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EDITORIAL

Publishing Negative Results of Neurobiological Studies in Mental Disorders Will Advance Knowledge in Pathophysiology

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Across all disciplines and most strongly in biomedical science, the proportion of publications reporting positive rather than negative results increased from 1990 to 2007, by which time 85% of published studies, mainly in the United States and Europe, confirmed the primary hypothesis.[1] This publication bias is assumed to have increased again in the last decade because of the higher likelihood that articles with statistically significant results will be accepted for publication in high-impact journals and that authors of such articles will receive third-party funding. In addition, researchers often do not publish contradictory or negative results because of time constraints and they assume that such results may not be highly cited in their scientific field.[2] However, advanced scientific knowledge can only be gained when research results are made available and reproduced,[2] and the scientific community needs to be informed about non-confirmatory results, inconclusive experiments, and unexpected findings that lead to the rejection or correction of hypotheses.[3]

One barrier to publishing negative results is the impact of power calculations and the application of statistical methods. In human neurobiology studies that analyze data statistically, 2 kinds of error may occur: A type I error, in which the null hypothesis is falsely rejected (false positive), and a type II error, in which the null hypothesis is not rejected, even though the alternative is true (false negative). Although the likelihood of obtaining false positive findings is controlled in the vast majority of studies ($P < \alpha$, usually $\alpha = .05$), the likelihood of obtaining false negative findings (β) is not often controlled, especially if no priori power analysis is performed.[4] Therefore, positive results are often better quantified than negative results, leading to a publication bias in favor of the former. Nevertheless, we believe that to advance scientific knowledge, negative results must be verified in large samples or in several independent studies that can then be summarized and analyzed by meta-analyses.

This issue of *Schizophrenia Bulletin* contains the study by Min et al,[5] who systematically searched for central nervous system-related DNA and RNA viruses in 1569 postmortem brains of patients with schizophrenia, bipolar disorder, autism spectrum disorder, and controls by high-throughput next-generation sequencing technology.[5] This is the largest postmortem study on this topic published to date. Min et al. occasionally detected Herpesviridae (EBV, CMV, HHV-6A, HHV-6B, HHV7), Polyomaviridae (JCV, HPyV6), Retroviridae (HIV, HERV-K113), Flaviviridae (GBV-C, HCV), Parvoviridae (B19V, V9, AAV), and Adenoviridae (HAdV-C) in the samples but found no significant differences between the diagnostic groups. This is a clear negative result that suggests that there is no association between persistent viral infection and major mental disorders. The study is limited by the inclusion of fewer brains from patients with schizophrenia and bipolar disorder than from healthy controls and especially by the small number of brains from patients with autism spectrum disorder, so the results should be confirmed in independent samples. However,

confirmatory studies may be difficult to realize because of the low availability of postmortem brains from patients with mental disorders.

This negative result is surprising considering the recent increased interest in the field of immune alterations in mental illness. A thorough literature search reveals that the results of the study by Min et al[5] are in agreement with those of previous smaller human postmortem brain studies, although the titles of some earlier studies are misleading and formulated as if the study results were positive. An example of such a study is "Infectious agents associated with schizophrenia: A meta-analysis" by Arias et al.[6] Here, the positive finding was obtained from a mixture of in vivo studies on antiviral antibodies in blood and postmortem studies on viral DNA and RNA in brain tissue. If one examines the results of the individual studies in detail, it becomes clear that some antiviral antibodies were detected more frequently in the blood of patients than in that of controls but that viral DNA or RNA was not found more often in the brains of patients than in those of the controls and that there were very few positive cases overall. Thus, the idea of a persistent brain infection as one of the concepts of the "mild neuroinflammation hypothesis of mental disorders" does not hold true for the tested viruses. However, viruses do not necessarily influence the course of mental diseases by persisting in the brain. Even if a virus is eliminated by the immune system, transient disturbances of neurotransmission may occur, eg, by proinflammatory cytokine-induced modulation of the tryptophan-kynurenine pathway or by cross-reaction of antibodies against neurotransmitter receptors.[7]

It should be borne in mind that by their very nature, postmortem studies analyze processes in more advanced disease stages than in vivo studies do and thus do not necessarily explain disease etiology. The elevated antiviral immunoglobulin G antibodies found by Arias et al[6] in the blood of some patients with schizophrenia are more likely to represent "serum scars" after viral infection before or during the onset of the mental illness, eg, during neurodevelopmental periods, than persistent infection. Large epidemiological studies have provided evidence that infectious agents may play a role as risk factors for the manifestation of mental disorders.[8] However, this potential role does not appear to be linked to specific pathogens.[8] Rather, we can assume that infectious agents may trigger the onset or worsening of mental illness, similar to the role of psychosocial stressors or drugs in the vulnerability-stress concept. Therefore, neuroinflammation may influence the individual course of the disease in vulnerable individuals who are predisposed to developing the disease because they have genetic and/or perinatal brain developmental risk factors.

In the past few decades, researchers have proposed that the cellular immune system and alterations of the density or activation of microglia are involved in schizophrenia. A first meta-analysis with 181 patients and 159 controls showed an increase in microglia density, but this increase was seen only in a few study cohorts and was not a generalized and stable effect.[9] Overall, the heterogeneous results are not surprising because cell density measurements may be affected by variations in tissue shrinkage after fixation and staining procedures. A more recent meta-analysis in a larger cohort of 238 patients with schizophrenia and 252 controls that excluded studies with overlapping samples considered different microglia markers, such as HLA-DR, CD68 (as markers of activated microglia and macrophages), and Iba1, which is also expressed in non-activated microglia. This study found no changes in the density or morphology of microglia in schizophrenia.[10] Consistent with previous genome-wide transcriptome analyses, expression of specific microglia genes was decreased in schizophrenia. Thus, the study strengthens the hypothesis that immune-related gene expression alterations overlap in mental disorders, but the results are in contrast to those in classical neurodegenerative diseases with confirmed neuroinflammation, such as Alzheimer's disease.[10]

In mental disorders, subtle alterations of the immune system may be involved in pathophysiological processes other than those in neurodegenerative diseases. The immune system may be involved only in a subgroup of patients with mental illness, so its specific role in individual patients should not be overestimated, just as the influence of stress depends on personal vulnerability and can favor the development of a gastric ulcer in one person and mental illness in another, for example. The role of long-term psychopharmacological treatment should also be considered because some drugs, eg, antipsychotics, have anti-inflammatory properties. Altogether, subgroups of patients with neuroimmunological abnormalities need to be better characterized with respect to clinical symptoms, cognitive performance, and longitudinal outcomes. Furthermore, neurobiological pathways involving immune alterations have to be identified. Although psychoimmunology has become very fashionable in recent years, we encourage researchers and editors to publish also negative results on this topic so that hypotheses can be proved and are not erroneously confirmed because of publication bias.

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Conflict of Interest

JS and AS declare no conflicts of interest. PF is a coeditor of the German Association for Psychiatry,

Psychotherapy and Psychosomatics (DGPPN) schizophrenia treatment guidelines and a coauthor of the World Federation of Societies of Biological Psychiatry (WFSBP) schizophrenia treatment guidelines; he is on the advisory boards and receives speaker fees from Janssen, Lundbeck, Otsuka, Servier, and Richter.

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