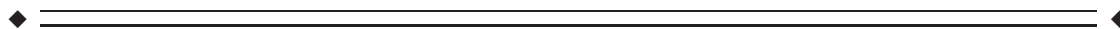


Imaging Genetics and Development: Challenges and Promises

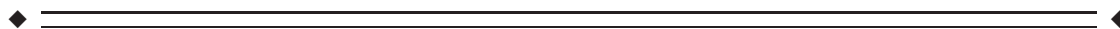
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Abstract: Excitement with the publication of the human genome has served as catalyst for scientists to uncover the functions of specific genes. The main avenues for understanding gene function have been in behavioral genetics on one end and on the other end, molecular mouse models. Attempts to bridge these approaches have used brain imaging to conveniently link anatomical abnormalities seen in knockout/transgenic mouse models and abnormal patterns of brain activity seen in humans. Although a convenient approach, this article provides examples of challenges for imaging genetics, its application to developmental questions, and promises for future directions. Attempts to link genes, brain, and behavior using behavioral genetics, imaging genetics, and mouse models of behavior are described. Each of these approaches alone, provide limited information on gene function in complex human behavior, but together, they are forming bridges between animal models and human psychiatric disorders. *Hum Brain Mapp* 31:838–851, 2010. © 2010 Wiley-Liss, Inc.

Key words: imaging; genetics; development



INTRODUCTION

The publication of the human genome has set the stage for new opportunities to begin to understand gene function. The two main avenues taken to accomplish this feat include human behavioral or psychiatric genetics, where genes are linked to complex behaviors or clinical diagnosis and mouse molecular genetics, where genes can be linked to alterations in cell physiology and gene expression. The

difficulty in reconciling these two avenues of research has been referred to as a “translational bottleneck” [Hyman and Fenton, 2003]. As information accumulates on the human and mouse sides of the bottleneck, there is growing demand for, and hence much opportunity, for expertise in levels of analysis, that lie between purely behavioral and purely cell biological levels of analysis. Attempts to bridge these behavioral and molecular approaches have used brain imaging to conveniently link anatomical abnormalities seen in knockout/transgenic mouse models and abnormal patterns of brain activity seen in certain patient populations. Although a convenient and rapidly growing approach (see Fig. 1), this article provides examples of challenges for imaging genetics, its application to developmental questions, and promises for future directions.

Technological advances in brain imaging techniques such as blood oxygenation level-dependent functional magnetic resonance imaging (fMRI), have allowed researchers to noninvasively assay brain function within discrete brain circuits supporting specific cognitive and emotional processes. Genetic influences on behavior are mediated by the impact of their molecular and cellular effects on brain development and function. As such,

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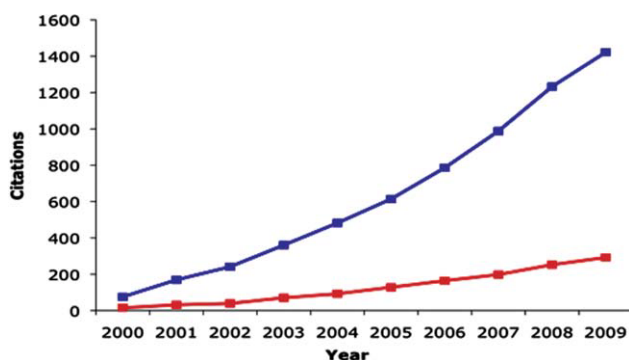


Figure 1.

Cumulative citations for neuroimaging genetic papers (in blue) and developmental neuroimaging genetic papers (in red) over the past decade.

genetic imagers have argued that the functional effects of candidate variants on brain structure and function may be more readily measurable than the cognitive and emotional processes supported by these substrates, and thus, functional polymorphisms in genes weakly related to behaviors or psychiatric syndromes may be more strongly related to the function of specific neural systems [Casey and Durston, 2006; Fossella and Casey, 2006]. Currently, 25,000 genes have been identified in the human and mouse genomes. Each of these genes can contain up to 100 polymorphic sites, some of which can impact the expression of those genes in a region-dependent manner. Thus, in an individual with a brain that can be measured at the 1–3 mm³ voxel level and whose dynamic networks are capable of an infinite variety of computational states, a one-to-one statistical mapping of genetic variants to brain function, is challenging, but may be a convenient initial step.

The challenge of linking genes, brain, and behavior becomes even more daunting when we consider gene function in a dynamically changing system, such as the developing brain. In the excitement to use the human genome project to uncover the functions of specific genes, researchers often ignore a fundamental factor: the gradual process of ontogenetic development. Claims about gene/behavior relations are typically based on a phenotypic end-state and grounded in the neuropsychological tradition of studying adults whose brains were fully and normally developed until a brain insult later in life. The developing brain, of course, is different in that brain regions and circuitry are not specialized at birth. Many years are required for the specialization of neural networks as a result of the complex interaction with the environment in gene expression and the resulting phenotype. Karmiloff-Smith [2006] has argued that “because ontogenetic development and timing play such important roles in development, a tiny impairment in the start state of the brain of a child with a genetic disorder may affect several brain regions, some more profoundly and others more

subtly, giving rise in the phenotypic end-state to what appears to be a domain-specific outcome.” This neuroconstructivist perspective is an important one to keep in mind when interpreting simple gene-behavior associations in typical development and in developmental disorders.

The objective of this article is to provide a framework whereby future genetic studies can be constrained and evaluated for validity and relevance with respect to existing data with a converging methods approach. These methods include the combined use of behavioral and imaging genetics together with molecular mouse models of gene function. The consideration of genotypes as dynamic in different environmental and developmental conditions or contexts, rather than static, is underscored.

Genetic Terms and Definitions

Definition of genetic terms may be useful to a predominantly imaging audience in setting the framework for discussing challenges and potentials of genetic imaging studies, beginning with the coined phrase of imaging genetics (see Table I). Imaging Genetics refers to the use of brain imaging to evaluate phenotypic variation in brain morphometry and physiology as a function of genotypic variation [Hariri et al., 2006]. Phenotypic variation or a phenotype is an observable trait or characteristic such as behavior, development, morphology, or physiology whereas a genotype is the inherited genetic code. Not all individuals with the same genotype behave in the same way because behavior is modified by environmental and developmental contexts. Thus, we consider a phenotype to result from the genotype, environment, and development. As such genetic effects are not static, but rather dynamic in changing environments and developmental periods.

Most genetic studies have been designed, not from a whole-genome perspective [Fossella and Casey, 2006], but rather from having selected from the genome, a candidate gene. The candidate gene study design is appropriate when substantial amounts of converging evidence suggest that a particular gene contributes to heritable variation in behavior or risk for psychopathology. Heritability is the extent to which variation in a trait among members of a population is determined by inherited genetic variation.

For candidate gene association studies, the individual nucleotides that vary from one individual to another are the most important attribute. These variable sites arise from errors in genome replication, which in humans, are rare, but occurs often enough in our genome of three billion nucleotides, so that approximately 100 base-pair changes accumulate per generation per genome. Most of these minor base pair changes are lost over long time scales as generations come and go. However, some of these changes continue to be passed on and even spread across populations. These ancient deoxyribonucleic acid (DNA) changes, known as polymorphisms or single nucleotide polymorphisms (SNPs) are presently found in

TABLE I. Definition of genetic terms

Terms	Definition
Heritability	The extent to which variation in a trait (such as cognitive performance) among members of a population is determined by inherited genetic variation.
Genome	The aggregate genetic information of an organism comprised of chromosomal and mitochondrial DNA in eukaryotes. The human genome consists of ~3.2 billion nucleotides.
Gene	The functional unit of DNA. Generally refers to protein-coding units but can also include non-protein coding regulatory units.
Locus	A specific location in the genome. Can be a specific nucleotide or a larger region such a gene or cytogenetic band.
Polymorphism	Heritable trait that occurs in multiple forms in populations. Usually refers to variants in DNA but can refer to phenotypic traits. Traditionally refers to genetic variant that occurs in more than 1% of a population with mutation used for more rare variants.
Allele	One form of a polymorphic trait.
Genotype	The composition of a particular region of the genome. Generally refers to the combination of the maternal and paternal alleles of a polymorphism.
Phenotype	An observable trait that results from the combined effects of genetic and environmental factors.
Endophenotype	An endpoint for genetic association or linkage studies that is between the genome and clinically-defined phenotypes.
Haplotype	A series of alleles that occur on the same chromosome. Usually inferred statistically from genotype data at multiple loci on a chromosome but can be determined through molecular cloning.
Linkage disequilibrium	A significant deviation from random assortment between the alleles of polymorphisms at two or more loci.
SNP	Single nucleotide polymorphism. Results from a point mutation during DNA replication which is almost always biallelic; the most common category of polymorphism.
VNTR	Variable number of tandem repeats polymorphism. Also referred to as a length polymorphism. Alleles of a VNTR differ by the number of identical or similar repeat elements.
GWAS	Genome wide association study. A genetic mapping study is one in which the entire genome is interrogated for significant association between individual polymorphisms and a target phenotype. Currently multiple genomic technologies allow screening the entire human genome at 500,000–1,000,000 SNP's using a single "chip" for each subject.

slightly more than 1 out of every 1,000 nucleotides in the genome. These changes then lead to an enormous amount of DNA sequence variability across human populations.

Mouse models have been developed to relate changes in gene expression to behavior. One of the most common approaches is to look at relationships between gene dosage and behavior. By breeding knockout mice (–/–) with wild-type mice (+/+), heterozygote animals (–/+) with one working copy of the gene of interest are produced. Often, such changes in the amount of gene activity lead to variations from the wild type. Knockout mice contain the same, artificially introduced mutation in every cell, abolishing the activity of a preselected gene. The resulting mutant phenotype (e.g., behavior or anatomy) may provide some indication of the gene's role in the mouse, and ultimately, in humans. Knockout mice are produced by a technique called gene targeting that involves the replacement of one gene sequence, with a related sequence that has been modified to contain a mutation.

A transgenic mouse contains additional, artificially-introduced genetic material in every cell that can lead to a gain of function. The extra genetic material is often described as foreign DNA, but it can come from any source, including another mouse. A knock-in involves the insertion of a protein coding DNA sequence at a particular

locus in an organism's genome. The difference between knock-in technology and transgenic technology is that a knock-in involves a gene inserted into a specific locus, and is a "targeted" insertion. Genes are silenced or "knocked down" by short pieces of double-stranded RNA. Viral vectors can be used to insert interfering RNA into stem cells or neurons to modify the activity of genes in selected tissues.

Challenges for Genetic Studies

With a definition of terms, challenges of and potential solutions for future genetic studies can more easily be discussed. The challenges include imprecise phenotypes, lack of known functionality of genetic variation, underpowered studies, and stratification effects.

Imprecise phenotype

Although the major psychiatric and developmental disorders display a substantial heritable component, very few genetic associations to these phenotypes have proven to be reliable. The majority of allelic associations with neurobehavioral phenotypes are not consistently replicated. For instance, a common variable number of tandem repeats

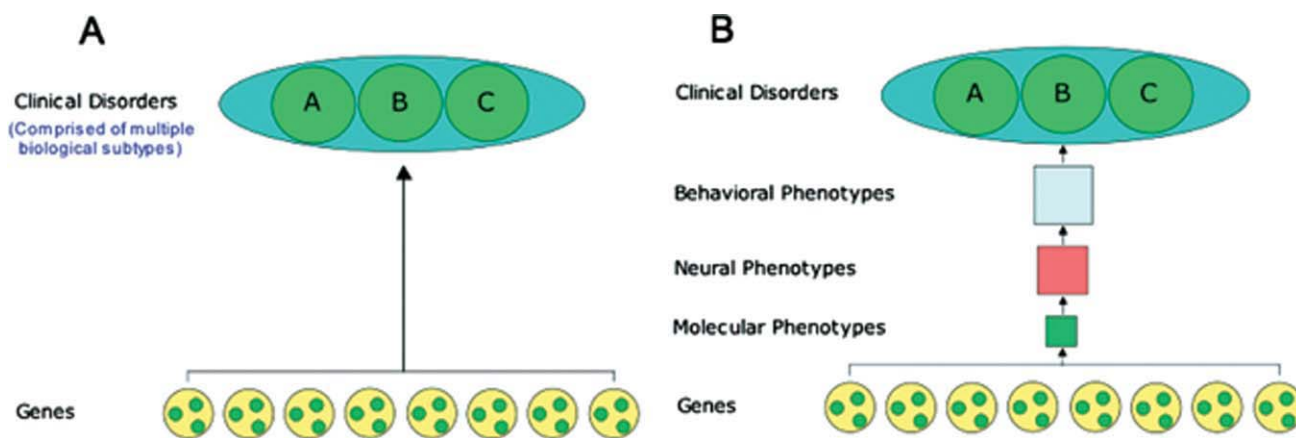


Figure 2. Phenomenological (A) versus biological (B) approach to behavioral genetics [Casey et al., 2009].

(VNTR) polymorphism in the gene for the serotonin transporter (the serotonin transporter-linked polymorphic region or 5-HTTLPR) has been heavily studied and associated with anxious personality traits, amygdala response to emotional stimuli, and susceptibility to depression in the face of environmental stressors. However, a recent meta-analysis of 24 studies (comprised of more than 3,000 subjects) of the association between the serotonin transporter-linked polymorphic region and anxiety-related personality traits found “that the effect, if present, is small [Munafò et al., 2005].” A more recent metaanalysis of the role of the 5-HTTLPR in gene–environment interactions for risk of depression has reported no evidence of association [Risch et al., 2009]. Genetic risk is likely distributed across many allelic variants in psychiatric illness, thus the effect size of any single risk factor for disorders will be small and difficult to reliably identify.

Endophenotypes are heritable, distinct endpoints in biology such as anatomy, biochemistry, and behavior that reflect discrete components of pathophysiologic processes. These refined phenotypes have been proposed as attractive targets for human genetic studies because they are less biologically complex than disease phenotypes and can be more objectively and reliably ascertained than categorical disorders. These attributes suggest that genetic correlation with a specific endophenotype should prove more reliable than associations with disease phenotype, but to date, this is often not the case.

To ensure the utility of endophenotypes, candidate gene studies must be focused on validated endophenotypes that are comprised of basic biological processes, relate more closely to the biology of the candidate gene, and are more precisely measured than categorical disorder phenotypes (see Fig. 2). Specifically, endophenotypes must fulfill several criteria: (1) reflect a biological process that is a component of the more complex disorder phenotype; (2) be more biologically simple than the disorder phenotype to ensure that the effect size of any particular risk factor is relatively

large; and (3) the biology of the endophenotype must be understood well enough that it can be related to specific candidate risk factors including genetic, environmental, and developmental ones. These criteria are particularly important for functional imaging genetic studies where the findings are dependent upon validity of the behavioral activation paradigm.

Functionality over replication of genetic effects

It is useful when investigating a candidate gene to understand how to classify variable sites according to functionality and frequency. Some variable sites lie within a gene and affect the structure of the protein (e.g., brain derived neurotrophic factor or BDNF). Other sites lie outside the coding sequence, and yet are able to influence the amount of expression of the encoded protein (e.g., 5-HTTLPR). Still other sites lie inside or outside of a protein-coding region, and yet, have no effect at all on the encoded protein structure or expression.

Unfortunately, many gene association studies are carried out using such nonfunctional polymorphic sites. A case in point presented by Fossella et al. [2006] provides a cautionary example of how simple gene, brain and behavior associations from genetic imaging studies can be over interpreted. They examined a well-studied genetic polymorphism referred to as the TaqIA restriction fragment length polymorphism (RFLP), which resides downstream from the dopamine D2 receptor (DRD2) gene. A number of electrophysiological and PET imaging studies have been performed on genetic variants of DRD2. Only recently has it been shown that this polymorphism lies 10 kb downstream of the coding region of DRD2 and occurs in Exon 8 of the novel kinase gene, named ankyrin repeat and kinase domain containing 1 (ANKK1) gene, resulting in a glutamate-to-lysine (E713K) substitution within the 11th ankyrin repeat. As yet, expression of the ANKK1 gene has not been detected in the developing or adult mammalian

brain. Thus, it is surprising, that so many gene association studies have been reported for a polymorphism for this gene. Fossella et al. show an association between this genetic variant and patterns of brain activity during attentional control studies.

Clearly future studies will be needed to understand how a polymorphism that lies downstream of the coding region of gene and in a gene as yet, undetectable in the developing and adult mammalian brain, can impact brain and behavior. One explanation for the existing literature and their finding would be that the TaqIA polymorphism in ANKK1 is in linkage disequilibrium or correlation with upstream polymorphisms in DRD2 and lies within a haplotype block, a series of alleles at multiple loci along a chromosome that spans the overlap between the two genes. This example, however, underscores the caution that should be taken in making simple associations between brain activity and a polymorphism in terms of the gene-behavior association. The need to examine functionality, despite replication, is thus warranted.

Overestimation of genotypic effects

One-to-one statistical mappings of genetic variants to brain function or behavior is limited in the context of thousands of genes with up to 100 variable sites within each of these genes in an individual with a brain whose dynamic networks are capable of an infinite variety of computational states. The problems in gaining sufficient power or effect sizes to detect genotypic effects on behavior or on risk for psychopathology would seem insurmountable. Nonetheless, initial studies have suggested as much as 20% of phenotypic variance could be explained by a single genotype. Hariri et al. provide a cautionary note in this regard.

In 2002, Hariri et al. used BOLD fMRI to evaluate the impact of the 5-HTTLPR on the physiological response of the human amygdala to biologically salient environmental stimuli. In this initial study, healthy adult volunteers from two independent cohorts ($n = 14$ in each sample, 7 per group) were divided into equal groups based on their 5-HTTLPR genotype, with the groups matched for age, gender, IQ, and task performance. During BOLD fMRI scanning, the subjects performed a simple perceptual processing task involving the matching of fearful and angry human facial expressions [Hariri et al., 2000]. The fMRI data showed that subjects carrying the low expressing 5-HTTLPR S allele exhibited significantly increased amygdala activity, as indexed by the BOLD signal, in comparison with subjects homozygous for the L allele [Hariri et al., 2002]. In fact, the difference in amygdala activity between 5-HTTLPR genotype groups in this study was nearly fivefold, accounting for 20% of the total variance in the amygdala response during this experiment, an effect size greater than any previously reported behavioral associations. This initial finding suggested that the increased anxiety and fearfulness associated with individuals pos-

sessing the 5-HTTLPR S allele may reflect the hyperresponsiveness of their amygdala to relevant environmental stimuli.

A subsequent metaanalysis by Hariri and coworkers [Munafò et al., 2008] showed that the magnitude of the reported associations between amygdala activation and the serotonin transporter gene linked polymorphic region (5-HTTLPR) was less substantial. Although their analysis provided support for the association of the 5-HTTLPR polymorphism and amygdala activation, it also suggested that most studies lack sufficient statistical power to make such claims. Increasing the sample sizes of future imaging genetics studies will allow a more accurate characterization of any true effect size and afford adequate power to examine the impact of multiple polymorphisms that likely work in concert to affect gene function and, in turn, bias neural processes mediating emotional related behavior.

Stratification effects

One of the most critical issues in designing candidate gene association studies is a consideration of the frequency of the polymorphic alleles under investigation. Allele frequencies can be extremely rare and vary by population. For example, the frequency of the E4 allele of Apolipoprotein E (APOE) ranges from 5% in Taiwan and Sardinia to 40% in Pygmies [Corbo and Scacchi, 1999; Siest et al., 1995]. It is desirable to choose a polymorphism whose alternate alleles exist at relatively high frequencies within and across populations. Choosing an allele that is present in only 5% of the population creates practical difficulties, from a recruitment perspective, but also can be problematic to interpret. Because allele frequencies can vary by population, it is important to monitor ancestral heritage and population admixture in study samples to avoid spurious associations between a polymorphism and a trait that might simply relate to different prevalence of that polymorphism in different ethnic groups in the sample.

Ethnic heterogeneity is an important concern in genetic association studies where subjects are categorized by phenotype and associations are then identified through statistical differences in genotype distributions. In such a case, phenotypically neutral polymorphisms may appear associated due to different allele frequencies in ethnic groups that are unevenly represented in cases and controls or across a quantitative variable. The problem of population stratification is analogous to that encountered when there is uneven representation of genders across genotype groups in a sample which may result in apparent differences by genotype, that are actually due to the effects of gender. Uneven representation of ethnicities can have the same effect (effects due to ethnicity rather than genotype).

For example, Keen-Kim et al. [2006] recently suggested that the association between a rare non-coding variant in *Slitrk1* with Tourette Syndrome may be due to population stratification effects. The association was reported the year before [Abelson et al., 2005] because it was seen in 2 out

of 348 Tourette Syndrome chromosomes (0.6%) but not once in a sample of nearly 5,000 control chromosomes with a significance of $P = 0.0056$. The following year, Keen-Kim et al. showed that the prevalence of this mutation was higher in Ashkenazi Jews than other ethnic groups (0.7% in patient cases, 0.8% in parents of cases, and 0.2% in controls). Using a family-based design they found that when a parent of a child with Tourette Syndrome possessed the risk allele they were almost as likely to pass on the nonrisk allele (two of five transmissions) to their affected child as the risk allele (three of five transmissions) meaning there was no association with Tourette Syndrome in that study. Further, they suggest that the previous report had more Ashkenazi Jews in patient cases than controls. This example demonstrates the potential risks of population stratification and also points to a benefit of family-based designs in that they automatically control for population stratification.

Stratification in population-based studies can be identified and adjusted for using genotype information from unlinked ancestry informative markers. Other than family-based designs, genetic studies where the design employed is to select subjects based on genotype—rather than phenotype—and then test for phenotypic differences, are not sensitive to classic stratification error. Moreover matching genotypic groups on ethnicity, as well as other factors such as gender and age further prevent spurious results. Yet, few genetic imaging studies have matched on ethnic diversity in their samples or had sufficient power to statistically control for such effects.

Similar concerns arise in genetically modified mice. Specifically, it has been shown that there are strain differences in the efficacy of standard animal behavior protocols [Balogh and Wehner, 2003; Bolivar et al., 2001; Brinks et al., 2008; Waddell et al., 2004]. These differences may be due to strain differences in pain sensitivity, learning, or other variables, but genetic background effects may lead to spurious results. Studies that do not account for ethnic variability in humans or genetic background in mice cannot make strong about genotypic effects.

New Directions for Genetic Research

Attempts to bridge behavioral and molecular genetics through brain imaging has provided a convenient link between abnormal patterns of brain activity seen in certain patient populations and anatomical abnormalities seen in knockout/transgenic mouse models. Genetically modified mice provide useful model systems for testing the role of candidate genes in behavior and imaging studies provide neuroanatomical evidence to validate cross-species translation. The extent to which such genetic manipulations in the mouse and the resulting phenotype can be translated across species, from mouse to human, is beginning to be assessed more directly. A new direction for genetic research is to exploit behavioral paradigms being used

with mice, and adapt them for use with humans in the imaging environment. Such an approach provides a direct move toward translating the function of genes in the context of human behavior. In highlighting the promise of imaging genetics, we provide a concrete example of how parallel human and mouse genetic studies can address many of the challenges of genetic studies.

We describe research that focuses on the impact of a polymorphism in the brain-derived neurotrophic factor (BDNF) gene. BDNF is a member of the neurotrophins a unique family of polypeptide growth factors that influence differentiation and survival of neurons in the developing nervous system. In adults, BDNF is important in regulating synaptic plasticity and connectivity in the brain. Recently, a common single nucleotide polymorphism in the human BDNF gene, resulting in a valine (Met) to methionine (Met) substitution in the prodomain of the peptide (Val66Met), has been shown to lead to memory impairment and risk for psychiatric illness. An understanding of how this naturally occurring polymorphism affects behavior and neuroanatomy is an important first step in linking genetic alterations in the neurotrophin system to definable biological outcomes in humans.

Functionality

BDNF Val66Met is common in human populations with a prevalence of 20–30% [Shimizu et al., 2004] and because it codes for an amino acid substitution has a high likelihood of affecting the biological properties of the BDNF peptide. Indeed, it has been demonstrated that the Met allele leads to reduced activity-dependent secretion of BDNF from hippocampal neurons in culture [Egan et al., 2003; see Fig. 3A]. This effect is due to a reduced affinity of the variant (Met) BDNF for sortilin an intracellular trafficking molecule [Chen et al., 2005].

The BDNF_{Met} allele has been associated with impairment in select forms of learning and memory [Egan et al., 2003] and susceptibility to psychiatric disorders [Neves-Pereira et al., 2002; Ribases et al., 2003, 2004; Sen et al., 2003; Sklar et al., 2002]. Given the established role of BDNF in promoting learning and memory [Desai et al., 1999; Korte et al., 1995; Patterson et al., 1996], it is likely that impaired BDNF secretion, due to expression of the BDNF_{Met} allele, may have pleiotropic effects (e.g., a single gene impacting multiple phenotypic traits) in a variety of BDNF-dependent processes.

Recently, a unique inbred genetic knock-in mouse strain was developed [Chen et al., 2006] that expresses the variant BDNF allele to recapitulate the specific phenotypic properties of the human polymorphism in vivo. All inbred mouse strains contain a Valine 66 residue in BDNF. The BDNF_{Met} mouse is a transgenic knock-in of a methionine residue at this position that mimics the human polymorphism. This model is unique in that it is the only animal model that fully recapitulates the established phenotypic effects of a common human polymorphism expressed in

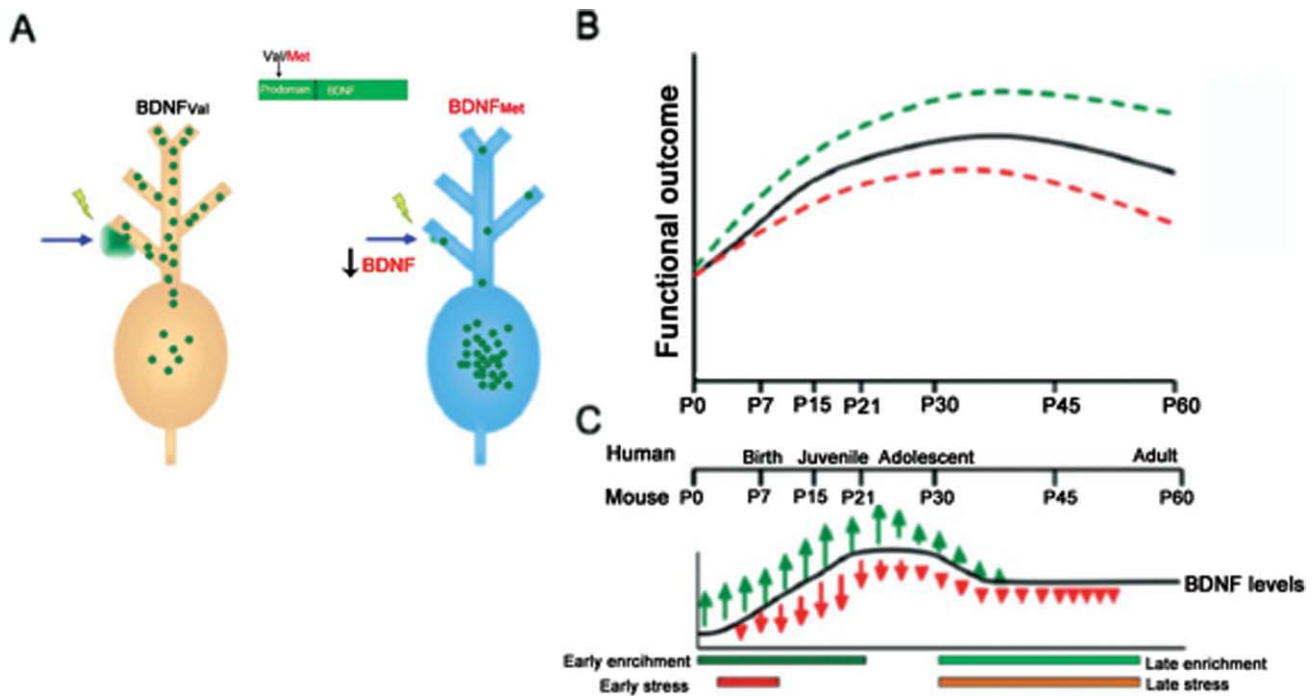


Figure 3.

Model of impact of BDNF across development. (A) The genetic variant BDNF Val66Met leads to an amino acid substitution in the BDNF prodomain (Val to Met at position 66) that results in decreased activity-dependent secretion of BDNF from neurons. Thus, this trafficking defect leads to a decrease in the availability of biologically active BDNF. (B) This model predicts that BDNF levels will have different functional consequences across development. As the variant BDNF (Val66Met) has decreased secretion throughout this period, we anticipate that there will be functional deficits, evident even in childhood, but (C) these deficits

will become diminished by adolescence when BDNF levels peak. In addition, BDNF levels will be modulated by environmental stressors. Carriers of the Met allele will have decreased secretion and less neurotrophic support for plasticity and change, whereas Val allele carriers will show greater change, including both positive and negative effects on hippocampal structure and function, but potentially greater neurotrophic support for plasticity and resilience once a stressor is removed [Casey et al., 2009].

the brain. Unlike traditional transgenic mouse models which alter the quantitative expression of targeted genes throughout development or at selected times, this model introduces the single polymorphic amino acid into the murine genome, thereby providing a precise physiologic model of the polymorphic effect of human BDNF Val66-Met. Such testable mechanistic approaches cannot be applied to other frequent polymorphisms related to behavior. For example, the 5-HTTLPR is postulated to be a regulatory polymorphism but its activity has not been consistently identified. Furthermore, the 5-HTTLPR genetic alteration cannot be fully recapitulated in transgenic mice because the regulatory element that is polymorphic in humans does not exist in nonprimate species [Lesch et al., 1997]. The mouse model of BDNF Val66Met has been validated by studies that have found that animals carrying the Met allele manifested phenotypes (hippocampal size and hippocampal-dependent learning) that matched differences in humans expressing the BDNF_{Met} allele, as compared to individuals with the Val/Val genotype [Chen et al., 2006].

Constrained model of genotypic effects

We have developed a model with testable hypotheses for how gene- or environment-related alterations in BDNF levels will have a significant impact on behavioral and neuroanatomic changes that vary with age. Such an approach can move the field away from simplistic notions of risk alleles, recognizing that an allele may be a risk factor during one period of development and a protective factor during another. Because the variant BDNF_{Met} allele shows decreased regulated secretion, we predict that there will be functional deficits or biases in BDNF dependent forms of learning when physiologic levels of BDNF are low (Fig. 3B,C). However, when BDNF levels peak [e.g., during adolescence, Katoh-Semba et al., 1997] this trafficking deficit may yield only minor differences in these measures in stable environments. During periods of increased physiologic expression of BDNF, the lower secretion conferred by the BDNF_{Met} allele may actually be protective and lead to risk for individuals in adolescence without

this allele [e.g., BDNF_{Val/Val} in substance abuse; see meta-analysis by Gratacos et al., 2007].

This model also encompasses nongenetic factors. Early environmental risk factors including physiological or psychological stress result in decreased neurotrophic support to certain BDNF-rich regions like the hippocampus [Smith et al., 1995]. The additional deficit in neurotrophic support in carriers of the Met allele may result in increased vulnerability to stress, and thus put them at greater risk for psychiatric disorders (e.g., anxiety, depression, and schizophrenia) that have been associated with stress. During other developmental windows when BDNF levels are high, carriers of the Val allele may be at greater risk for other psychiatric disorders given that stress can increase BDNF in the amygdala and ventral striatum, areas implicated in bipolar disorder [e.g., Geller et al., 2004] and substance abuse [Liu, 2005; Matsushita et al., 2004]. Thus, it is important to consider changes in the level of BDNF across development and the opposing effects that stress has on BDNF levels in brain regions that support very different forms of learning.

Promise for development

This model distinguishes itself by underscoring the importance of development in examination of genetic effects on behavior. First, BDNF is a molecule that is essential for developmental processes including, neuronal plasticity [Barde et al., 1987; Bramham and Messaoudi, 2005; Leibrock et al., 1989; Liao et al., 2007; Lu, 2003; Rattiner et al., 2005; Thoenen, 1995; Tongiorgi et al., 2006; Yamamoto and Hanamura, 2005]; regulation of both short-term synaptic function and long-term activity-dependent synaptic consolidation [Barco et al., 2005; Black, 1999; Katz and Shatz, 1996; Lohof et al., 1993; Lu and Chow, 1999; McAllister et al., 1999; Patterson et al., 1996; Poo, 2001; Thoenen, 1995]; potentiation of synaptic transmission [Kang and Schuman, 1995; Levine et al., 1995; Lohof et al., 1993]; modulation of long-term potentiation (LTP) *in vitro* and *in vivo* [Korte et al., 1995; Messaoudi et al., 2002; Patterson et al., 1996]; and induction of morphological changes in dendritic spines [Gomes et al., 2006; McAllister et al., 1995]. Thus, BDNF has a role in (1) synaptic plasticity; (2) inducing changes in synaptic morphology; and (3) mediating cell survival and cell proliferation during development. These functions serve to underscore the importance of considering BDNF in any neurodevelopmental disorder of learning.

Second, BDNF availability changes across development (Fig. 1B,C). Although these changes have been shown to differ by region [Hofer et al., 1990; Katoh-Semba et al., 1997; Maisonpierre et al., 1990; Webster et al., 2006], rodent studies suggest that changes in BDNF levels across development approximate an inverted U-shape function [Ivanova and Beyer, 2001; Silhol et al., 2005]. In humans, BDNF mRNA levels in cortical regions increase approximately one-third from infancy to adulthood. They are rela-

tively low during infancy and childhood, peak during young adulthood, and are maintained at a constant level throughout adulthood. The increase in BDNF at this critical time in human development may have important implications for the etiology and treatment of the severe mental disorders that tend to present during this time [Webster et al., 2002]. The BDNF Val66Met mouse model is able to recapitulate this regional and temporal complexity as the single nucleotide polymorphism occurs in the protein coding sequence and leaves the regulatory elements of the gene unaffected, thus maintaining the normal regional and temporal expression of this gene.

Precision of (endo)phenotype

Genetically influenced forms of learning that lie at the core of neurodevelopmental disorders include those that capture the difficulties some individuals have in: (1) recognizing signals of safety or danger (cued learning); and (2) learning to adjust behavior when actual associations no longer exist (extinction). Unlike disease states, the tasks that examine these types of learning can be assessed equivalently in typically and atypically developing humans and mice. Although most studies have emphasized the role of BDNF in learning and memory processes supported by the hippocampus, high levels of BDNF mRNA and protein are expressed in the amygdala [Conner et al., 1997; Yan et al., 1997] suggesting another important potential site for BDNF-mediated plasticity. In studies focusing on the hippocampus, BDNF has been shown to facilitate long term potentiation (LTP) at hippocampal CA1 synapses [Figurov et al., 1996; Korte et al., 1995; Patterson et al., 1996] and BDNF mRNA levels have been found to increase following induction of LTP [Barco et al., 2005; Bramham et al., 1996; Castren et al., 1993; Pang and Lu, 2004; Patterson et al., 1992, 1996; Radecki et al., 2005; Zakharenko et al., 2003]. The activity-dependent secretion of BDNF enhances the molecular mechanisms of synaptic restructuring needed to support LTP. We have shown [Chen et al., 2005] that the Val66Met mutation in the BDNF gene leads to a decrease in this regulated secretion of BDNF, suggesting that carriers of this allele would have compromised BDNF-dependent synaptic modulation. In humans, Val/Met individuals have repeatedly been shown to have a smaller hippocampal volume relative to individuals who are homozygous for the Val allele (Val/Val) [Bueller et al., 2006; Pezawas et al., 2004; Szeszko et al., 2005].

In this context, we provide data that focus on simple measures that reflect adaptation to environmental change/stress (e.g., fear conditioning) and that appear to lie at the very core of a number of clinical disorders [Charney and Manji, 2004; Duman et al., 1997; Nestler et al., 2002; Pine, 2007]. Importantly, these measures can be tested across species and throughout development and have known underlying biological substrates. Using such measures across development and under varying degrees of stress,

will ultimately allow us to examine vulnerability and protection of each BDNF allele (Val and Met), in an attempt to understand gene X environment interactions across development. Mouse and human data are presented to illustrate this multilevel approach for understanding gene function. The objective of this study was to test if the Val66Met genotype could impact extinction learning in our mouse model, and if such findings could be generalized to human populations. For preliminary gene X development and gene X environment findings see Casey et al., [2009].

Cross species validation of genetic results

We examined the impact of the variant BDNF on classic fear conditioning and extinction paradigms [Soliman et al., 2010]. Approximately 70 mice and 70 humans were tested. The mice include 17 BDNF^{Val/Val}, 33 BDNF^{Val/Met}, and 18 BDNF^{Met/Met}. The human sample included 36 Met allele carriers (31 BDNF^{Val/Met} and 5 BDNF^{Met/Met}) and 36 nonMet allele carriers group-matched on age, gender, and ethnic background. To avoid spurious allelic associations, we balanced demographic factors, including age, gender, and ethnicity across genotype categories [Soliman et al., 2010; Supporting Information Table S1]. We also performed ethnicity-specific analyses and found that the effect of the Met allele on extinction and conditioning, as measured by change in SCR with time, was not driven by any single ethnic group (extinction: $F(3,64) = 0.32, P < 0.81$) or (conditioning: $F(3,64) = 0.69, P < 0.56$). Fear conditioning consisted of pairing a neutral cue with an unconditioned aversive stimulus until the cue itself took on properties of the unconditioned stimulus (US) of an impending aversive event. The extinction procedure consisted of repeated presentations of the cue (i.e., conditioned stimulus or CS) alone.

There were no effects of BDNF genotype on fear conditioning in mice or humans as measured by freezing behavior to the conditioned stimulus in the mice ($F(2,65) = 1.58, P < 0.22$) and by skin conductance response in humans to the cue predicting the aversive stimulus relative to a neutral cue ($F(1,70) = 0.67, P < 0.42$). However, both the mice and humans showed slower extinction in Met allele carriers than in nonMet allele carriers as shown in Figure 4A,B below. Moreover, human functional magnetic resonance imaging data provide neuroanatomical validation of the cross-species translation. Specifically, we find alterations in frontoamygdala circuitry, known to support fear conditioning and extinction in previous rodent [LeDoux, 2000; Milad and Quirk, 2002; Myers and Davis, 2002; Quirk et al., 2003] and human [Delgado et al., 2008; Gottfried and Dolan 2004; Kalisch et al., 2006; LaBar et al., 1998; Phelps et al., 2004; Schiller et al., 2008] studies, as a function of BDNF genotype. Met allele carriers show less ventromedial prefrontal cortical (vmPFC) activity during extinction relative to nonMet allele carriers (Fig. 4C), but greater amygdala

activity relative to nonMet allele carriers (Fig. 4D). These findings suggest that cortical regions essential for extinction in animals and humans [Milad and Quirk, 2002; Milad et al., 2007; Phelps et al., 2004] are less responsive in Met allele carriers during extinction. Moreover, amygdala recruitment, which should show diminished activity during the extinction [Phelps et al., 2004] remains elevated in Met allele carriers.

The mouse model provides the ability to directly test for dose-dependent effects of the Met allele (Fig. 4A) that is problematic in human studies given the rarity of humans homozygous for the Met allele. Moreover, the mouse model provides the ability to test the effects of the Met allele in both a controlled genetic and environmental background not feasible in humans. Thus, any behavioral differences observed in the mouse can be reliably assigned to the effects of the Val66Met polymorphism. Together with the human behavioral findings, these data provide confidence of the effects of BDNF Val66Met across species. With the added human imaging data, the effects are shown to be biologically valid as they directly map onto known circuits involved in fear conditioning and extinction.

The findings are exciting as they provide an example of bridging human behavioral and imaging genetics with a molecular mouse model. Each of these approaches alone, provide limited information on gene function in complex human behavior, but together, they are forming bridges between animal models and human psychiatric disorders. Specifically, the findings of impaired extinction in mice and humans with the BDNF Met allele, suggests a role in anxiety disorders showing impaired learning of cues that signal safety versus threat, and in the efficacy of treatments that rely on extinction mechanisms such as exposure therapy.

Mouse strain effects

As noted, previous reports have reported strain differences in the efficacy of standard fear conditioning protocols and the rate of extinction Balogh and Wehner, 2003; Bolivar et al., 2001; Brinks et al., 2008; Waddell et al., 2004]. These differences may be due to strain differences in pain sensitivity, learning, or other variables. The results above are based on data collected from C57BL/6J mice. To directly compare the contribution of the Val66-Met polymorphism to fear extinction across genetic backgrounds, we used identical fear conditioning and extinction paradigms in a sample of Val66Met Swiss Webster mice. Fear extinction occurred more rapidly in Swiss Webster mice compared to C57BL/6J mice. Despite these differences, the Swiss Webster homozygous BDNF Met allele mice, like the C57BL/6J Val66Met mice, showed slower extinction to the condition stimulus to than wildtype (Val/Val) mice [see Soliman et al., 2010; Supporting Information].

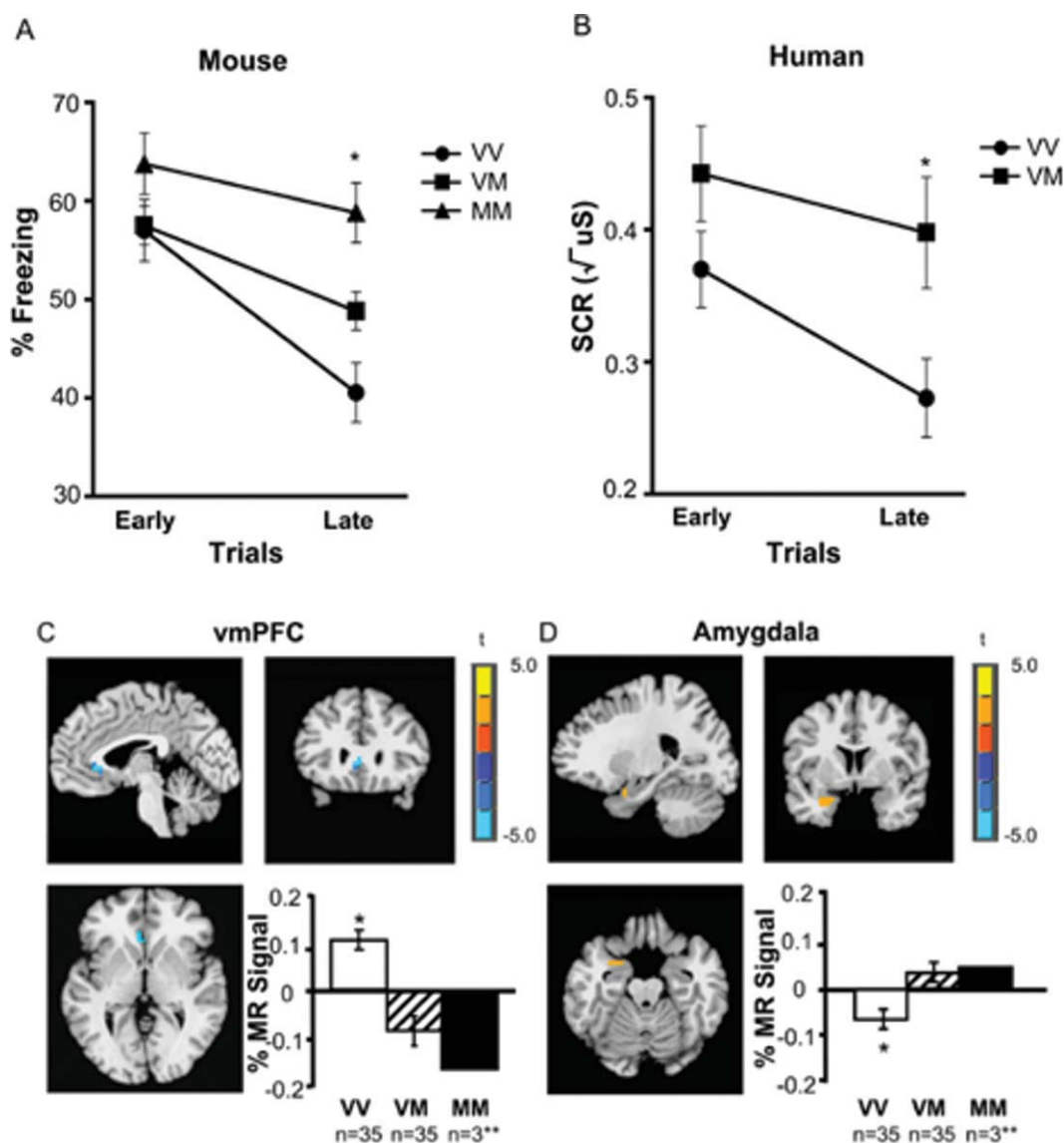


Figure 4.

Altered behavior and neural circuitry underlying extinction in mice and humans with BDNF Val66Met. Impaired extinction in Met allele carriers (Val/Met and Met/Met) as a function of time in 68 mice (**A**) and 72 humans (**B**) as indexed by percent time freezing in mice and skin conductance response (SCR) in humans to the conditioned stimulus when it was no longer paired with the aversive stimulus. (**C**) Brain activity as indexed by percent change in MR signal during extinction in the ventromedial prefrontal cortex (vmPFC) by genotype ($xyz = -4, 24, 3$), with Met allele carriers having significantly less activity

than Val/Val homozygotes [VM < VV = blue], image threshold $P < 0.05$, corrected. (**D**) Genotypic differences in left amygdala activity during extinction ($xyz = -25, 2, -20$) in 70 humans, with Met allele carriers having significantly greater activity than Val/Val homozygotes [VM > VV = orange], image threshold $P < 0.05$, corrected. * $P < 0.05$. **MM were included in the analysis with VM, but plotted separately to see dose response. All results are presented as a mean \pm SEM. VV = Val/Val; VM = Val/Met; MM = Met/Met [Soliman et al., 2010].

Future genetic approaches

Although the majority of imaging genetic imaging studies have focused on candidate genes, comparisons of gene expression between individuals or experimental groups

has been greatly facilitated in the past decade by the introduction of genome-wide microarrays or “gene-chips.” A genome-wide association study is defined as any study of genetic variation across the entire human genome that is designed to identify genetic associations with observable

traits or the presence or absence of a disease or condition. Whole genome information, when combined with clinical and other phenotype data, offers the potential for increased understanding of basic biological processes and those affecting human health, improvement in the prediction of disease and treatment, and ultimately the realization of the promise of personalized medicine.

A potential use for combining imaging genetics with genome-wide microarray methods is to use a biological signature in much the same way that behavioral signatures such as social responsiveness have been used in genetic research on autism [Duvall et al., 2007]. A neural signature specific to a disorder such as functional coupling between ventromedial prefrontal cortex and amygdala may serve to identify risk genes involved in distinct disorders but with a common pathway in frontolimbic circuitry. Numerous cross-site imaging initiatives are underway to begin to provide sufficiently large samples of scans needed to pursue this approach.

SUMMARY

With the continued excitement of the publication of the human genome, scientists will no doubt continue to uncover the functions of specific genes. These discoveries will be augmented by connecting major avenues of genetic research across disciplines, using different approaches that bridge animal models with human behavior and evolving imaging methods with genetic technologies. These approaches will provide a more unified understanding of neural mechanisms involved in human behavior and its disruption in psychopathologies. Such an approach may open up new avenues for therapeutic intervention for clinical populations at the pharmacological, genetic and behavioral levels and identify windows of development that may be most optimal to treatment.

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