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Heterogeneity and hypothesis testing in neuropsychiatric illness

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Abstract

The confounding effects of heterogeneity in biological psychiatry and psychiatric genetics have been widely discussed in the literature. We suggest an approach in which heterogeneity may be put to use in hypothesis testing and may find application in evaluation of the Crespi and Badcock (C&B) imprinting hypothesis. Here we consider three potential sources of etiologic subtypes for analysis.

Coming to grips with the problem of heterogeneity has been a long-standing challenge to neuropsychiatric research. Etiologic and phenotypic forms of heterogeneity have the potential to sap experimental designs of their statistical power to detect group differences among diagnostic and control groups, and to frustrate attempts to identify genes associated with neuropsychiatric diagnostic status (Fanous & Kendler 2005; McClellan et al. 2007).

Heterogeneity and its consequences hold broad implications for hypothesis testing in the field, including theses put forward by Crespi and Badcock (C&B) regarding genomic imprinting. The authors acknowledge that their topics of study, autistic- and psychotic-spectrum disorders, have multiple causes that are not necessarily related to imprinting. Nonetheless, they posit a lawful tendency for imprinted genes with maternal expression to underlie the psychosis spectrum, whereas paternally expressed genes contribute to the autism spectrum. Will these effects of imprinting cohere in the face of heterogeneity?

Typically, discussions of heterogeneity in neuropsychiatry strategize to surmount obstacles to discovery. In contrast, our commentary discusses a way that one might turn etiologic heterogeneity to an advantage, for example, finding application in testing the C&B imprinting hypothesis. We limit our comments to this aspect of the target article, and our examples derive primarily from autism research.

A rigorous test of their hypothesis requires multiple instances of imprinting to evaluate whether parent-of-origin effects are statistically associated with diagnosis. In the absence of statistical inference, the imprinting hypothesis is speculative and narrowly based.

However, combining across a collection of these instances – utilizing an etiologically heterogeneous assortment conditions associated with a diagnostic spectrum – one could determine whether predicted imprinting effects indeed assort with diagnosis. How readily can multiple instances of conditions be attained? We outline three approaches in the following paragraphs.

First, it seems reasonable that known genes for a disorder might provide a test of this hypothesis. However, our current stage of knowledge is limited. Except for syndromic forms in which mutations in a single gene have been specified, no genes have been unambiguously associated with autism (Sebat et al. 2007). Perhaps with the advent of large-scale whole-genome association studies (Ropers 2007), solid findings of single genes may appear for autism and psychosis. Also, the power to detect these genes may be increased by the use of strategies such as stratification on phenotypic subtypes and incorporation of broader

phenotypes (Geschwind & Levitt 2007; Matthysse et al. 1992; McCaffery & Deutsch 2005; Spence et al. 2006). Once reliable single-gene effects are documented, the imprinted subset would be relevant vis-à-vis the authors' hypothesis.

Second, until these single genes are identified, potentially informative tests may be derived from identified genetic syndromes associated with neuropsychiatric phenotypes. This approach to testing the imprinting hypothesis was adopted by C&B. Take, for example, the contrast of Prader-Willi and Angelman syndromes – two imprinted forms of a genetic disorder (deletion of 15q11-q13; Christian et al. 1999). The authors adduced a pattern in which autistic symptomatology in Angelman syndrome, as opposed to psychotic features in Prader-Willi syndrome, is associated respectively with dysregulation of imprinted genes towards paternal and maternal origin.

This experimental strategy lends itself to a test of the imprinting hypothesis. Many genetic syndromes display autistic and/or psychotic symptomatology. Are there enough to test the imprinting hypothesis at hand? To address this question, first one might ask whether these syndromes yield bona fide cases of neuropsychiatric illness that meet standard diagnostic criteria. We have found that conditions associated with autism reveal many syndromes that do meet the “gold standard” of the Autism Diagnostic Inventory (revised, ADI-R; Lord et al. 2001), among them Angelman (Peters et al. 2004), velocardiofacial/diGeorge (deletion of 22q11.1; Fine et al. 2005), fragile X (Clifford et al. 2007), and Smith-Lemli-Opitz (Tierney et al. 2001), as well as Cowden disease (PTEN mutation; Butler et al. 2005), Mobius sequence (Johansson et al. 2001), neurofibromatosis (Havlovicova et al. 2007), and tuberous sclerosis (Smalley 1998). A test of the C&B hypothesis would also require a priori identification of imprinting for a genetic marker.

Third, a newly emerging class of genetic markers may prove useful in testing the association of parent-of-origin effects with neuropsychiatric illness. These are alterations in DNA copy number (*copy number variants*, or CNVs; Iafrate et al. 2004; Sebat et al. 2004), and they are assayed at a level of resolution higher than conventional cytogenetic methods that have been used to identify karyotypic abnormalities in autism (Wassink et al. 2004). Recently, germline mutations have been found to be remarkably prevalent among individuals with autistic-spectrum disorders (Sebat et al. 2007). The genomic regions of these CNVs correlated with autism were highly heterogeneous and included de novo mutations of single genes. In general, variations may be de novo or familial, for example, in which the proband and one parent share the CNV.

The array-based technology to detect these CNVs is now providing yet more resolute assays, and even more CNVs are expected to be identified. Some of these may overlap, deriving from genomic regions that are particularly vulnerable (Perry et al. 2006). Sebat et al. (2007) have already found variants identified by other investigators. Potentially, variants may emerge for autism and psychosis that are tractable to imprinting studies. This subset could be studied in association with neuropsychiatric phenotypes, yielding a quantitative test of assortment.

We have suggested an instance in which heterogeneous genetic markers could be useful in hypothesis testing. Heterogeneity may have other lessons to teach us, as well. How could a miscellany of etiologic factors converge on a common behavioral phenotype? The answer has proven elusive, although there are empirically based models that address the phenomenon. For autism, there have been explanations tied to neuronal connectivity (Just et al. 2004; Rippon et al. 2007), disordered neural excitation or inhibition (Rubenstein & Merzenich 2003), aberrant connections of neural networks (Geschwind & Levitt 2007), and neurodevelopmentally altered temporal binding (Brock et al. 2002; Deutsch 1998).

Understanding how a heterogeneous collection of causal factors emerges as a single diagnostic entity is key in delineating the biology of neuropsychiatric illness.

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