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Mouse models of gene-environment interactions in schizophrenia

Geetha Kannan, **Akira Sawa**, and **Mikhail V. Pletnikov**
Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD

Abstract

Gene-environment interactions (GEI) likely play significant roles in the pathogenesis of schizophrenia and underlie differences in pathological, behavioral, and clinical presentations of the disease. Findings from epidemiology and psychiatric genetics have assisted in the generation of animal models of GEI relevant to schizophrenia. These models may provide a foundation for elucidating the molecular, cellular, and circuitry mechanisms that mediate GEI in schizophrenia. Here we critically review current mouse models of GEI related to schizophrenia, describe directions for their improvement, and propose endophenotypes provide a more tangible basis for molecular studies of pathways of GEI and facilitate the identification of novel therapeutic targets.

Keywords

schizophrenia; gene-environment interaction; animal models; endophenotypes; translational neuroscience

Introduction

Genetic and environmental factors, as well as their interplay, contribute to individual differences in vulnerability to psychiatric disease (Kas et al., 2007; van Os et al., 2008). Geneenvironment interplay is a term that encompasses several models (Kendler and Eaves, 1986; Rutter et al., 2006). These include altering gene expression by environmental factors via epigenetic mechanisms, additive interaction between genetic and environmental factors, geneenvironment correlations or genetic control of exposure to the environment, and genetic control of sensitivity to the environment (Kendler and Eaves, 1986; Rutter, 2008; Rutter et al., 2006). Genetic moderation of individual susceptibility to the adverse or protective effects of the environment provides an explanation for most examples of what have been termed genotypeenvironment interactions (GEI) (Rutter et al., 2006). This review will focus on mouse models that mimic GEI etiologically relevant to schizophrenia.

GEI are difficult to assess in clinical studies (Heath et al., 2002; Uher, 2009). Animal models offer a means of elucidating the contribution of genes, environmental factors, and their interactions on pathogenesis of psychiatric disease (Rutter, 2002; Tecott, 2003). As technology has and continues to develop, a number of genetic models can be made to use in conjunction with environmental insults to look at GEI. However, the most useful models to

study human disease should incorporate genetic changes and environmental components that are etiologically relevant (Ayhan et al., 2009; Caspi and Moffitt, 2006).

As schizophrenia and related disorders are increasingly considered as disorders that include etiologies associated with brain development, rodent models with manipulation in genes involved in neurodevelopment may be useful (Insel, 2011; Jaaro-Peled et al., 2009). In a similar vein, it is important to take developmental considerations into account when interpreting environmental effects that can be variable in different age groups. For schizophrenia, pre- and postnatal events that induce psychological stress seem to exacerbate symptoms in adulthood, infectious etiologies have predominantly been associated with prenatal exposure, and illicit use of drugs has been found to be relevant during early adolescence (Moffitt et al., 2005; Rutter, 2008).

We have also proposed that promising animal models of GEI would include etiologically relevant genetic and environmental risk factors that would have strong functional impact and converge on common signaling pathways (Ayhan et al., 2009). Thus, we will overview mouse models that combine genetic variations with psychological stressors (Bethus et al., 2005; Koenig, 2006; Markham and Koenig, 2011), immune activation (Brown et al., 2004; Patterson, 2007) and cannabis exposure (Caspi et al., 2005; Henquet et al., 2008; Henquet et al., 2005). The present review will critically evaluate the weakness of the current approaches and will suggest possible new directions in the development of GEI models with a particular focus on endophenotypic measures that are thought to be instrumental for mechanistic studies, more readily translatable to human conditions, and targetable by therapeutics (Battaglia et al., 2008).

Endophenotypes in animal models for GEI in schizophrenia

As it is impossible to faithfully create the key features of schizophrenia such as hallucinations and delusions in animals, a more tractable and promising approach that has been gaining attention is to model brain circuitry, cellular, and molecular alterations associated with the disease. Such alterations can be broadly termed as endophenotypes (Amann et al., 2010). In the context of GEI animal models, the main advantage of endophenotypes is that such abnormalities can be objectively measured in patients and faithfully replicated in animals to help decipher the underlying mechanisms of GEI. Here, we briefly overview several endophenotypes that are relevant to schizophrenia and may be utilized in basic studies of GEI.

a. Behavioral endophenotypes

Despite the obvious reservations about reproducing human emotion and cognition in animals, some behaviors are conserved in humans, primates, and rodents. Changes in some evolutionarily preserved behaviors are observed in patients and can be experimentally induced in animals, including hyperactivity, impaired pre-pulse inhibition (PPI) of the acoustic startle response, deficient social interaction, and cognitive deficits (Kas et al., 2007). Although these behavioral alterations are not specific to schizophrenia, their objectivity and reproducibility make them useful endophenotypes. For example, as PPI is diminished in patients with schizophrenia (Braff et al., 2001), testing for PPI impairment remains a critical component of any animal study of schizophrenia (Geyer M., 2002; Powell et al., 2012; Swerdlow et al., 1992). Similarly, given that cognitive deficits are debilitating and the least treatable abnormalities in schizophrenia (Keefe, 2008; Reichenberg et al., 2006), there is a growing appreciation for developing more sophisticated tests to evaluate cognitive processes, including working memory and attention (Arguello and Gogos, 2006; Kellendonk et al., 2009). Behavioral endophenotypes have been widely used in animal models of major psychiatric diseases and animal models of GEI. Still, more work is needed

to develop translatable and reproducible behavioral endophenotypes for negative symptoms and cognitive deficits of the disease (O'Tuathaigh et al., 2010).

b. Electrophysiological endophenotypes

Patients with schizophrenia display deficits in processing external stimuli from the environment (Barch and Ceaser, 2012; Rissling and Light, 2010; Silverstein and Keane, 2011). These deficits can be assessed with auditory event-related potentials (ERPs) methodology. Reductions in N100 or mismatch negativity, changes in theta and gamma frequency have been proposed as electrophysiological endophenotypes relevant to schizophrenia (Ford et al., 2007; Thaker, 2008; van der Stelt and Belger, 2007). Such endophenotypes can now be successfully measured in animals (Amann et al., 2010; Ehrlichman et al., 2009; Ehrlichman et al., 2008). Abnormal functional inter-regional connectivity has also been implicated in the pathophysiology of schizophrenia (Schmitt et al., 2011; Uhlhaas, 2012). Newly developed tools enable us to study functional connectivity in laboratory animals. For example, reduced synchronization of neural activity between the hippocampus and the prefrontal cortex during a working memory task was found in a mouse model of the 22q11.2 deletion (Sigurdsson et al., 2010). Another electrophysiological method that is being developed to examine the pathophysiology of cognitive impairment is stimulus specific response potentiation. This tool can be used to assess long-lasting, experience dependent plasticity in the primary visual cortex of rodents (Cooke and Bear, 2012). These techniques are only beginning to be utilized in animal models of psychiatric disease but hold the significant promise of objectively evaluating effects of GEI at the circuitry level particularly in combination with *in vivo* imaging.

c. Brain imaging endophenotypes

The introduction of neuroimaging has revolutionized brain research, providing significant insights into the pathophysiology of psychiatric diseases (Lancelot and Zimmer, 2010; Nenadic et al., 2012; Shepherd et al., 2012; Vyas et al., 2012). Adaptation of neuroimaging to rodents has enabled researches to observe *in vivo* longitudinal changes at the organ, cell, and molecular levels (Lancelot and Zimmer, 2010; Poole et al., 2011). Magnetic resonance imaging (MRI) has been used to assess volumetric changes in the lateral ventricles and brain regions in several animal models for schizophrenia (Denic et al., 2011; Dijkhuizen and Nicolay, 2003; Hikida et al., 2007; Pletnikov et al., 2008). The animal variant of positron emission tomography (PET), micro-PET, has been helpful in assessing neurochemical changes (e.g. receptor binding) that resemble PET findings in patients (Sossi and Ruth, 2005). The simultaneous use of MRI and micro-PET in rodent models of schizophrenia may provide valuable information on changes in receptor density and neurotransmitter and metabolite concentration due to specific genetic or environmental manipulations (Lancelot and Zimmer, 2010). The significant advantages of *in vivo* neuroimaging are longitudinal monitoring of the brain alterations of GEI and the treatment effects in the same animal. However, the cost of neuroimaging is high and likely deters wider use of this technology. Also, low resolution of the images may make subtle changes difficult to assess, requiring the use of traditional histological methods.

d. Histological endophenotypes (GABA neuronal changes and spines)

Histological analysis provides insight into specific cell modifications (e.g. number or morphology) that still are unavailable with *in vivo* imaging. Decreased immunoreactivity of parvalbumin positive gamma-aminobutyric acid (GABA) interneurons in the cortex and hippocampus are commonly observed in postmortem brains of patients with schizophrenia (Gonzalez-Burgos and Lewis, 2008). This histological hallmark of the disease has been reported for many animal models of schizophrenia and is a promising endophenotype for GEI research. Similarly, abnormal maturation, morphology, and functions of dendritic

spines and synapses have been associated with major psychiatric diseases (Penzes et al., 2011). Although specific dendritic or synaptic abnormalities for schizophrenia have not been found, understanding the molecular underpinnings of synaptic pathology has been suggested to be a promising direction for future research on identifying new therapeutic targets (Hyman, 2012; Pratt et al., 2012).

Current animal models of GEI relevant to schizophrenia

A number of animal models of schizophrenia have focused on manipulating genes in order to determine their contributions to disease. However, it is becoming increasingly apparent that genetic manipulations alone do not faithfully reproduce many endophenotypes of schizophrenia. Combining genetic risk factor with environmental adversities such as psychological stress, immune challenge, or drug exposure, provides a better approach to modeling the complexity and heterogeneity of schizophrenia. Here we review the GEI models grouped according to the type of the environmental factor used.

a. Psychological stress

Pre- and post-natal psychological stress has been implicated in the etiology of schizophrenia (Bradley and Dinan, 2010; Markham and Koenig, 2011). To better understand the role of stressful experience on the development of schizophrenia, a number of groups have exposed genetically modified mice to various types of stressful treatment either *in utero* or postnatal.

One group sought to determine whether a combination of prenatal variable stress and a point mutation in the synaptosomal-associated protein of 25 kDa (SNAP25) would lead to behavioral endophenotypes reminiscent of schizophrenia. They found that prenatal stress and the genetic mutation acted synergistically to produce deficits in sociability and social novelty that were not seen in unchallenged mutants or stressed control animals. In addition, prenatal stress and the point mutation additively increased PPI impairment already present in mutant mice (Oliver and Davies, 2009). This study was one of the first to demonstrate synergistic and additive effects of genetic and environmental factors on behavioral endophenotypes relevant to schizophrenia. However, the model has the limitations for molecular mechanistic studies of interactions between the point mutation in the *Snap 25* gene in the mouse embryo and an array of signaling pathways activated by stress in pregnant dams as the point mutation may or may not have functional effects on the intracellular stress response signaling. In addition, it is important to note that while this GEI model revealed impaired PPI, the point mutation in the *SNAP25* gene has not been associated with schizophrenia. Rather, a reduction in SNAP25 expression has been found in the postmortem samples (Thompson et al., 2003).

The interaction of postnatal stress and deletion of a schizophrenia risk gene, *Neuregulin-1* (NRG1) has been looked at by a few groups. Desbonnet and associates (2012) examined the behavioral effects of interactions between repeated social defeat during adolescence (postnatal day 35–45) and deletion of NRG1. Independent of adolescent stress, mice deficient in NRG1 showed increased activity levels, deficits in PPI, and a reduction in social novelty preference. Interestingly, adolescent stress alone did not have an impact on the behaviors. Rather, a combination of adolescent stress and NRG1 deletion was needed to observe decreased activity, poorer spatial working memory, and diminished sucrose preference (Desbonnet et al., 2012). In NRG1 mice, stress also influenced spleen cytokine response to concanavalin A and altered expression of mRNAs encoding for brain cytokines and brain derived neurotrophic factor. In contrast, acute restraint stress during adulthood (3–4 months and 6–7 months of age) led to different effects. Older NRG1 mutants were less susceptible to the effects of stress on anxietyrelated behaviors than younger mutants (Chesworth et al., 2012). These studies highlight that varying the time of exposure to

stressful events leads to differential behavioral alterations in the same transgenic mouse model. A caveat of the NRG1 deficient model is lack of tissue specificity. NRG1 has an important role in cardiac development (Lemmens et al., 2007), with homozygous deletion or NRG1 leading to embryonic death from cardiac problems in mice (Stefansson et al., 2002). It is conceivable that NRG1 heterozygous mice may have heart problems that affect brain functions. Future studies with this model should try to delineate the specific mechanisms of GEI (e.g. a role of NRG1 in the stress signaling pathways).

Our group has recently provided a mechanistic insight into how a mild isolation stress during adolescence affects the mesocortical projection of dopaminergic neurons in which DNA hypermethylation of the tyrosine hydroxylase gene is elicited only if stress is combined with expression of a promising genetic risk factor, mutant *Disrupted-In-Schizophrenia-1* (DISC1) under the prion promoter. These molecular changes could contribute to several neurochemical and behavioral deficits that are blocked by a glucocorticoid receptor antagonist. We propose that the biology and phenotypes of the mouse model resembled those of psychotic depression (Niwa et al., 2013).

b. Immune Challenge

Viral infections have been implicated in the pathogenesis of schizophrenia (Brown and Derkits, 2010; Sham et al., 1992; Torrey and Yolken, 2003). One possible mechanism whereby viral infections can contribute to schizophrenia is via secretion of pro-inflammatory factors by the host in response to infection (Patterson, 2007). Notably, elevated levels of maternal serum cytokines have been associated with children who later develop schizophrenia (DeLisi and Wyatt, 1982; Miller et al., 2009; Patterson, 2007). Mouse models of immune activation due to viral infection use the viral mimic polyinosinic:polycytidylic acid (poly I:C). Poly I:C is a synthetic analog of double-stranded RNA and, similar to a live virus, interacts with toll-like receptor (TLR) 3 expressed by cells of the immune system (Amarante and Watanabe, 2010).

Prenatal immune activation with poly (I:C) has been used in a few models of GEI. One study combined prenatal administration of poly (I:C) during early gestation stage (embryonic day 9, E9) and expression of mutant human *Disrupted in Schizophrenia 1* (DISC1) and evaluated behavioral, brain imaging, spine density, and molecular markers that could be relevant to psychiatric disease. The initial hypothesis of the authors was that a combination of prenatal immune activation and mutant DISC1 would synergistically produce stronger phenotypes consistent with aspects of schizophrenia. However, prenatally exposed mutant DISC1 mice showed a set of neurobehavioral alterations that were not consistent with schizophrenia and were more similar to affective disorders, including increased anxiety and depression-like behaviors, decreased social interaction, decreased volumes of amygdala and periaqueductal gray, and altered responses to acute retrain stress (Abazyan et al., 2011). Taking advantage of the inducible system in this transgenic mouse model, the authors also demonstrate that life-long expression of mutant DISC1 is required to produce the affective behaviors in mice. An unexpected outcome of the study was an emergence of previously unseen behavioral and brain abnormalities in mutant DISC1 mice. This result illustrates an important consideration for evaluating GEI models. Combining genetic and environmental factors can lead to the development of new phenotypes rather than exacerbation of pre-existing abnormalities. A potential caveat of the study is the predicted timing of expression of mutant DISC1 in selective neuronal population. Although the authors show expression of mutant DISC1 as early as E9 when poly (I:C) treatment is applied, additional studies are needed to better understand the regional and time-related expression pattern of this mutant protein during early gestation to inform future molecular studies with this model. A more detailed evaluation of timing of GEI in this model would be

important as well, including the question if turning off expression in adult mice might improve some brain and behavior alterations.

Meyer's group also examined interactions between prenatal immune activation during late gestation stage (E17) and the heterozygous deletion of nuclear receptor related 1 (*Nurr1*), gene that encodes for a transcription factor involved in dopaminergic neuronal development through activation of tyrosine hydroxylase (Vuillermot et al., 2012). The combination of the genetic and environmental factors was found to not only exert additive effects on increased locomotor activity and deficits in sensorimotor gating, but also to produce synergistic effects on attentional shifting and sustained attention. In addition, *Nurr1* deficiency in conjunction with prenatal poly (I:C) administration lead to improper development of prefrontal cortical and ventral striatal dopamine systems. This was suggested via reduced dopamine receptor 2 (D2R) in the nucleus accumbens and reduced tyrosine hydroxylase and increased catechol-O-methyltransferase (COMT) in the medial prefrontal cortex (Vuillermot et al., 2012). These molecular deficits are consistent with findings in patients with schizophrenia (O'Tuathaigh et al., 2012). However, a caveat of this model is the limited human relevance of the deletion as only missense mutations in the *NURR1* gene have been associated with schizophrenia and manicdepressive disorder (Buervenich et al., 2000). Still, given a leading role of DA dysregulation in the pathophysiology of schizophrenia, one can foresee future research with this model of GEI in identifying the molecular mechanisms whereby NURR1 and the innate immune response from poly (I:C) administration work together to modulate maturation of DA neurons.

Postnatal immune activation has also been studied in conjunction with DISC1. One study looked at neonatal (postnatal days 2–6) treatment with poly (I:C) in transgenic mice constitutively expressing mutant DISC1 (Ibi et al., 2010). Focusing on behavior and histological measures, they found that DISC1-poly (I:C) exposed mice showed reduction in working memory, increased susceptibility to MK-801 induced hyperactivity, and a decrease in parvalbumin positive GABA neurons in the medial prefrontal cortex. These deficits were not observed in mutant DISC1 mice without immune stimulation or control immune stimulated mice, suggesting synergistic effects. The authors focused on the characterization of schizophrenia-like behaviors in poly I:C/DISC1 mice and did not provide a detailed description of possible neurobehavioral effects following the single factor exposure. In a follow-up study, cognitive impairment and MK-801 induced activity observed in poly I:C/DISC1 mice were improved by administration of clozapine (Nagai et al., 2011). The ability of currently approved antipsychotic medication to ameliorate behavioral endophenotypes suggests that this GEI mouse model may be useful in the development of new therapeutics. A limitation of this model is that there is insufficient epidemiological support for childhood viral infection to increase the risk for schizophrenia. From a methodological standpoint, the model needs to control for possible maternal effects. For example, if poly (I: C) administration of pups resulted in sickness behavior, nursing dams may have treated sick and healthy pups differently, which may confound the outcomes of the study.

Use of poly (I:C) administration in mouse models has distinct advantages in reproducibility, reliability, and simplicity. However, there are disadvantages. The route of administration (e.g. intraperitoneal or intravenous) used does not mimic the route by which pregnant women are most likely to get infected by a virus (e.g. respiratory). The elicited immune response may differ from that produced by a live viral infection (Fatemi and Folsom, 2009). To overcome the limited immune response due to poly (I:C), some have used live microorganisms to model human infections in animals (Fatemi et al., 2009; Kneeland and Fatemi, 2013). A potential issue of this approach is our limited options for using live pathogens in rodents. Many relevant infections are species-specific and thus do not involve the same mechanisms of invasion, replication and dissemination (e.g., herpes simplex virus

1). In addition, C57BL/6 mice that are predominantly used in genetic models easily succumb to human pathogens (e.g., influenza virus or *Toxoplasma gondii*), complicating longitudinal behavioral studies. A possible solution to this issue is to transfer the mutation in question to less susceptible Balb/C mice by a series of backcrosses or to treat animals with attenuated pathogens to better mimic the specificity and magnitude of immune activation.

c. Drug abuse

Cannabis use may precipitate schizophrenia in genetically susceptible individuals (Henquet et al., 2008). In order to mimic this interaction, animal models utilize Δ-9-tetrahydrocannabinol (Δ^9-THC) that is the principal psychoactive component of the cannabis plant (Waller, 1971). A functional variant in the catechol-O-methyltransferase (*COMT*) gene (i.e., a switch in the amino acid methionine to valine in position 158) leads to greater COMT activity in the prefrontal cortex and resultant dysfunctions in humans (Chen et al., 2004). It has been found that adolescent cannabis exposure in individuals with the genetic variant in the *COMT* gene synergistically increased risk of psychosis (Caspi et al., 2005).

The interactions between COMT and cannabis exposure were evaluated in a series of elegant studies by John Waddington and colleagues. They found that COMT knock out (KO) mice chronically exposed to THC during adolescence exhibited behavioral and histological alterations reminiscent of schizophrenia (Behan et al., 2012; O'Tuathaigh et al., 2010). COMT KO mice exposed to THC as adolescents showed decrease spatial working memory and anxiety, which were not seen in mice treated with THC in adulthood (O'Tuathaigh et al., 2010). Adolescent exposure to THC in COMT KO mice led to decreased size of dopaminergic neurons in the ventral tegmental area and parvalbumin positive GABA neurons in the prefrontal cortex, consistent with the similar endophenotypes in patients with schizophrenia (Behan et al., 2012). These results further indicate the importance of timing of GEI and the utility of endophenotypic measures. As with many GEI models described, a caveat of this mouse preparation is a deletion of the *Comt* gene rather than a knock-in expression of the Val158Met polymorphism seen in humans (Papaleo et al., 2012).

A number of studies were performed to assess the effects of acute and chronic THC treatments in adolescent and adult heterozygous Nrg1 KO mice, with a focus on behavior endophenotypes (Boucher et al., 2007a; Boucher et al., 2007b; Boucher et al., 2011; Long et al., 2012a; Long et al., 2012b). A greater decrease in locomotion, elevated anxiety and in lightdark box test and enhancement of PPI were more pronounced after acute THC treatment in adult NRG1 male mice compared to wild type littermates. A follow-up study did not find significant effects of acute THC in female mutant mice, indicating a major role of sex in modulating neurobehavioral outcomes of GEI in many mouse models. The same group of investigators also evaluated the behaviors in NRG1 mice chronically treated during adolescence with THC and found few effects (Long et al., 2012a). When adult mice were given chronic injections of a cannabis constituent cannabidiol (CBD) that has anxiolytic and antipsychotic properties (Long et al., 2012b), it did not alter the pre-existing behavioral abnormalities but selectively enhanced social interaction in in NRG1 heterozygous (HET) mice. In addition to behavior, this group looked at receptor profiling versus autoradiography. They found that chronic THC exposure during adolescence increases binding in NRG1 KOs and decreases binding in wild-type of serotonin receptors in the cortex (Long et al., 2012b). The study also highlights a recurrent issue with GEI models, namely, that GEI do not always lead to exacerbation of pre-existing conditions but can be protective or produce new changes that were not originally present in mutants. A potential weakness of this model is lack of human studies on association between cannabis and genetic variants in the *NRG1* gene as well as our poor understanding of molecular interrelatedness between this gene and CB

receptors signaling. Still, this type of interplay could provide interesting information on GEI relevant to schizophrenia (Karl and Arnold, 2013).

Future directions

Recent progress in psychiatric genetics and epidemiology has facilitated the development of animal models of GEI relevant to schizophrenia. Although these models have provided some important insights, many caveats of recent preparations need to be addressed in the future studies. We would like to group the current issues into the methodological and technological ones. The former group calls for improvement of design and analysis of the existing models, while the latter one requires new technological approaches.

a. Design weaknesses

The field of animal models of GEI is still in its infancy but is rapidly expanding. As it continues to expand, it can learn from the studies of genetic models of psychiatric disease to avoid the known pitfalls. These include varying mouse background strains, history of breeding (e.g. inbred, outbred), and housing conditions. We believe that utilization of standard endophenotypic measures may not only help minimize variability in effects of GEI but also bring in new model organisms to study the molecular mechanisms of GEI across species (e.g., worms, fruit flies, zebrafish).

Another issue is the under appreciation of sex-dependent effects. Many models demonstrate sex-dependent alterations, yet most GEI models focus on a single sex. Future research in GEI models should address if sex-specific abnormalities result from the effects of GEI or their individual components on actions of gonadal hormones or from the modulatory influence of sex hormones on the pathways involved in GEI. Advancing our knowledge of the underpinnings of sex differences in psychiatric disorders could help uncover risk/protective factors and develop better treatments.

A promising direction in optimizing GEI model is to develop behavioral tests that are based on the natural murine behavioral repertoire (Desbonnet et al., 2012). It has been proposed that more accurate behavioral evaluation of animal models of neuropsychiatric disorders should include species-specific behavioral approaches (Baker, 2011). Rodent specific tests will complement clinically relevant paradigms by providing more sensitive tests for analysis of underlying pathology (Desbonnet et al., 2012)

Further, schizophrenia is increasingly considered a disorder of brain development, animal models with manipulation in genes involved in neurodevelopment are going to be most informative (Insel, 2011; Jaaro-Peled et al., 2009). It is important to take developmental considerations into account when interpreting environmental effects that vary across different time points (Moffitt et al., 2005; Rutter, 2008). In the past, addressing time-dependent interaction in GEI models has been achieved by changing the time when genetically modified animals are challenged with an environmental adversity. Future studies should also attempt to regulate timing of the effects of a specific mutation as exemplified by a recent study with inducible expression of mutant DISC1 in mice prenatally exposed to maternal immune activation (Abazyan et al., 2011).

Combining an environmental challenge with a genetic mutation can produce both protective and adverse effects. Instead of predicted synergistic effects, a GEI design can, for example, result in phenotypes previously unseen in unchallenged mutant mice (Abazyan et al., 2011; Behan et al., 2012; O'Tuathaigh et al., 2010). Such results should be anticipated by designing future GEI experiments to avoid a trap of a limited set of pre-planned tests used to "capture" specific disease-related phenotypes. Appearance of new brain and behavioral

phenotypes, particularly while using the genetic mutation implicated in various psychiatric conditions, could inform us about the role of environment in bringing about diverse clinical outcomes in patients with the same mutation. The Scottish pedigree with the disruption of DISC1 due to the chromosomal defect is a most prominent example of such a possibility (Blackwood et al., 2001).

The focus of most published GEI research has been on risk factors. However, the contribution of protective factors is also important and has so far been relatively neglected, although there are some exceptions. Identification of genes conferring resilience to schizophrenia-related abnormalities is a new emerging research to uncover unrecognized molecular targets (Mihali et al., 2012). New models using neurodevelopmental factors of resilience are clearly needed to advance this promising research. In this context, the role for environment enrichment in ameliorating/rescuing genetically produced abnormalities has been recently reviewed (Takuma et al., 2011). Combining this type of preventive “therapy” with current GEI models would be interesting in determining whether environmental enrichment can overcome effects caused by aversive environmental insults (e.g. psychosocial stress, infection, drug use) and offer a novel approach to treatments of the cognitive and negative symptoms that resistant to the current antipsychotics (Laviola et al., 2008; Pratt et al., 2012).

b. Technology development

The methodologies to manipulate the mouse genome at different levels of its organization (DNA, RNA regulatory sequences) are constantly improving. Simple knockout and transgenic technologies while remaining the workhorse of mouse genetics produce artificial systems inconsistent with the molecular pathology of schizophrenia. New models with mutations in regulatory elements in candidate genes with more subtle and temporally specific expression changes or human genetic variants knock-in models will better reflect the complex genetic and molecular mechanisms of schizophrenia (Papaleo et al., 2012). Therefore, time-dependent, circuitry- or cell-specific manipulations to target mRNA and/or proteins should be utilized.

There is growing appreciation that combining multiple genetic mutations or several environmental factors in a single model could be more informative. Recent studies from John Waddington’s and Urs Meyer’s groups are an intriguing example of how one can proceed with more complex but etiologically relevant models of not only G × E but G × G and E × E interactions (Giovanoli et al., 2013; ICOSR, 2013). In addition to conventional breeding approaches, newer technologies include plasmid-based cell-type-specific and inducible expression systems using *in utero* gene transfer by targeting multiple genes (Taniguchi et al., 2012). A complementary methodology is to suppress the expression of target genes using RNA interference (RNAi) knockdown technology (Mello and Conte, 2004). Several susceptibility genes can be knocked down simultaneously in mice carrying multiple siRNA expression transgenes.

Most studies have focused on neuronal functions of susceptibility genes. However, these genes are also expressed by glial cells (Iijima et al., 2009; Prevot et al., 2003). Given, growing interest in the role for glia cells in mediating the effects of stress and microbial pathogens, GEI models with cell-specific perturbation of candidate genes are also needed. A recent study has provided the first evidence for the potential role of DISC1 in astrocytes, connecting DISC1 and serine racemase in modulating NMDA receptor functions (Ma et al., 2013).

In conclusion, GEI animal models have already begun to provide new insights into the etiological complexity and heterogeneity of schizophrenia. We believe GEI animal models

will continue to be a crucial tool to advance our knowledge about this debilitating disease and help searching for new treatment options.

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