

## Why Schizophrenia Epidemiology Needs Neurobiology—and Vice Versa

John J. McGrath<sup>1-4</sup> and Linda J. Richards<sup>2,5</sup>

<sup>2</sup>Queensland Brain Institute, University of Queensland, St Lucia 4076, Australia; <sup>3</sup>Queensland Centre for Mental Health Research, The Park Centre for Mental Health, Wacol, Queensland 4076, Australia; <sup>4</sup>Department of Psychiatry, University of Queensland; <sup>5</sup>School of Biomedical Sciences, University of Queensland, St Lucia 4076, Australia

Schizophrenia epidemiology can provide us with valuable information to guide research directions. However, while epidemiology is useful for generating candidate risk factors, it can not always deliver studies that prove causality. We argue that the field needs more translational research that links schizophrenia epidemiology with molecular, cellular, and behavioral neuroscience. Cross-disciplinary projects related to candidate genetic or nongenetic risk factors not only can address the biological plausibility of these factors, but they can serve as catalysts for discovery in neuroscience. This type of cross disciplinary research is likely to be more efficient compared to clinically dislocated basic neuroscience. Examples of this type of translational research are provided based on (a) the impact of prenatal nutrition and prenatal infection on brain development and (b) understanding the causes and consequences of agenesis of the corpus callosum. We need to build shared discovery platforms that encourage greater cross-fertilization between schizophrenia epidemiology and basic neuroscience research.

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### Recently Identified Risk Factors in Schizophrenia

In this volume, Xu et al<sup>1</sup> present evidence linking prenatal exposure to famine with an increased risk of schizophrenia. The findings are consistent with 2 other studies.<sup>2,3</sup> Severe famine is associated with adverse outcomes at many levels—the striking drop in birth rates during

the famine is a stark testament to this.<sup>1</sup> Why do neonates who are lucky enough to survive this extreme nutritional stressor go on to have an increased risk of developing schizophrenia? Which aspects of brain development are less able to buffer this type of perturbation?

Like many hypotheses in medicine, the first clues linking prenatal famine and schizophrenia emerged from ecological studies. These studies have compared the risk of schizophrenia for birth cohorts who were in utero during the famine vs control cohorts who were not exposed to prenatal famine. Because ecological studies rely on indirect assessment of the exposure, stronger evidence from additional types of research is needed. Reliable case-control level data on prenatal diet during famines are not so readily available. Randomized controlled trials of exposure to famine are neither feasible nor ethical. Clearly, we do not need any additional evidence to confirm that famines have disastrous immediate and long-term consequences for a wide range of health outcomes. So, where to next for this type of research? Focused clinical research looking at the physiological consequences of prenatal exposure to famine can help.<sup>4</sup> But the utility of these clues is best addressed in experimental research based on animal models. What are the consequences of prenatal protein and calorie restriction to brain development? Do data from animal models recapitulate clinical findings?<sup>5</sup> This work is now underway, and the early results are promising. For example, Palmer et al<sup>6</sup> examined a rat model of prenatal protein deprivation. They identified a range of behavioral and neurochemical findings that linked the prenatal exposure to altered dopaminergic and glutaminergic neurotransmission in the adult offspring of the model.

### Public Health and the Search for Candidate Exposures

Nutritional exposures are of particular interest from a public health perspective because they can offer opportunities for public health interventions.<sup>7,8</sup> Similarly, prenatal infection, an exposure that has long been on the radar screen of schizophrenia epidemiology, offers the chance of prevention via vaccinations or other public health measures.<sup>9</sup> Even if these exposures are thought to explain only a small proportion of all cases, if the public health interventions are cheap and safe, there is a strong case to support this type of research. From

<sup>1</sup>To whom correspondence should be addressed; Queensland Centre for Mental Health Research, The Park Centre for Mental Health, Wacol, Queensland 4076, Australia; tel: +61-7-3271-8694, fax: +61-7-3271-8698, e-mail: john\_mcgrath@qcmhr.uq.edu.au.

the perspective of “dollar per disability-adjusted-life-year averted,”<sup>10,11</sup> these interventions could be sound investments.

None of the nutritional or infectious candidate risk factors currently have a sufficiently coherent and robust evidence base to allow us to make public health recommendations. It is unlikely that we will ever reach a level of evidence that could be classified as “beyond reasonable doubt,” but it is feasible that we will reach a level of evidence consistent with the “preponderance of evidence.”<sup>12</sup>

### The Fertile Intersection Between Schizophrenia Epidemiology and Developmental Neurobiology

In addition to translational research that progresses information from epidemiology to the arena of public health, the research community also benefits from translating these clues into the field of neurobiology. Also appearing in this volume, Kinney et al<sup>13</sup> examine hypotheses that might explain the latitude gradient in the prevalence of schizophrenia. They leverage these clues in order to explore hypotheses related to prenatal vitamin D deficiency<sup>14</sup> and/or prenatal infection.<sup>15</sup> Both these candidate exposures demonstrate how information from epidemiology can lead to new discoveries in developmental neurobiology.

With respect to developmental vitamin D deficiency as a risk factor for schizophrenia, while data on season of birth, latitude gradient, and migrant studies are tantalizing, the evidence from analytical epidemiology remains scant.<sup>16,17</sup> In contrast, rodent models of developmental vitamin D deficiency have provided many findings that may be of interest to schizophrenia research (eg, enlarged ventricles, altered sensitivity to glutamatergic agents).<sup>18–20</sup> This research has subsequently stimulated interest in the role of this neurosteroid in adult brain function.<sup>21,22</sup> Similarly, while epidemiological research linking prenatal infection and schizophrenia has been slowly accumulating evidence over the decades, the pace of discovery has been accelerated considerably by developmental neurobiology groups using animal models.<sup>23,24</sup> In particular, Feldon and colleagues have undertaken an impressive array of studies that have elucidated connections linking prenatal immune activation and altered brain development.<sup>25</sup> The discoveries are now able to feedback into more focused and hypothesis-driven analytical epidemiology.

Neurobiology is currently an intensely fertile area of research, and the pace of discovery is astounding. For the schizophrenia researcher, trying to engage with neuroscience is akin to “sipping from a fire hose.” Despite this, we argue that it is critical that schizophrenia epidemiology is firmly anchored to a neurobiologically informed framework. While clinical research is clearly important, animal models can play a key role in unraveling the biological mechanisms linking early life disruptions

to later neuropsychiatric disorders. The field is now mature enough to appreciate that animal models will never recapitulate the full phenotype of clinical disorders that impact on higher cognitive function. However, animal models provide an experimental platform that allows researchers to focus on more substrate-pure neurobiological correlates of clinical syndromes.<sup>26</sup>

Apart from the need to test our candidate exposures in animal models, how can schizophrenia research best guide neuroscience research? Schizophrenia researchers have the skills to generate candidate exposures and to identify neuroanatomical, neurochemical, or behavioral phenotypes of interest to clinical research. Animal models can help explore the neurobiological correlates of intermediate phenotypes of interest to our field (eg, differential sensitivity to glutamatergic or dopaminergic agents). Rodent models, zebra fish, or invertebrates such as *Drosophila* and *Caenorhabditis elegans* can provide powerful and efficient research platforms to explore key research questions for both genetic and nongenetic risk factors<sup>27–29</sup> and to help identify the function of genetic candidates.

### Clinical Syndromes Catalyze Discovery Pathways

Like neuroscience, epidemiology is a broad discipline. As discussed above, clues from *risk factor epidemiology* (eg, prenatal nutrition and infection) have catalyzed novel discovery pathways for developmental neurobiology. *Descriptive epidemiology* also plays an important role in defining key parameters such as the incidence and prevalence of a disorder. *Genetic or molecular epidemiology* applies family- or population-based frameworks to the assessment of genetic factors that influence health and disease. Increasingly, epidemiological research is incorporating genetic variations into disease model (eg, Mendelian randomization<sup>30</sup>). The intersection between genes and the environment has been the focus on a recent collection of articles appearing in *Schizophrenia Bulletin*.<sup>31–34</sup>

While large population-based studies are important for risk factor research, simple case series studies remain a key foundation stone for neuroscience. Observant clinicians are central in the identification of neuropsychiatric syndromes. The next section of this commentary will briefly discuss how neuroscience has leveraged clues from case series of disorders that share a common anatomical feature—agenesis of the corpus callosum (ACC). It is one of many examples of how the simplest form of epidemiological study, the case series, can galvanize research.

ACC is associated with a large number of human congenital disorders, with phenotypes ranging from severe motor and sensory deficits as well as mental retardation, to more mild symptoms involving learning difficulties, language deficits, and social-behavioral problems.<sup>35</sup>

Like schizophrenia, ACC is a heterogeneous group of disorders. Unlike schizophrenia, it is characterized by a prominent neuroanatomical feature. In recent years, a large number of mouse mutants that display ACC have been identified, and thus, some progress has been made in identifying genes and the underlying mechanisms involved (reviewed in Lindwall et al<sup>36</sup>). Understanding the basic biology of the system is essential for understanding the pathology.

Because ACC is a gross structural abnormality in the brain, it is relatively easy to diagnose and study in both humans and animal models. However, neuropsychiatric conditions such as schizophrenia and autism are much more complex and thus much more difficult to unravel. These disorders involve aspects of brain function and circuitry that are more uniquely human and less amenable to modeling in animals. However, in the case of autism, translational and basic research has revealed new insights into the genetic and molecular basis for this disorder (reviewed recently in Geschwind,<sup>37</sup> Walsh et al,<sup>38</sup> Kelleher and Bear<sup>39</sup>). Unexpectedly, research related to understanding the pathogenesis of ACC is now cross-linking with autism research. For example, an inbred mouse strain that is associated with ACC<sup>40</sup> displays a range of behaviors associated with the clinical phenotype of autism (eg, altered vocalisation, repetitive and stereotypic behaviors, altered social behavior).<sup>41–43</sup> Curiously, a recent report of a transgenic mouse engineered to express a truncated Disrupted in Schizophrenia 1 (DISC1) gene was also reported to have a partial ACC.<sup>44</sup> As is typical for scientific discovery, research stimulated by one research question can unexpectedly uncover answers for a completely different question.

Autism, like schizophrenia, is associated with many different genetic disruptions, which in turn, impact on apparently disparate cellular mechanisms including synaptic dysfunction, metabolism, RNA splicing, and neuronal migration.<sup>37</sup> Much work needs to be done in order to link these molecular and cellular features to neuroanatomy and behavioral neuroscience. Here, the advances in imaging technology continue to provide new insights into brain structure and function. With respect to understanding disruption of white matter tracts such as ACC, these techniques have particularly important. Magnetic resonance imaging (MRI) techniques known as “diffusion tensor imaging” (DTI) have been used to study the brain in humans and animal models.<sup>45–47</sup> In simple terms, DTI allows for the axon tracts of the brain to be color coded depending on their orientation. This can provide a 3-dimensional view of the axon tracts of the brain and their anatomical projections in relation to one another. Axonal tracts can be viewed in any orientation, making this type of analysis extremely powerful for identifying gross defects in axon tract morphology. One problem with DTI is that it is not good at resolving areas where axonal bundles cross one another. Newer imaging techniques such as

high angular resolution diffusion imaging (HARDI) coupled with Q-ball reconstruction overcome the fiber crossing problem and allow unprecedented anatomical precision with which to study the brain. In cases of partial ACC, it has been shown that cortical axons passing through the callosal remnant are diverse in their origins between different patients.<sup>48</sup> Even patients who seem to have similar callosal remnants by anatomical scans have vastly different axonal connection patterns.<sup>48</sup> Such patients even show ectopic “sigmoid” bundles previously found in ACC patients by DTI analysis.<sup>49</sup>

This imaging work demonstrates one way in which new and powerful anatomical techniques can be applied to studying brain connectivity in relation to function and dysfunction. In schizophrenia research, valuable insights have been gained from studying brain anatomy throughout development by structural MRI and observing differences in brain regional activation through functional MRI (reviewed in Paus et al<sup>50</sup>). Newer techniques such as HARDI/Q-ball offer the possibility of understanding brain connectivity in schizophrenic patients and ultimately some insight into the anatomical basis of this disease.

### Building-Shared Research Platforms

The neuroanatomical and neurohistochemical correlates of schizophrenia are much more subtle than ACC.<sup>51</sup> Within the context of the heterogeneity of this poorly understood group of brain disorders, this is to be expected. But, regardless of this heterogeneity, research inspired by the epidemiological clues such as prenatal nutrition and prenatal infection are more likely to lead to the identification of informative pathways compared with clinically dislocated basic neuroscience research. Similarly, on its own, epidemiology will never be able to address the bio-complexity underpinning a poorly understood group of disorders like schizophrenia.

Schizophrenia epidemiology can provide us with valuable information to guide research directions. These clues are much too important to waste. But, while epidemiology is useful for generating candidate risk factors, it cannot always readily deliver studies that prove causality.<sup>52</sup> The best returns will come from linking schizophrenia epidemiology with molecular, cellular, and behavioral neuroscience. Cross-disciplinary projects related to candidate genetic or nongenetic risk factors can address the biological plausibility of these factors and can also provide a road to new discoveries in neuroscience. We need to build shared discovery platforms that encourage greater cross-fertilization between schizophrenia epidemiology and basic neuroscience research.

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