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Translational neuroscience of schizophrenia: seeking a meeting of minds between mouse and man

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Abstract

Understanding the etiology of developmental brain disorders such as schizophrenia is critical for achieving advances in treatment and requires novel research strategies that control for individual variation in genetic background, environmental challenges and expression of phenotype. SYSGENET, a European systems genetics network for the study of complex genetic human diseases using mouse genetic reference populations, drew together in Helsinki a cross-disciplinary group of clinical and basic scientists and mouse geneticists to debate, formulate and prioritize a strategy for future research based on mouse models. The main conclusions of this meeting are summarized here.

Introduction

Schizophrenia is a psychotic disorder characterized by positive and negative symptoms, including reality distortion (delusions and hallucinations), psychomotor poverty (poverty of speech, social withdrawal and blunting of affect) and disorganisation (inappropriate affect and thought disorder), together with cognitive deficits; negative symptoms and cognitive deficits are the primary determinants of poor functional outcome. Lifetime risk for schizophrenia is about 1 % and, because of its onset in early adulthood and severity, it causes considerable disability to patients and high

socioeconomic costs to society. Schizophrenia is a complex disease influenced by both genetic and environmental factors, with heritability estimated at approximately 80%. In recent years, several susceptibility genes have been identified in human cohorts, leading to new insights into pathogenetic mechanisms of this neurodevelopmental disorder [1-3]. However, current medications for schizophrenia are only partially effective; for example, while reducing psychotic symptoms, they are minimally effective in improving cognition [4] and induce a range of potentially life threatening long-term side effects. Therefore, there is a need for better treatment practices for schizophrenia that target positive and negative symptoms and improve cognitive deficits. Increased understanding of the neurobiological mechanisms underlying schizophrenia should facilitate this task.

Merging knowledge from genetics, genomics, epidemiology, physiology and pharmacology, including both pre-clinical and clinical studies, is needed to resolve core aspects of schizophrenia. In this regard, studies using animal models can contribute considerably to a better understanding of the biological processes that underlie disease risk and development. Experimental animal models will also be required for the development of new, etiology-directed medication. The mouse is well placed to address most of these requirements, by virtue of its advanced genetics, understanding of developmental and behavioral biology, short generation time and low cost [5]. In particular, the effects of targeted, experimental and natural genetic variations and their phenotypic consequences can be studied in mouse populations or in genetically engineered lines, such as mouse gene knock out lines [6;7]. However, to provide real insight and to be of meaningful translational value, it is first essential to identify the core biological phenotypes and mechanisms that underpin schizophrenia. This challenge was the focus for the targeted workshop of SYSGENET on cross-species studies of schizophrenia.

Core features of schizophrenia

Compromised function of the nervous system in schizophrenia is evident, on a population basis, as early as the first year of life in terms of slightly delayed developmental milestones such as walking [8]. Manifestations become more evident during early adolescence (ages 11-16 years) and are characterized by cognitive decline and social withdrawal. During late adolescence/young adulthood (16-25 years), the first symptoms of psychosis appear, most typically hallucinations and delusions. Thus, the first material signs of the illness are not the more obvious psychotic symptoms but, rather, the slow but steady decline in cognitive functioning that precedes the onset of psychosis by an average of

nine years [9;10] and may continue to progress thereafter [11]. Indeed, cognitive dysfunction constitutes not only one of the core features of the illness [10] but also one of the most intractable to treat [4]. Although the words 'dementia praecox' as Kraepelin named the disease he delineated in 1895 may not carry a hopeful message, the 'dementia', i.e. the cognitive decline, reflects a core process in the illness we currently call schizophrenia.

Not only does cognition worsen during the early course of schizophrenia; there is accompanying loss of cerebral grey matter in excess of what is seen with normal ageing. Brain imaging studies in healthy humans have revealed that the developing and ageing human brain undergoes dynamic changes in volume over time. Specifically, absolute brain volume increases up to early puberty and, thereafter, declines before the age of 20 [11]. Then, there is a short episode with slight growth followed by a decline, until reaching a stable brain volume around the ages of 25-40. In contrast, at illness onset brain volume in schizophrenia patients is already reduced relative to matched healthy controls; subsequently such patients exhibit an accelerated decrease in brain volume, especially in frontal and temporal cortical brain areas [11]. This progressive reduction in cortical volume in schizophrenia can occur independent of medication [12], is heritable [13] and correlated with poor prognosis and psychosis severity [14]. Cannabis use, the strongest known environmental risk factor for schizophrenia thus far, worsens grey matter brain volume loss in schizophrenia [15;16].

A recent study aimed to reduce phenotypic heterogeneity in a large sample of psychosis patients, their relatives, and community controls using latent class analysis to analyze variation in Comprehensive Assessment of Symptoms and History (CASH) lifetime-rated symptoms [17]. This study revealed that variation in five continuous dimensions (disorganization, positive, negative, mania, and depression) was accounted for by the presence of seven homogeneous classes (Kraepelinian schizophrenia, affective psychosis, manic-depression, deficit non-psychosis, depression, healthy, and no symptoms). This analysis showed that almost all (85%) of these schizophrenia patients were assigned to the Kraepelinian schizophrenia class, while the remaining patients were assigned to the affective psychosis class. Analyses of symptom variation revealed that this prominent difference within the patient sample is based on the distinction between low versus high levels of disorganization and negative symptoms, rather than on the level of positive, psychotic symptoms. Levels of disorganization and negative symptoms are also associated with diminished cognitive performance and poor outcome, suggesting that these may be relevant dimensions to model in mice. Together, these findings indicate that schizophrenia is a complex neurodevelopmental disease of cognitive decline associated with progressive brain loss, negative symptoms and the emergence of psychotic symptoms that lead to diagnosis. The presence of measurable physiological parameters should make it possible to study well-defined

phenotypes in mouse populations and mutants and to determine the genetic basis for premature, disease-related brain loss.

Human genetic findings

Although the heritability of schizophrenia is high, at about 80% [18], it has been challenging to identify predisposing variants for schizophrenia in humans. Early studies utilizing linkage mapping, cytogenetic analysis and candidate gene studies identified several putative susceptibility genes, including the dopamine receptor 2 (*DRD2*) (www.szgene.org), neuregulin 1 (*NRG1*) [19;20], dysbindin (DTNBP1) [20] and disrupted in schizophrenia 1 (*DISC1*) [21].The recent common variant-common disease hypothesis has stimulated a number of genome wide association studies (GWAS) and resulted in the identification of variants with weaker effects, including ZNF804A [22]. Several international consortia, including SGENE, the ISC (International Schizophrenia Consortium) and the MGS (Molecular Genetics of Schizophrenia Collaboration), detected their strongest association signals in the MHC region, which seem to come from two partially independent signals: one in the large histone gene cluster on chromosome 6, near the major histocompatibility complex (MHC) class I region, and another one near the NOTCH4 gene, which also tags classical HLA alleles DRB1 and HLA-B [23-25]. They also identified other loci, including *TCF4* and *Neurogranin* [23]. Follow-up analyses, including studies by the Psychiatric Genetics Consortium (pgc.unc.edu), and other groups (e.g., [26;27]) are gradually identifying further GWAS-significant loci.

Several rare, *de novo* copy number variants (CNVs) have also been recently associated with schizophrenia, including deletions at 15q13.3, 1q21.1, neurexin 1, 17p12 [28;29] and duplications at 16p11.2, 16p13.1 and VIPR2 [30-32]. Interestingly, CNVs at some of these loci associate also with other phenotypes, including bipolar disorder [25], autism spectrum disorders (e.g., [33]), cardiovascular diseases such as aortic dissection and teratology of fallot [34] and obesity [35]; these findings have important implications with regard to potential DNA diagnostics, understanding disease etiology across disorders, and treatment development. However, while important disease pathways are beginning to emerge, more than 90% of the heritability of schizophrenia remains to be explained and larger samples and sequencing-based approaches are expected to shed light on this issue. Interestingly, the occurrence of *de novo* CNVs and recent rare point mutations may explain why psychiatric diseases with a reduced fecundity, such as schizophrenia and autism, remain frequent in the human population [36].

Genetic mouse models

The contribution not only of common risk variants, but also of deleted or duplicated *de novo* CNV regions in schizophrenia suggest that transgenic animals, such as knock-outs or knockins for these specific gene regions, may provide genetic causality for schizophrenia. Most mutants studied thus far do not model schizophrenia *per se*; rather, they assess the functional roles of genes associated with risk for schizophrenia (e.g. *Disc1*, *DTNBP*1 and *Nrg1*) or putative endophenotypes (e.g. *Comt* deletion in relation to cognitive phenotypes). Furthermore, studying the 'mental health' of mice is challenging. There may be an unappreciated role for ethological, species-specific behaviors in more naturalistic settings, to include components of the mouse ethogram such as nest building. Current approaches [37;38] have more commonly involved trans-species models of positive symptoms (e.g. pre-pulse inhibition), negative symptoms (e.g. social behavior), and cognitive dysfunction (e.g. working memory). In the case of ENU-induced amino acid substation *Disc1* mouse models, the behavioral phenotypes and pharmacological rescues are allele dependent [39]. Certain features of schizophrenia, such as poverty of speech, may be uniquely human. However, anhedonia, asociality and avolition are at least theoretically accessible in both humans and animals and can be studied in relation to genetic risk factors for schizophrenia [40] For example, *Nrg1* mutant mice have selective disruption to social novelty preference [41]. However, the uncertainty regarding the clinical concept of schizophrenia means that the predictive validity for such mutant phenotypes and their pathobiology is similarly uncertain. Subsequent studies, using cross-sectional MR imaging, showed that *Nrg1* mutant mice have slightly *smaller* total ventricular volume and cerebellum when compared to wild type controls [42]. Furthermore, gene $\times$ environment (G $\times$ E) interactions are also evident; for example, interactions (i) between maternal immune activation and *Nrg1* mutation, (ii) between adolescent social defeat and *Nrg1* mutation, and (iii) between adolescent cannabis exposure and *Comt* mutation have been shown to regulate subsequent mouse phenotypes such as social behavior and working memory over young adulthood [43]. Such studies elaborate clinical findings by indicating that the overall psychosis phenotype may be influenced by the interaction of risk genes with both biological and psychosocial environmental adversities operating at critical time points across the developmental trajectory.

Clinical relevance

Genetic information has the potential to aid clinicians in risk prediction and diagnostics. Both of these approaches are routinely carried out for monogenic diseases. Certain rare genomic lesions have high predictive value, such as the t(1;11) disruption of *DISC1* [44], or the VCSF deletion locus on chromosome 22q11 [45]. Unfortunately, GWAS studies in schizophrenia published to date have generated low values for the relative risk provided by the identified risk alleles. For example the SGENE consortium reported odds ratios of around 1.2 for their most important findings, with the risk-conferring allele being very prevalent, around 80-90% in most cases [25]. Translated into a predictive test, this has very low specificity and no clinical utility. Combining genetic risk factors to create a genetic risk score has been attempted in other complex phenotypes, leading both to negative results for height [46] and cardiovascular traits [47] and to statistically significant but clinically unresolved positive findings for type 2 diabetes [48] and coronary heart disease [49]. The current genetic findings do not allow clinically relevant tests to be performed, but such tests may be possible when the genetic landscape of schizophrenia becomes better characterized.

However, clinical studies in schizophrenia can inform on the most relevant phenotypes for studies in mice. When constructing a mouse to model aspects of schizophrenia, it is important to distinguish whether one seeks to model some rare and severe trait, potentially with quantitative endophenotypes, or a trait that is present quantitatively in the human population. Traditionally, a stress-diathesis model has prevailed. This model views disease state as dichotomous but the underlying risk phenotypes as quantitative. There is also evidence to suggest that the actual phenotype of psychosis is common and quantitatively distributed in the population, with no point of rarity. First of all, over 3% of individuals have a diagnosed psychotic disorder [50]. Moreover, brief auditory illusions when falling asleep/waking up are common and normal; many widowed persons have sensory impressions of their loved ones; experimental sleep deprivation and sensory deprivation can cause hallucinations in experimental settings. When psychosis proneness in the population is assessed by a questionnaire (the Perceptual Aberration Scale), less than half of individuals report no psychotic-like experiences and the number of psychotic-like experiences in the population follows a Poisson distibution, with no point of rarity [51]. These findings do not support the dichotomous view of psychosis in itself. The research community seems divided on this dichotomous/continuous debate, and whichever eventually turns out to be true, it is something to keep in mind when constructing mouse models for this disorder.

Another important issue to consider is which human phenotypes can be modeled in the mouse and which are human specific [52]. Recent cognitive neuropsychiatric models of psychosis emphasize the role of attention disturbances

and inappropriate incentive learning in the development of disturbances of thought, such as delusions. It is clear that the more proximal phenotypes, such as sensory gating abnormalities, would be a better target for mouse modeling than human specific, distal phenotypes like delusions of reference. The most critical question is where on this proximal – distal scale to put traits such as disruption to social behavior and brain imaging abnormalities. These considerations suggest that the phenotypes to be modeled in the mouse should be a reliably detectable, physiological measure of proximal processes in the etiological chain of events in psychosis.

Modeling clinical relevant phenotypes in mice

This overview of our current knowledge towards understanding the neurobiology of schizophrenia signals an urgent need for animal models that also take into account the genetic complexity of those small effect size genetic variants contributing to the development of core features of the disease. One main conclusion from this workshop was that schizophrenia is a complex neurodevelopmental disease associated with early, progressive cognitive decline in association with accelerated reduction in brain volume of cortical areas and, possibly, the cerebellum. Manifestation of the diagnostic symptoms of psychosis during late adolescence may result from the effects of moderate to high-risk mutations such as CNVs in some individuals, and interaction of a large number of low risk polymorphisms with several environmental adversities at critical time points across the developmental trajectory of the brain. Future studies should aim at developing specific, standardized phenotypes to be measured longitudinally, to include cortical thickness and other structural phenotypes, cognitive phenotypes, pre-pulse inhibition, sociability/social novelty preference, and habituation of exploratory behavior in a novel environment. These phenotypes will need to be assessed in standardized contexts of G $\times$ G and G $\times$ E interaction and measured longitudinally to address both multi-factorial and progressive aspects such as reduction in brain volume and cognitive decline. Here, mouse model systems will be of great value.

Mouse models can inform study of the etiology of schizophrenia

Targeted therapies for schizophrenia are desperately needed, but their development and effective use requires understanding of the molecular mechanisms involved in the etiology of schizophrenia. Genetics offer an ideal route to the molecular basis of schizophrenia, as any identified genes can potentially be linked to their functions from the

cellular to the behavioral level. Mouse models can be very informative both in the identification of susceptibility genes and in understanding their biological function [5;37]. In addition to revealing the function of the gene in a defined genetic background [38], single gene knock-out mouse models of candidate genes for schizophrenia allow studies of G × E interaction [43]. Furthermore, the same mice can be used in detailed analysis of gene expression, proteomic, and neuronal phenotypes both in intact brain and cellular models, in order to provide additional tools for drug development and testing, for example using approaches such as connectivity mapping [53]. Some of these models, such as those relating to positive symptoms, can be validated with existing drugs used to treat schizophrenia, to provide some predictive validity [39]. However, others, such as those relating to negative symptoms and cognitive dysfunction which respond poorly even to clozapine, cannot be validated in this way [52].

On the other hand, clinically relevant mouse models of schizophrenia will complement the human genetic efforts to identify small effect susceptibility variants, as human GWAS fail to capture more than a modest fraction of the measured heritability [54]. Quantitative trait locus (QTL) mapping using mice has been used to identify susceptibility genes for endophenotypes of schizophrenia, including ventricular size [55] and prepulse inhibition [56-59] in F_2 animals and chromosome substitution strains. However, the relatively small number of highly polymorphic alleles in these strains might not be optimal in identifying genes for complex diseases such as schizophrenia.

At the core of the SYSGENET network (http://www.helmholtz-hzi.de/sysgenet/) is the use of mouse genetic reference populations (GRPs). An important new GRP that will soon become available is the Collaborative Cross (CC), comprising a large panel of recombinant inbred (RI) strains derived from a genetically diverse set of eight founder strains and designed specifically for complex trait analysis [60;61]. The CC population also includes wild derived mouse strains and thus represents a greater degree of genetic variation than currently available GRPs. This should result in larger phenotypic and behavioral variations that are relevant to schizophrenia and other neurodevelopmental and behavioral disorders. GRPs also offer the intrinsic advantages of access to brain tissue at any chosen developmental time point, before or after behavioral testing and/or environmental or pharmacological intervention. Polymorphisms within susceptibility genes may lead to differences in gene function or expression; these differences can be studied with gene expression, proteomic and metabolomic analyses, allowing identification of novel gene regulatory networks perturbed in schizophrenia. Therefore, the set of clinically relevant phenotypes discussed above should be measured in the CC population. Longitudinal quantitative measurements of brain morphology (e.g. cortical thickness), sensory processing and behavioral phenotypes (e.g. social behavior) in GRPs will allow the systematic modeling of clinically

relevant phenotypes of schizophrenia at different developmental stages and under controlled genetic and environmental conditions (see Box 1).

Conclusions

All participants concluded that there is a real opportunity and need to interface preclinical and clinical research in order to better understand the etiology of schizophrenia and related developmental brain disorders. Crucially, the laboratory mouse can play a vital role in both constructing and testing hypotheses of direct translational relevance. As a complement to the established armamentarium of mouse genetic techniques and tools, the Collaborative Cross has an important contribution to make.

Box 1. Core features of schizophrenia for mouse modeling.

Schizophrenia		**Mouse**
Critical developmental stages	----	Age-matched developmental stages
Disease progression	----	Longitudinal phenotypic assessment
Environmental factors	----	Maternal infection/stressful events/cannabis use
Candidate genes	----	Targeted mouse mutants, “humanized” genetic models
Genetic background/epistasis	----	Collaborative cross/crossing mutant lines
Phenotypes:	----	Cognitive decline
		Cortical thinning
		Sensory processing (e.g., pre-pulse inhibition)
		Social behavior

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