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Special Lecture

Current and Future Animal Modeling of Schizophrenia

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Abstract

During the last 100 years, how far has the biological investigation of schizophrenia, its scientific diagnostics, or its modeling progressed? Clinical and/or basic medical studies on schizophrenia, have proposed more than 100 etiologic and pathogenic hypotheses, suggesting the roles of various genes, intestinal bacteria, infectious immunity, and stress exposure, etc. The number of these hypotheses are still increasing and broadening and the confusion of the schizophrenia etiology is rather deepening. One of the reasons stems from the theory of molecular and cellular reductionism as well as from the concept of RDoC. In spite of the theory, the current neuroscience hardly illustrates the integrative system of higher brain function, which performs strong nonlinear processing with multi-variates of sensory inputs and their memory traces. With the limited neuroscience knowledges, therefore, we are not able to fully illustrate any of psychotic symptoms with the current neurosciences. At present, we believe that the selection of the most proper animal model(s) relevant to the schizophrenia pathology or physiology is the most important step. Analysis of the model animal that mimics the cytokine storm famous for COVID-19 and RS virus infection has revealed the possibility that mice or rats exposed to the cytokine EGF may model hallucinations, communication deficits and soliloquy after maturity. The EGF-treated model is known to exhibit similar pathophysiology in various electroencephalogram tests (ASSR, MMN, P300, ABR etc.) as well. Rats seem to have

higher intelligence and emotions and perform various games and tricks with human players. Thus we are scientifically reverse-translating the characteristics of the patient's symptomatology and pathophysiology into rodent scales with the modern technology. Such as the fact that the cause of gastric cancer was Helicobacter pylori, we must keep in mind that the biggest problem hidden in academic challenges is the curse of existing concepts and knowledges.

Keywords: schizophrenia, RDoC, cytokine storm, reverse-translation, animal modeling

Introduction

Not only in the U.S. but also in Japan, short-term results, i.e., the development of diagnostic and therapeutic methods for psychiatric disorders, are increasingly demanded in order to obtain funding for basic medical research. In recent years, cognitive brain research appears to slow down as its focus has shifted to the study of the complex neural network systems, such as higher brain functions. We conducted a quantitative analysis of the current status of global schizophrenia research in terms of the number of publications using PubMed, dividing it into clinical and basic research. The number of clinical patient studies of schizophrenia began to increase significantly in the early 2000s, peaking at 6,000 papers in 2015, and then started to decline. On the other hand, research on animal models of schizophrenia also increased explosively around 2000, and then declined significantly after peaking in 2015 with 782 papers. What is the reason for this? It does not seem to be simply because of the decrease in research funding for basic medical research.

In contrast to schizophrenia research, the number of clinical research papers on Alzheimer's disease has continued to increase, and exceeded 13,000 papers by 2020. Research on animal models of Alzheimer's disease have also shown a similar trend of continuous increase. It is not clear whether the headway in biological research of schizophrenia is due to the lack of neuroscientific progress in cognitive neuroscience over the past 20 years, or whether it is due to the large amount of money being invested in the other fields as a result of societal demand. However, it may be true that schizophrenia research is in decline as a result.

I. Global Network of Brain Functions

The Research Domain Criteria

(RDoC) is a new diagnostic axis of the schizophrenia construct that is being promoted mainly in Europe and the United States. It was proposed by Dr. Insel, former director of the National Institutes of Mental Health (NIMH) in the United States. In order to promote diagnostics and neuroscience research on psychiatric disorders, he stated that it is necessary to study everything from genes to circuits and behavior, assuming functional disorders in the six brain functional domains based on neuroscience theory and discriminating corresponding disorders for each functional domain 7). It is also believed that the classification of diseases based on this concept is consistent with the classification of therapeutic drugs into efficacy groups, but is this really true?

The six functional domains of RDoC are: negative function, positive function, cognition, sociality, wake-sleep control, and the perceptual-sensory system. This RDoC domain concept divides the cognitive brain function hierarchy, which is located in the third layer of the seven-layer brain function structure in Figure 1, into six, but this functional reductionism concept itself is also inconsistent. In actual brain functions, especially in their developmental processes, the interactions among these domains are strong, and the domains cannot develop functions by themselves 17). For example, the function of the social domain in autism spectrum disorder is strongly influenced by other domains, such as the perceptual-sensory domain for facial expression recognition, the negative function domain for worry and fear emotions, and the cognitive domain for memory and learning abilities, to develop and acquire the appropriate functional processing style.

In current research on the higher-order association cortex of the brain, many argue that instead of a domain-type global network that assumes functional domains and domain clusters, it is better to consider an integrated global network that is much larger and has a higher degree of freedom (Figure 2) 3). In other words, they believe that the information-processing network of the higher brain does not have a reductionistic structure such as functional domains, but rather shares information more or less widely with the entire brain. However, such an integrated global network is closer to the basic structure of artificial intelligence (AI), which poses the difficult problem that human cognitive functions and emotions may not be explained by a meaningful causal or linear theory. In other words, our current reductionistic academic systems may not be able to understand them. Thus, there is a possibility that the understanding of schizophrenia

symptoms and its diagnosis may be markedly improved by studying artificial intelligence that is similar to the human brain structure.

II. Understanding the Brain through Neuroscience

The operating principles of brain functions in each domain defined in RDoC are still largely unresolved. Neuroscience began in the early 1900s when Drs. Golgi and Cajal described the neural circuits in the brain, so neuroscience research, like schizophrenia research, is only about 100 years old (Figure 3). Although the functions of the peripheral nervous system have been elucidated to a marked extent, the cerebral mechanisms of higher brain functions, such as language and text comprehension, consciousness, and attention control, which are typical of schizophrenia, are still largely unknown. These cognitive and associative functions have not yet been fully reproduced by artificial intelligence. In fact, although the projection pathways in the peripheral nervous system and spinal cord have been identified for the somatosensory sensations of "pain," "temperature," and "itch," we do not even know how these sensations are discriminated and encoded in the cerebrum.

However, recent studies on such sensory information have revealed that the information in the brain, which is supported by memories such as "predictive coding" and "top-down", markedly affects the actual sensory processing from the peripheral nervous system 1). In other words, it has become clear that memory and the five senses strongly influence real sensory perception, such as the perception of "hotness" at the mere sight of a flame. This is also consistent with the integrated global network hypothesis of information processing in the brain mentioned earlier.

III. Animal Models of Schizophrenia

To the present, hypotheses and models of schizophrenia have been developed mainly on the basis of their relevance (construct validity) to genetic association analysis, epidemiology, and drug abuse data in schizophrenia. These hypotheses and models have been evaluated mainly using intermediate phenotypes representing so-called animal behaviors. However, many of them, such as social behavior assessment and prepulse suppression, are widely applicable to any disease, such as schizophrenia, autism, and PTSD, and have become evaluation indices with low disease specificity (surface validity) 11). As a result, the disease specificity of model animals has also become lax, and the number of

model animals called schizophrenia models has exceeded 100 species (Fig. 4). A typical example of an animal model that has problems regarding disease specificity is a model of maternal viral infection developed by the administration of polyinosinic-polycytidylic acid (Poly I:C), which is double-stranded RNA. It has been used as a model of schizophrenia and autism. Of course, if you point out that schizophrenia and autism share a common neuroscientific basis in the form of a spectrum, I do not have words to argue against it. In any case, the gap between the current diagnostic criteria based on human psychopathology and these animal models is immeasurable. In addition, recent studies revealed that the intensity of cognitive-behavioral deficits in Poly I:C-treated models of maternal infection varies markedly depending on where the animals were purchased 12). In other words, the influence of external environmental factors may be stronger in models of psychiatric disorders related to inflammatory immunity than we have imagined. This fact is consistent with a paper claiming that intestinal bacteria hold the key to pathogenesis in a model of maternal infection developed by Poly I:C administration 4).

In the current situation, the approach that will bring about a breakthrough in the study of schizophrenia models may be the application of human data to animal models, based on recent advances in functional brain imaging research and multi-point EEG analysis. In other words, rather than trying to explain auditory hallucinations using immature neuroscience knowledge, would it not be a shorter path to accumulate scientific evidence of the patient's pathophysiology and model or analyze them? Changes in Blood Oxygen Level Dependent (BOLD) signals and EEG activity and their connectivity abnormalities, which are associated with auditory hallucinations and hallucinations, have been attracting attention 9)13). These EEG-related physiological indexes and BOLD signals can be measured in rodents, and the mechanisms of their changes can be explored from a neuroscientific perspective. They are also quantifiable indicators that can be used to determine their relationship with psychological symptoms in human patients. In the past, translational research, in which neuroscience data derived from animals were applied to humans, was popular, but in the future, reverse translational research, in which human disease physiology data or psychological symptoms are applied or extrapolated to animals, is expected to become popular.

IV. Fetal and Neonatal Inflammation and Schizophrenia Models

For many years, we have been exploring the neuroscientific relevance and causal relationship between inflammatory cytokines and schizophrenia by generating various animal models 15)20). Indeed, series of inflammatory cytokines are strongly induced in the body following maternal infection or perinatal disorders and are known to disrupt brain development. The phenomenon in which such cytokines are expressed in large amounts in the body is called a "cytokine storm" and has become well-known as a cause of death in patients with pneumonia due to novel coronavirus infection (COVID-19) (Figure 5). Our studies have shown that among the cytokines involved in cytokine storms, a cytokine called epidermal growth factor (EGF) causes the most potent and sustained cognitive behavioral changes when administered to animals 14)18). Postnatal administration of EGF to mice, rats, and monkeys results in abnormalities of various behavioral indicators after sexual maturation, including impaired prepulse inhibition, impaired social behavior, stereotypical behavior, and irritability. Surprisingly, EGF is the cytokine most intensely activated in COVID-19-induced pneumonia and neonatal RS virus infection, and has the strongest pathological association with the severity of COVID-19-induced pneumonia 5)19). Although it is only a hypothesis, our animal model study suggests the risk of a cytokine storm caused by COVID-19 in pregnant women and newborns.

However, this alone is far from sufficient to suggest that EGF-treated animals that can mimic COVID-19 are valid models of schizophrenia, as mentioned above. We are currently investigating whether human pathophysiological indicators can be reproduced in various animal models of inflammation-induced schizophrenia, incorporating the concept of reverse-translation described earlier. The EGF-treated model rat was surprisingly found to show almost homologous abnormalities on comparison with schizophrenia patients in a number of auditory physiological responses [auditory steady-state response (ASSR), mismatch negativity (MMN), auditory brainstem response (ABR), and positive event-related potential (P300)] 6)10). We are also working on the reverse-translation of human psychopathology and functional brain imaging abnormalities. Although research is still in the validation phase, it is becoming clear that this model also exhibits frequent soliloquy, abnormal frontal-temporal cortical communication, hyperactivity in the auditory cortex, and auditory fear-emotional hypersensitivity after maturity.

In the future, we would like to use these animal models for calcium imaging of whole-brain activity and optogenetic intervention of the auditory and somatosensory cortices to reproduce auditory hallucinations and discrimination disorders, to more directly address the causes of psychopathological symptoms and physiological brain imaging changes in psychiatric disorders. Do rats and monkeys have pathological conditions similar to schizophrenia? Many psychiatry clinicians have stated that schizophrenia is a human higher-brain dysfunction represented by the language system that does not exist in animals. Of course, it is not possible to reproduce psychiatric symptoms in animals in a strict sense that is applicable to the Diagnostic and Statistical Manual of Mental Disorders (DSM) or the International Statistical Classification of Diseases and Related Health Problems (ICD). However, the author believes that hallucinogenic brain activity in the rat, social fear behavior in the mouse, and vulnerability changes in psychological stress in the monkey exist in their own fashion. In particular, if you think that rats do not have advanced cognitive abilities or emotionality, you are mistaken. In recent years, several reports have appeared in scientific journals such as Science, showing that rats can play, laugh, and play hide-and-seek with humans. If time permits, try searching for "Rat, Trick" on the Internet. You will be surprised at the unexpectedly high cognitive abilities and emotional behavior of rats. If rats are intensively trained as pets, they not only exhibit abilities that rival those of dogs, such as tightrope walking, coin collecting, outdoor rearing, and ball picking, but also show behaviors such as jealousy and affection toward their owners 8)16). It may be shortsighted to consider that mice do not have emotions such as affection simply because we humans cannot distinguish facial expressions of mice. The latest computer image analysis technology can discriminate such changes in facial expression in mice. In fact, it has been proven that mice have emotions and facial expressions in their own way 2). Based on these facts, it may not be a far-fetched idea that "the prototype of human auditory hallucination exists in mice".

Conclusion

It has been about 100 years since research on schizophrenia began. Animal model studies of schizophrenia using neuroscience have come to a standstill in recent years. On the other hand, patient-based studies are leading to the accumulation of many clinical and medical findings, and hypotheses

are being consolidated due to progress in brain imaging research, breakthroughs in physiological measurement techniques, and the expansion of patient diagnostic and intervention methods, beginning with transcranial magnetic stimulation (TMS) and other therapies. Since many of these patient data and measurement techniques can be reverse-translated to animals, it may be possible to conduct more evidence-based neuroscience research, perhaps even auditory hallucination research, through the selection of animal models in the future.

We have no conflicts of interest to disclose in connection with this paper.

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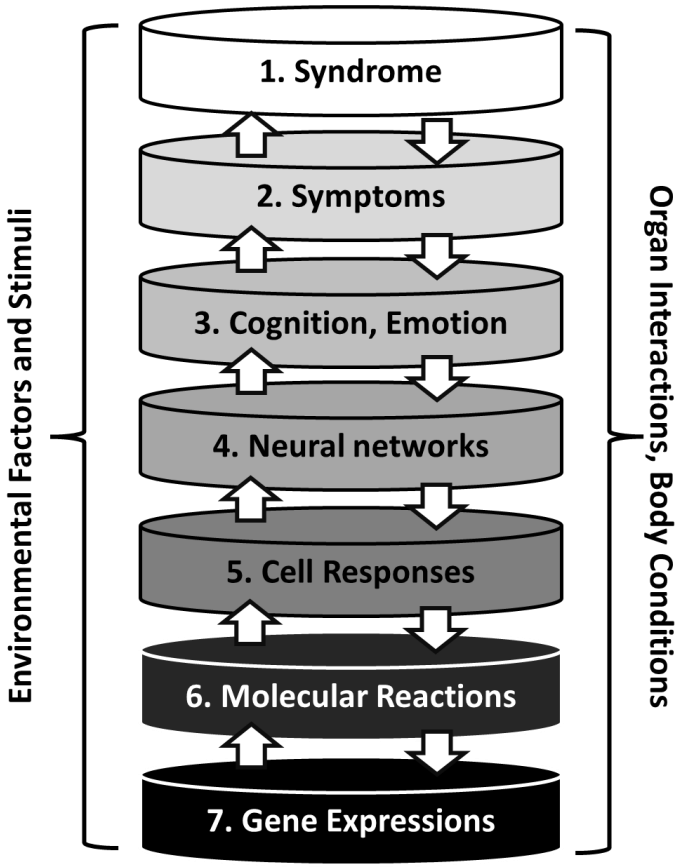

Figure 1: Integrated Operating Principle of Brain Function in Psychiatric Disorders

This diagrammatic representation of abnormal operating principles of the brain function in psychiatric disorders is divided into seven hierarchical levels (scales). Each hierarchy is regulated by another hierarchy and is also affected by external environmental factors (sunlight, temperature) and internal environmental factors (immunity, nutrition, endocrinology), resulting in nonlinear and complex behavior, which requires research from a multi-scale perspective.

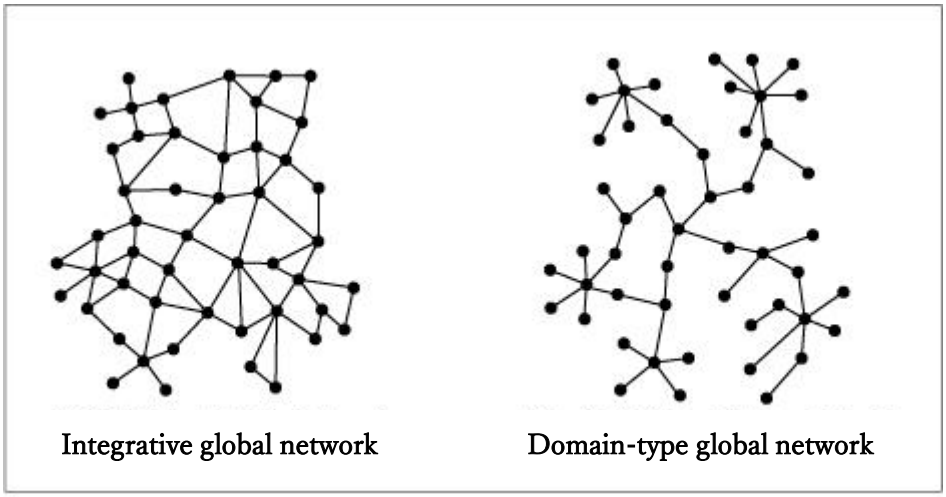

Figure 2: Global Network Hypothesis
The information network in the brain consists of clusters or domains for each sensory, emotional, and cognitive function; however, how much freedom do they have to communicate with each other? Depending on the level of freedom of the information connections, it has been hypothesized that there are domain-type global networks with information clusters and integrated global networks with larger degrees of freedom. Although it is known from neuroanatomy that the domain structure of perceptual input exists in hardware, it does not necessarily mean that higher-order information is processed in the same way. (Adapted with modifications from Reference 3)

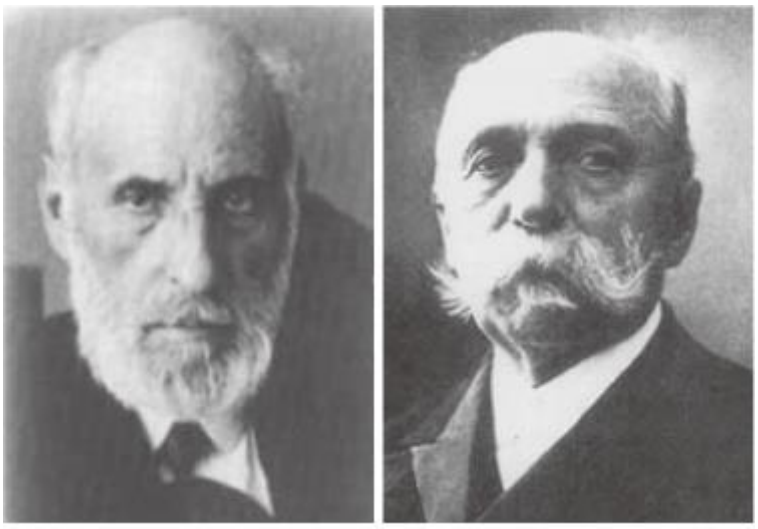

Figure 3: Drs. Cajal (left) and Golgi (right)
Santiago Ramón y Cajal (1852-1934) and Camillo Golgi (1843-1926), who at the time were disputing whether a physical gap existed in the connections of nerves, so-called synapses, were both awarded the Nobel Prize in Physiology or Medicine in 1906 for their discoveries and contributions to neuroanatomy. This coincided with the time when Dr. Kraepelin began describing and studying schizophrenia.

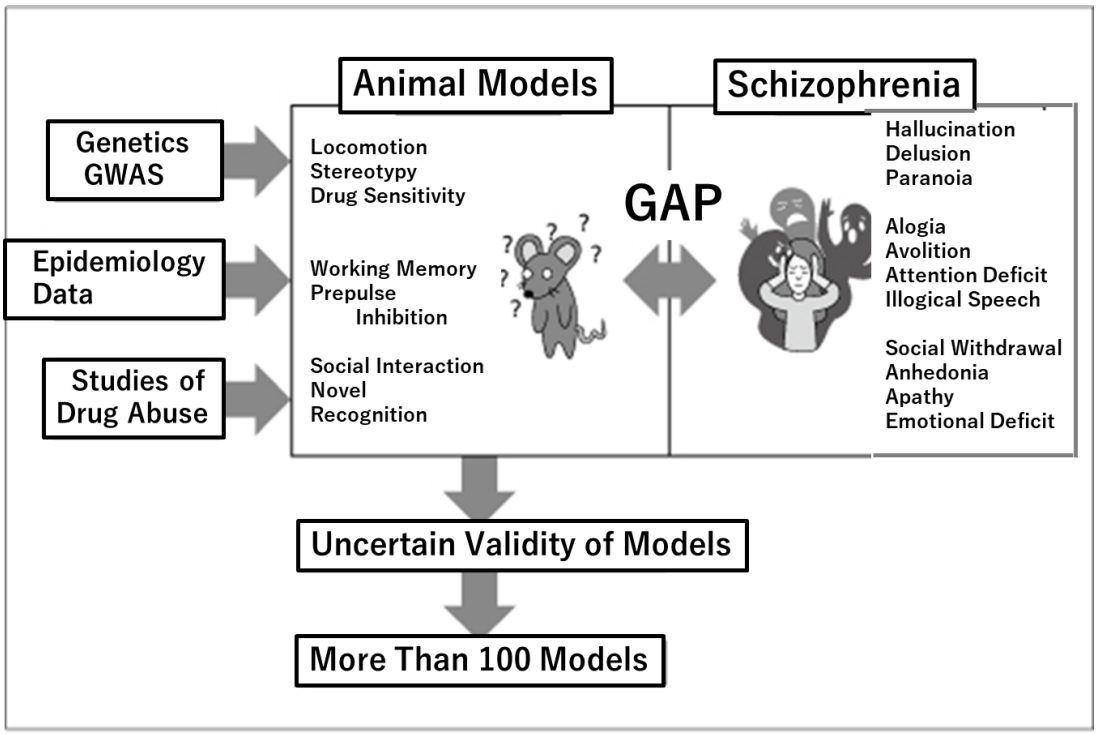

Figure 4: The large gap between schizophrenia patient symptoms and phenotypes in animal models

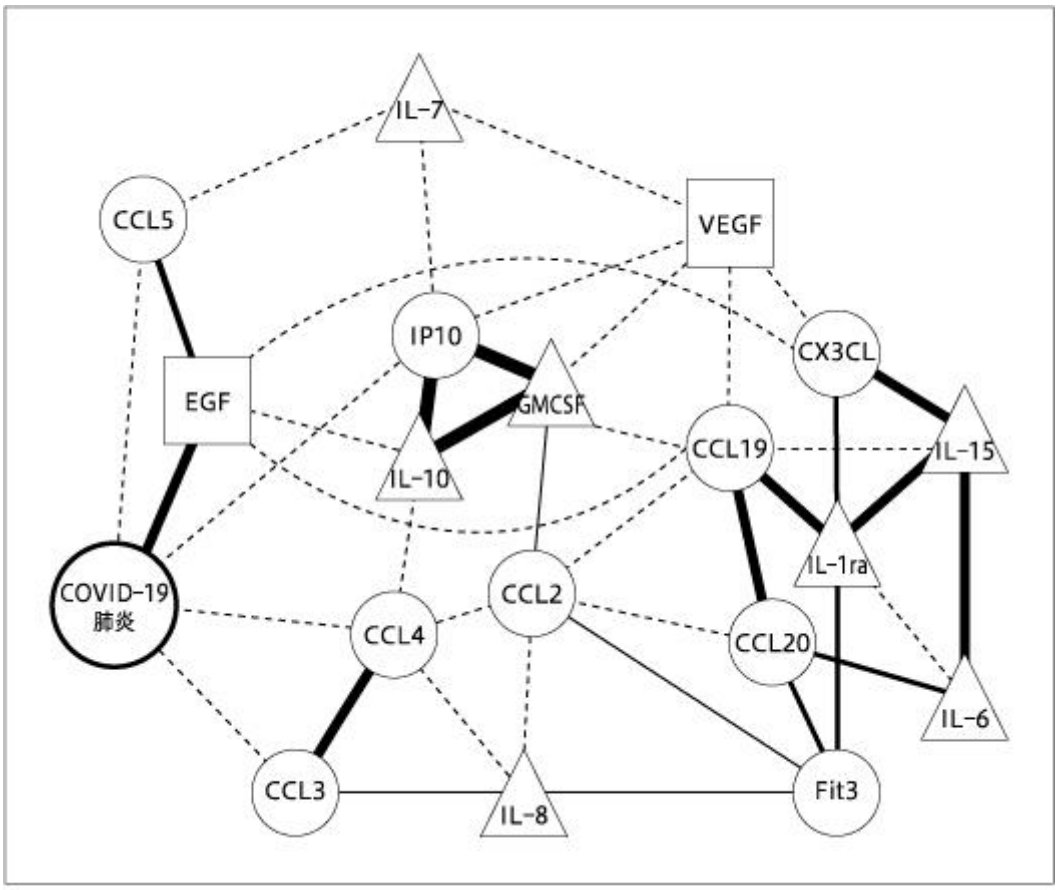

Figure 5: Association of individual cytokines and chemokines with acute lung inflammation in the COVID-19 cytokine storm

The correlation intensities between pneumonia severity by COVID-19 and various inflammatory cytokines and chemokines induced in the serum are schematically illustrated in the figure, limiting them to significant correlations. The cytokine that showed the strongest primary correlation with pneumonia severity was epidermal growth factor (EGF), and chemokines such as CCL5 were observed as secondary factors. (Adapted with modifications from Reference 11)