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REVIEW ARTICLE

Translational pediatrics: clinical perspective for Phelan–McDermid syndrome and autism research

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Phelan–McDermid syndrome (PMS) is a rare genetic disorder presenting with developmental delay, epilepsy, and autism spectrum disorder (ASD). The segmental deletion of chromosome 22q13.3 affects the copy number of *SHANK3*, the gene encoding a scaffolding protein at the postsynaptic density. Biological studies indicate that *SHANK3* plays crucial roles in the development of synaptic functions in the postnatal brain. Notably, induced pluripotent stem (iPS) cells have enabled researchers to develop brain organoids and microglia from patients and to explore the pathophysiology of neurodevelopmental disorders in human cells. Single-cell RNA sequencing of these cells revealed that human-specific genes are uniquely expressed during cortical development. Thus, patient-derived disease models are expected to identify as-yet-unidentified functions of *SHANK3* in the development of human brain. These efforts may help establish a new style of translational research in pediatrics, which is expected to provide therapeutic insight for children with PMS and broader categories of disease.

Pediatric Research (2022) 92:373–377; https://doi.org/10.1038/s41390-021-01806-x

IMPACT:

- Phelan–McDermid syndrome is a prototypic model for molecular studies of autism spectrum disorder.
- Brain organoids are expected to provide therapeutic insight.
- Single-cell RNA sequencing of microglia may uncover the functional roles of human-specific genes.

INTRODUCTION

In the past decade, next-generation sequencing and bioinformatic approaches have brought us an unprecedented amount of knowledge on the genetic background of neurodevelopmental disorders, including autism spectrum disorder (ASD).[1] Child neurologists are currently challenged to contemplate how these genes function in the developing brain before and after birth. Understanding the molecular functions of ASD-associated genes has markedly aided in deciphering the pathogenic mechanisms underlying neurodevelopmental disorders in childhood. Thus, establishing disease models is essential for identifying therapeutic targets for ASD and related disorders.

In the present report, we focus on one such disorder—Phelan–McDermid syndrome (PMS)—and discuss the indispensable role of disease models in the evolution of translational medicine.[2]

ASD AND PMS

Trio-based family studies have also identified a variety of copy number variations that occurred de novo in ASD patients at significantly higher frequencies than control populations.[3] The segmental deletion of 22q13.3 occurs in a telomeric region of the long arm of chromosome 22 and is known to cause PMS.[2] Patients with PMS manifest muscular hypotonia, speech delay, ASD, and epilepsy.[2] Genetic studies recurrently detected entire or interstitial deletions of *SH3 and ankyrin repeat domain containing 3* (*SHANK3*), a gene located at the far telomeric region of 22q13, in patients with PMS (Fig. 1). Thus, *SHANK3* has been considered to play a central role in the neurodevelopmental phenotype of PMS.[4]

As one of the SHANK family proteins (SHANK1, SHANK2, and SHANK3), *SHANK3* encodes a scaffolding protein at postsynaptic density.[5] Greater attention has been drawn to the functional role of *SHANK3* since Durand et al.[6] reported that seven patients with non-syndromic ASD carried pathogenic variants in *SHANK3*. This report and following studies supported the notion that the deletion of *SHANK3* had a pathogenic effect in cases with PMS.[7] The prevalence of *SHANK3* mutations is reportedly 0.5–0.7% in patients with ASD at present,[8] increasing to 2.1% for cohorts of patients with ASD and intellectual disability.[9] In contrast, much lower frequencies of mutations have been reported for *SHANK1* (0.04%) and *SHANK2* (0.17%) in patients with ASD.[8] Thus, the pathogenic effects of mutations in *SHANK1* and *SHANK2* remain unclear, whereas mutations in *SHANK3* are likely to have a higher penetrance than those in *SHANK1* and *SHANK2*. These population studies suggest that *SHANK3* might have indispensable functions in the developing brain.

MOLECULAR STUDIES IN MICE

Shank3, the murine homolog of *SHANK3*, encodes at least six isoforms of the protein (Shank3a–f) through differentially

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Received: 29 September 2021 Accepted: 5 October 2021
Published online: 26 October 2021

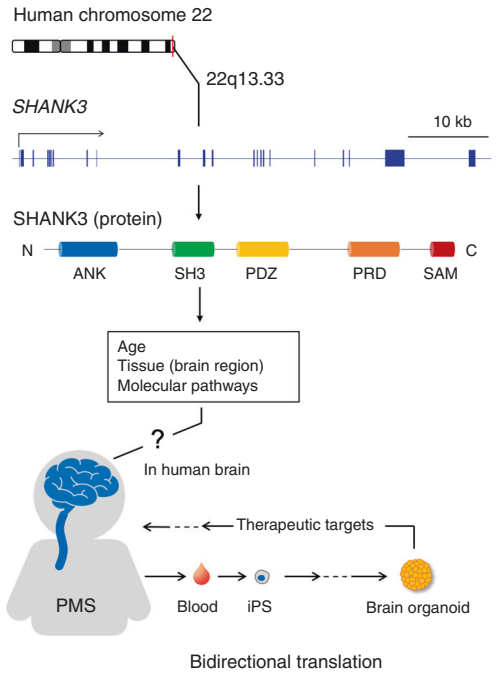

Fig. 1 Clinical relevance of molecular studies and brain organoids from patients with Phelan–McDermid syndrome. Human *SH3 and multiple ankyrin repeat domains 3* (*SHANK3*) is located at chromosome 22q13.3 (top). The gene consists of 23 exons and encodes a postsynaptic protein SHANK3 (middle). The 180 kDa full-length isoform contains ankyrin repeat domain (ANK), Src-homology 3 (SH3), PDZ, proline-rich domain (PRD), and SAM domain from its N-terminus to C-terminus. Multiple isoforms are expressed from the murine homolog, *Shank3*. Little is known, however, for the age- and tissue-dependent regulation of transcription and alternative splicing in the human brain (square). To investigate the molecular pathways in the human brain, iPS cells and brain organoids can be established from a patient with Phelan–McDermid syndrome (PMS and left-to-right direction). The right-to-left direction represents the backward translation for molecular studies to find therapeutic targets for children with PMS.

activating transcription and alternative splicing.[10] Currently available mouse strains harbor partial or whole deletions of coding exons in the murine *Shank3* gene.[11] Partial loss of the coding exons affects the expression profile of Shank3 isoforms and is known to cause unique behavioral phenotypes, depending on the deleted region. Thus, the functional domains of Shank3 and the protein isoforms are considered to play unique roles in the developing brain. This concept might be applicable to the human brain, as the gene structure and functional domains of SHANK3 are generally conserved across species. However, little is known about the spatiotemporal expression of SHANK3 isoforms or their specific roles in the human brain (Fig. 1).

Recently, mice with the conditional deletion of *Shank3* in peripheral somatosensory neurons reproduced the autistic behaviors and hypersensitive tactile responses observed in children with ASD.[12,13] Experimental data show that *Mecp2*, the Rett syndrome (RS)-associated gene,[14] has a regulatory role in the interplay of the sensory neuron with the GABAergic interneurons. Although functional interactions between *SHANK3* and *MECP2* have been hypothesized in earlier studies,[12,15] they might cooperatively regulate the development of neural circuits involving GABAergic neurons (Fig. 2). These data provided insight into the pathogenic mechanisms and an alternative point of therapeutic intervention for hypersensitive phenotypes in children with ASD.

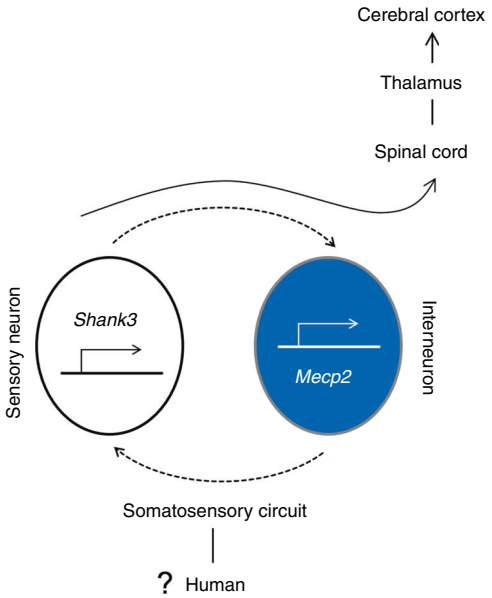

Fig. 2 The interplay between SHANK3 and MECP2-expressing neurons in somatosensory circuits. Deletions of *SHANK3* cause PMS, while mutations in *MECP2* lead to Rett syndrome. This model shows that MECP2-expressing interneurons regulate the magnitude of inputs from SHANK3-expressing somatosensory neurons.[12,13] The functional cooperation of *SHANK3* and *MECP2* remains unproven in the human brain. However, the common mechanisms of both disorders may be partly attributed to aberrant circuits involving peripheral somatosensory neuron and GABAergic interneurons. Rett-like neurodevelopmental phenotypes are associated with mutations in 28 genes, including *SHANK3*.[51]

HUMAN PLURIPOTENT STEM CELLS

Transcriptomic profiling with single-cell resolution has begun to reveal new concepts in research on pediatric cancer and hematopoietic disorders.[16] In contrast, the human brain has been a difficult subject for in vivo and ex vivo monitoring of pathogenic mechanisms. A system for the direct conversion of patient skin fibroblasts into neurons partly compensates for such disadvantages,[17] while dopaminergic neurons derived from stem cells in deciduous teeth are instrumental for analyzing the cellular phenotypes associated with neurodevelopmental disorders.[18]

Induced pluripotent stem (iPS) cells from patients and iPS-derived brain organoids have provided an alternative way to visualize the developmental process of the human brain in a dish.[19,20] Thus, brain organoids are currently used to dissect the molecular pathology of rare diseases, such as PMS and RS.[21,22] In combination with the organoid system, single-cell RNA sequencing provides molecular insight into the differentiation process of neuronal progenitors.[23] Modified protocols were shown to effectively promote the formation of the laminated structure of the cerebral cortex and to reproduce a variety of region-specific brain organoids.[24]

The single-cell profiling of organoids revealed that 261 genes were found to be uniquely expressed during the development of the human brain and cortical organoids.[25] Among these, 223 genes (85%) were exclusively expressed in the human cerebral cortex and organoids but not in iPS cells or fibroblasts. Notably, 21 (8%) of the 261 genes overlapped with those located in chromosomal regions that showed human-specific duplication during the evolutional process of primates.[26]

These data suggested that human-specific genes regulate the complex process of human cortical development in cooperation with those conserved across mammalian species. Furthermore, the presence of human-specific enhancers might lead to the next proposition that the functional development of the human

brain might be organized with unique epigenetic programs in humans.[27,28]

IPS CELLS FOR MODELING PMS

The deleterious effects of mutations in *SHANK3* are reproducible in different species, from rodents to humans. However, based on the perspective described above, one could hypothesize that the human brain might have an additional layer of complexity in pathogenic mechanisms beyond animal models.

In this field, the targeted disruption of *SHANK3* in human neurons was unexpectedly found to cause an impairment in hyperpolarization-activated cation currents, a non-synaptic effect of *SHANK3* haploinsufficiency.[29] These data indicated that the human neurons successfully recapitulated the hyperexcitable phenotype of pyramidal neurons in *Shank3*-knockout mice. However, we have no answer yet to the question whether identical molecules are involved in such a reproducible phenotype in human and rodent neurons. Thus, the human-specific mechanism is also worth addressed in the future studies with patient-derived neurons and brain organoids.[30]

Of note, the iPS system has been recently shown to generate the assembly of different organoids, so called "assembloids."[30,31] Improved models will be useful resources for evaluating neural connectivity in vitro.[32] Nonetheless, either organoids or assembloids do not develop physiological neural circuits of the brain. Compensating for their disadvantage by multidisciplinary omics analyses may provide clues for understanding the acquisition process of human-specific brain functions and behaviors.[32,33]

MICROGLIA

We cannot close this overview without considering microglia, resident macrophages in the central nervous system.[34] Microglia are involved in various processes of the host defense against injury and infection.[35] In addition to their roles in inflammatory conditions, recent studies have characterized the versatile functions of microglia in the developing brain.[35] For example, microglia contribute to the establishment of gut–brain interaction through sensing chemokines and other signals secreted from the gut microbiota.[36] The microbiota-mediated homeostasis of microglia also plays a role in protecting neurons in epileptogenic circuits from overactivation and excitotoxicity.[37]

To date, microglia have been considered to regulate the excitation–inhibition balance of the neural network through synaptic pruning in both embryonic and postnatal stages.[38] Polarized microglia with M1 and M2 phenotypes might participate differently in the developmental process of neural circuits. However, single-cell RNA sequencing data did not necessarily support the concept that microglia are classified into either M1 or M2 polarity; rather, the data suggested that they were composed of populations with much greater diversity than previously expected.[39,40]

The frequency of inflammatory bowel disease in children with ASD is four times greater than that in other children.[41] Various conditions might be associated with the gastrointestinal problems of children with ASD; however, recent studies have pointed out the reciprocal interaction of immune dysregulation due to alteration of the gut microbiome among the microbiota, immunity, gut function, and behavior.[42] Indeed, microbiota transplanted from patients with ASD exaggerated the pathogenic condition of recipient mice, which accompanied the deregulation of the metabolomic and gene expression profiles in the brain.[43]

As a gatekeeper of the immune response in the brain, microglia play a pivotal role in both receiving signals from gastrointestinal tracts and regulating the homeostatic condition of peripheral organs.[36] Thus, it might be worth further investigating the functional roles of *SHANK3*, in addition to *MECP2*, in the neuron–microglia interactions in the developing brains of mice[44] and in organoids.[45]

The diverse phenotype and versatile functions of microglia extend our understanding of their roles in the human brain, encompassing the concept of self- and non-self-recognition systems of immune cells.[46] Thus, it is not surprising when future studies may find that primate brains have more diversely differentiated microglia than other mammalian species.[47] Also, it might be worth investigating whether patients with PMS harbor different subsets of microglia in their brains from children with typical development.

FUTURE PERSPECTIVE

Child neurologists envisage the human brain function by taking the history of developmental milestones from the parents of patients. Some start walking unaided after 12 months of age, and some do not. Some catch up from their language delay after 2 years of age but may experience hyper-reactivity to sensory inputs or problems with social skills at older age. Thus, each child follows a unique course in their acquisition of motor, language, and social skills after birth. In other words, human brains show various developmental traits from early infancy and potentially through life.

Bayley, Enjohji, and other standards of developmental quotient were established in the 1960s with enormous efforts in collecting data on performance skills from thousands of developing children in the United States and Japan.[48] Transcriptomic profiling of brain organoids may require similar efforts in order to fully describe the human brain functions. In this regard, accumulating data from different protocols and longitudinal observations of organoids at different ages will be necessary to elucidate how human brains acquire unique functions during embryonic and postnatal development.

Single-cell analyses of rodent brains might not necessarily represent the whole-cell populations that comprise primate and human brains. However, PMS models have provided the framework that is conserved across the mammalian brains.[49] Comparing the gene expression profiles of rodent brains and organoids might also be a resource for identifying the subset of genes that are commonly expressed in human brains and organoids.[50]

CONCLUSION

In this review, we emphasize the value of generating disease models from patient-derived samples. The state-of-the-art technique using iPS cells thus leads to more pediatricians becoming involved with translational research, which makes it possible to associate clinical questions closely with the findings of molecular studies. This approach may aid in identifying pathogenic mechanisms in the human brain, eventually leading to the acquisition of a greater degree of clinical knowledge concerning the management of children with PMS and other diseases. As described, *SHANK3* and hundreds of other ASD-associated genes are not always human-specific genes; however, they might have distinct roles in co-regulating the expression of genes that are essential for the physiological development of the human brain. Future discoveries of unknown variations in regulatory elements and combined analyses with brain organoids will provide further insight into the pathogenic mechanisms and therapeutic targets for PMS.

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ACKNOWLEDGEMENTS

We thank the patients and their parents for kindly cooperating with our studies and Kazuaki Nonaka (Professor Emeritus, Kyushu University), Masafumi Sanefuji, Chong Pin Fee, Yuko Ichimiya, Yuri Sonoda, Kousuke Yonemoto, Ryoji Taira, Fumihiko Fujii, and the laboratory members for their helpful discussions.

AUTHOR CONTRIBUTIONS

Y.S. conceptualized the study, drafted the initial manuscript, and revised the manuscript. C.P.S., S. Okuzono, and S. Ohga critically reviewed the intellectual content and revised the manuscript. All the authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

FUNDING INFORMATION

This study was supported by JSPS KAKENHI grant numbers JP19K08281, JP18H04042, JP21K10293; AMED under the grant numbers JP20ek0109411 and JP20wm0325002h; a Health and Labour Sciences Research Grant for Prion Diseases under the grant number JP20FC1054; The Japan Epilepsy Research Foundation, and Kawano Masanori Memorial Public Interest Incorporated Foundation for Promotion of Pediatrics. These funders had no role in the design and conduct of the study.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Patient consent was not required for this work.

ADDITIONAL INFORMATION

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