of subcortical structures such as amygdala and the cortical regions that regulate them during adolescence (39, 40). The relative inability of adolescents to extinguish learned fear associations that are no longer relevant may explain why a number of anxiety disorders have a peak of onset during that time (41).

Contextual fear learning displays even more dramatic differences across development than cued fear extinction. In mice, there is a discreet period during adolescence when contextual fear associations can be formed but cannot be retrieved and influence behavior (42). Contextual associations are formed during this time because they can be retrieved and influence behavior later on in adulthood. The unique developmental trajectories of components of cued versus contextual fear learning lead to an interesting disjunction in which adolescents are particularly sensitive to cued fear associations and insensitive to contextual fear associations (43).

BDNF Val66Met interacts with these different developmental trajectories in ways that can have paradoxical effects on behavior and risk for psychopathology. Because adolescence is a period of inefficient cued fear extinction learning, there is a floor effect and the additional quantitative reduction in BDNF signaling due to the BDNF^{Met} allele has a less apparent effect on behavioral phenotypes as there is no difference in anxiety-related behaviors as a function of BDNF genotype during this developmental stage (44). However, in adulthood the reduced efficiency of cued fear extinction learning due to the BDNF^{Met} allele is associated with the emergence of an anxiety-related phenotype in the open field and elevated plus mazes (20, 45). In contrast, BDNF^{Met/Met} mice have a selective deficit in acquiring contextual fear associations so that fearful contexts experienced during adolescence are not retrieved during adulthood the way they are in BDNF^{Val/Val} mice (44). These findings demonstrate that the relationship of the BDNF Val66Met polymorphism to anxiety may depend on the nature and timing of traumatic exposures that could prevent reliable identification of its role in human anxiety according to standard criteria.

Gene × Environment Interactions

Stress is a particular environmental exposure that can precipitate or exacerbate a range of psychiatric disorders (46). The physiologic mechanisms underlying stress responses are well conserved from mouse to human facilitating psychopathology-relevant assessment of gene × environment interaction in the BDNF Val66Met polymorphic mouse model (47). Adult male BDNF^{Val/Met} mice display normal hypothalamic-pituitary-adrenal (HPA) axis function under normal conditions however, chronic restraint stress causes exaggerated HPA axis responses as well as increased anxiety-related and depression-related phenotypes (48). These results demonstrate a BDNF Val66Met × stress interaction that may also help guide the design of human association studies of BDNF Val66Met although there may also be developmental stage-specific aspects of this interaction (49).

Gene × Gene Interactions

Complex neural and behavioral phenomena are the result of the coordinated actions of many genes. If multiple genes involved in a biological process contain functional polymorphisms, their effects can interact in ways that have implications for human association studies and personalized psychiatry. For instance, the prototypical endocannabinoid, anandamide, regulates conditioned fear extinction learning (50–52) and its primary catabolic enzyme, fatty acid amide hydrolase (FAAH) contains a common polymorphism in human populations (53). This polymorphism, FAAH C385A, (dbSNP ID:rs324420) codes for an amino acid substitution that reduces steady state levels of FAAH protein and activity *in vitro* and in T-lymphocytes from human subjects (53, 54). We have recently developed a FAAH C385A polymorphic knock-in mouse in the same manner as the BDNF Val66Met mouse and found that it has markedly increased anandamide levels in brain tissue (55). In contrast to the variant BDNF Met allele, the variant FAAH A allele enhances cued fear extinction learning in both the polymorphic mouse model and in humans (55). Because both the BDNF Val66Met and FAAH C385A polymorphisms affect cued fear extinction learning and assort independently in human populations, it is likely that association studies of the effects of either polymorphism are confounded by the effects of other. We are currently crossing BDNF^{Met} mice with FAHH^A mice to allow for systematic study of the interactions of these

polymorphisms that can then inform human association testing of interactions between these polymorphisms.

Implementation

Candidate gene studies of behavior have fallen out of favor because of high rates of non-replication in which very compelling initial associations display no effect in subsequent meta-analyses. A major source of false positives in candidate gene studies is due to *post hoc* analysis leading to the testing of multiple genotype-phenotype hypotheses (56). This point is borne out by the markedly divergent results of two large-scale meta-analyses of the same association, the serotonin transporter-linked polymorphic region (5HTTLPR) and gene $\times$ stress interactions in depression (57, 58). An initial meta-analysis that subjected data from a number of studies to a single analytic framework found no interaction effect (57) but a subsequent meta-analysis that included many more studies but also relied on each study's independent analysis by combining studies at the level of their significance tests identified a highly significant association (58). The additional studies considered in the latter analysis included very divergent definitions of exposure from common stressful life events to sexual or physical trauma or different medical conditions. Additionally, there was evidence of *post hoc* hypothesis testing as a number of studies reported results of multiple analyses without statistical correction or identified effects on particular subtypes of trauma or gender specific effects which may be valid but were not presented or justified as *a priori* hypotheses. The ease of performing *post hoc* analyses of complex association data sets and difficulties in performing replication analyses that actually test the initial hypothesis mean that statistical associations alone cannot validate genotype-phenotype relationships; other forms of validation are needed.

The vertically integrated translational approach provides for validation of polymorphic effects that do not involve the inevitable complexities of replication of human association studies. Mice recapitulating human polymorphisms provide construct validity for the effects of polymorphisms on behavior so long as the cross-species phenotypes are equivalent (14). Using this approach, we have identified parallel effects in mice and humans of the BDNF Val66Met on cued fear extinction learning which has implications for human anxiety disorders; we have also leveraged the strengths of the polymorphic mouse model to identify complexities in the effects of BDNF Val66Met that may explain why it is not strongly associated with human anxiety disorders. Nonetheless, the BDNF Val66Met polymorphic mouse model allows for extensive hypothesis testing that can guide and constrain the design and analysis of rigorous prospective human association studies for the identification of clinically relevant phenotypes other than diagnosis where the effect of a polymorphism is large enough to guide clinical practice.

Treatment Response

An area where genetic biomarkers may have great value is in targeting particular treatments to the individuals most responsive to them during their most responsive window of sensitivity (36). For instance, there is a large literature suggesting that BDNF is central to the mechanism of action of the selective serotonin reuptake inhibitors (SSRIs) (59, 60).

Consistent with the BDNF^{Met} allele's effects on activity-dependent secretion, BDNF^{Met/Met} mice are less responsive to the SSRI fluoxetine than BDNF^{Val/Val} mice, however, they are equally responsive to the norepinephrine transporter-specific drug desipramine suggesting that BDNF Val66Met could be used as a biomarker guiding the choice of pharmacologic therapy for depression and anxiety disorders (48).

Perhaps the most analogous application of BDNF Val66Met as a clinical biomarker is in exposure-based treatment of post-traumatic stress disorder (PTSD) and other anxiety disorders. The principles of fear extinction learning are at the core of exposure therapies for PTSD that seek to identify fearful symptom triggers and present them repeatedly in the absence of traumatic events (61, 62). Because the BDNF Met allele is associated with impaired cued extinction learning human carriers of that allele may be less responsive to exposure therapy as has been found (63). Current protocols for exposure therapy in PTSD call for a standard number of sessions but it may be that the necessary duration or intensity of treatment varies as a function of the BDNF Val66Met polymorphism.

Generalization

The vertically integrated translational approach is labor and resource intensive and must be applied to polymorphisms individually in contrast to whole genome association approaches. Nonetheless, the potential value of genetic biomarkers in clinical psychiatry is so great that such investment is warranted. Mouse models of additional human polymorphisms can be developed and phenotypic testing can be scaled up to encompass additional behavioral domains across development and environmental exposures.

A knock-in mouse has been developed to recapitulate a common nonsynonymous polymorphism in the mu opioid receptor (OPRM1), which has relevance for therapeutic opioid response as well as drug dependence (64–67) and likely other mu opioid-related behaviors. Additional common amino acid altering polymorphisms have been described in the catechol-O-methyl transferase gene (COMT) and dopamine D4 receptor (DRD4) and inconsistently associated with human behavioral phenotypes. These polymorphisms can and should be recapitulated in mice to clarify their effects on neural function and behavior (68). Recent technologies for genome editing should make the production of such mice even more efficient than knock-in techniques (69–71) and offer the potential to introduce multiple mutations simultaneously for studies of gene × gene interactions or haplotype effects (72).

Modeling regulatory polymorphisms that alter the expression of a gene rather than the structure of the gene product is more challenging because the sequence/function relationship is much less clear for non-protein coding polymorphisms and the sequences in which they occur are much less conserved meaning the introduction of human variants into the mouse gene may not recapitulate its effects in the human genome. However, the motivation for creating such mice is very high because most inter-individual variation is believed to be due to such polymorphisms (73) and virtually all significantly associated polymorphisms in behavioral GWAS occur in non-coding sequence (2). Application of a vertically integrated translational approach to genomic regions identified in GWAS studies can be expected to identify phenotypes that display larger effect sizes of disorder-associated polymorphisms

that will elucidate biological mechanisms underlying their associations to disorders and lead to clinically useful biomarkers. Recent advances in BAC transgenic technologies allow insertion of large portions of the human genome containing entire genes and their regulatory elements into mice. The two alleles of GWAS-identified regions and surrounding genomic material can be introduced onto a null mouse background and compared for their effects on gene expression, behavior, and a variety of additional intermediate phenotypes.

Phenotypic analysis of human polymorphic mice can also be scaled up to identify the full array of a polymorphism's behavioral effects. BDNF is broadly expressed in the brain where it is involved in a range of experience-related learning modalities. Thus the effects of BDNF Val66Met are likely pleiotropic impacting multiple behavioral domains that can interact with one another in contributing to clinically relevant phenotypes. Phenomic analysis of polymorphic mouse models can screen across a large range of neural, physiological, and behavioral domains to identify the full array of a polymorphism's phenotypic effects and their interactions with pathology-relevant factors (74, 75). In instances where complex human behavior is not directly relatable to mouse behavior (i.e., anxiety-like behavior versus self report anxiety inventories) vertical integration can be used to relate species-specific findings at one level of analysis to validated equivalent phenotypes such as conditioned fear responses at adjacent levels of analysis. Developing common polymorphisms as clinical biomarkers will always require association testing in human samples but the more these studies can be informed by characterization of a polymorphism's effects in mouse models, the more efficient and reliable they will be.

Lessons from the BDNF Val66Met mouse make it clear that a polymorphism can have effects on basic cellular processes that lead very quickly to complex and potentially paradoxical effects on higher level phenotypes. In this context, it is not surprising that there are no major genetic risk factors for psychiatric disorders. Emphasis on behavioral domains as opposed to psychiatric disorders facilitates identification of a polymorphism's effects, the way those effects vary as a function of development, experience, and co-occurring genetic factors, and the way they can confer risk for and protection from psychopathology. Larger samples in behavioral GWAS can enhance power to identify smaller and smaller contributions of common polymorphisms to disease risk but they can't be expected to identify any novel risk factors that have a clear enough risk effect to guide clinical practice. To make progress in personalized psychiatry the biology of polymorphisms must be incrementally matched to the biology of behavior, a process facilitated by the RDoC framework.

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Figure 1. Levels of analysis in behavioral domains
The vertically integrated translational approach (A) emphasizes biological effects of polymorphisms across levels of analysis and the modifying effects of development and experience using knock-in mouse model systems. Translating to humans is facilitated by the similar organization of the RDoC framework (B) across levels of analysis.