included in the LD bin of rs7603001. The r^2 LD value between rs7603001, the SNP that was associated with autism in our study, and the SNPs that were associated with autism in the GWAS[7] ranged between 0.25 and 0.28. The GWAS finding was thus replicated at the gene level, not at the level of specific SNPs.

In addition to genetic association, CNVs (gain and loss), mostly de novo, were observed at the *ZNF804A* locus of boys with autism who had a verbal deficit. Griswold and colleagues[8] and Talkowski and colleagues[9] have also reported CNVs at the *ZNF804A* locus in individuals with autism. Since the penetrance of CNVs is variable, it is not possible to predict the effect of these CNVs in the pathogenesis of autism. Copy number gain and loss were observed in autistic individuals, and similar CNVs were observed in unaffected parents. Furthermore, similar CNVs have also been observed in patients with other neuropsychiatric disorders,[32] suggesting pleiotropic effects. Future studies to correlate specific CNVs with detailed clinical characteristics and to assess their effects on neurodevelopment are warranted.

Impaired linguistic/verbal ability is a key cognitive defect in individuals with autism.[33,34] Based on our results, we suggest that ZNF804A could be a modulator of verbal traits in individuals with autism. There is ample evidence of the involvement of ZNF804A in the development of ToM,[10] which in turn, is closely intertwined with the development of linguistic/verbal abilities from infancy.[15–17]

Genetic, neuropsychological and neuroimaging studies have suggested that ZNF804A is involved in higher-order cognitive processes such as ToM,[10] working memory[35] and executive control of attention.[36] It has been found to play a pivotal role in the maintenance of functional connectivity in the brain.[37,38] We observed a reduced expression of *ZNF804A* in the ACG of individuals with autism compared with controls. The ACG, a brain region vital for cognitive and behavioural abilities, is involved in emotion formation and processing, learning and memory.[39,40] Downregulated expression of *ZNF804A* could lead to adverse effects on the cognitive processes associated with this gene.

Even though the previous studies on *ZNF804A* were focused on schizophrenia, overwhelming evidence suggests that the risk variants of this gene may be involved in the modulation of intermediate cognitive phenotypes associated with the disorder rather than the disorder itself.[10,35,36,38] Adult-onset schizophrenia and early-onset autism, despite being 2 clinically distinct, complex neurodevelopmental disorders, share several deficits in cognitive functioning.[41–43] A deficient ToM has been identified as a potential contributor to the social cognitive dysfunction in individuals with schizophrenia and autism,[44,45] and it could be a common factor mediating ToM-related key intermediate phenotypes in people with these disorders. Several studies have shown the association of *ZNF804A* variants with cognitive dysfunction in individuals with schizophrenia.[46–48] Interestingly, we observed a stronger association of *ZNF804A* in individuals with an autism subtype characterized by verbal deficits.

The protein sequence of ZNF804A shows a C2H2-type zinc-finger domain at its N-terminal end, suggesting that it may bind DNA and have a role in regulating gene expression.[18] ZNF804A has been found to modulate the expression of several genes implicated in the pathogenesis of schizophrenia.[18,49]

We examined the possible role of ZNF80A as a regulator of the expression of genes previously reported to be associated with verbal/linguistic abilities and/or social cognition. The expression of *SNAP25* was downregulated in *ZNF804A*-silenced cells compared with control cells. Furthermore, the expression of *SNAP25* was significantly reduced in the ACG of individuals with autism, and a strong positive correlation was observed between the expression of *ZNF804A* and *SNAP25* in the ACG.

SNAP25 is a presynaptic plasma membrane protein that is specifically and abundantly expressed in nerve cells. It participates in synaptic vesicle exocytosis through the formation of a soluble NSF attachment protein receptor complex[50] and plays a pivotal role in modulating calcium homeostasis.[51] SNAP25 is important for axonal growth and synaptic plasticity, 2 essential steps in the wiring of the central nervous system.[50,52] *SNAP25* variants have been found to modulate cognitive performances.[29,53,54] *SNAP25* is located in a chromosomal region (20p12–p11.2) with a previously suggested linkage to intelligence.[55] Moreover, polymorphisms in *SNAP25* have been associated with hyperactivity in individuals with autism.[56] However, at present, there is no literature linking ZNF804A and SNAP25.

Limitations

A replication study in a larger cohort of verbally deficient individuals with autism from different racial backgrounds would have been more informative. Further studies on the functional implications of *ZNF804A* CNVs and on the nature of the interaction between ZNF804A and SNAP25 in the pathogenesis of autism are warranted. The small number of postmortem brain samples used is another limitation of our study.

Conclusion

We suggest that *ZNF804A* could have a pivotal role in mediating the intermediate phenotypes associated with verbal traits in individuals with autism. It could be a common factor modulating the ToM-related intermediate phenotypes in individuals with schizophrenia and autism.

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Contributors: A. Anitha, I. Thanseem and K. Nakamura designed the study, acquired and analyzed the data and wrote the article. M.M. Vasu, K. Yamada, T. Ueki, Y. Iwayama and T. Toyota acquired and analyzed the data and reviewed the article. K.J. Tsuchiya, Y. Iwata and K. Suzuki analyzed the data and reviewed the article. T. Sugiyama, M. Tsujii, T. Yoshikawa and N. Mori designed the study and reviewed the article. All authors approved the final version for publication.

References

1. Meyer U, Feldon J, Dammann O. Schizophrenia and autism: Both shared and disorder-specific pathogenesis via perinatal inflammation? *Pediatr Res* 2011;69:26R-33R.
2. Carroll LS, Owen MJ. Genetic overlap between autism, schizophrenia and bipolar disorder. *Genome Med* 2009;1:102.
3. O'Donovan MC, Craddock N, Norton N, et al. Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* 2008;40:1053-5.
4. Riley B, Thiselton D, Maher BS, et al. Replication of association between schizophrenia and ZNF804A in the Irish Case-Control Study of Schizophrenia sample. *Mol Psychiatry* 2010;15:29-37.
5. Li M, Luo XJ, Xiao X, et al. Allelic differences between Han Chinese and Europeans for functional variants in ZNF804A and their association with schizophrenia. *Am J Psychiatry* 2011;168:1318-25.
6. Williams HJ, Norton N, Dwyer S, et al. Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. *Mol Psychiatry* 2011;16:429-41.
7. Anney R, Klei L, Pinto D, et al. A genome-wide scan for common alleles affecting risk for autism. *Hum Mol Genet* 2010;19:4072-82.
8. Griswold AJ, Ma D, Cukier HN, et al. Evaluation of copy number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. *Hum Mol Genet* 2012;21:3513-23.
9. Talkowski ME, Rosenfeld JA, Blumenthal I, et al. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell* 2012;149:525-37.
10. Walter H, Schnell K, Erk S, et al. Effects of a genome-wide supported psychosis risk variant on neural activation during a theory-of-mind task. *Mol Psychiatry* 2011;16:462-70.
11. Baron-Cohen S. The autistic child's theory of mind: a case of specific developmental delay. *J Child Psychol Psychiatry* 1989;30:285-97.
12. Yirmiya N, Erel O, Shaked M, et al. Meta-analyses comparing theory of mind abilities of individuals with autism, individuals with mental retardation, and normally developing individuals. *Psychol Bull* 1998;124:283-307.
13. Bora E, Yucel M, Pantelis C. Theory of mind impairment in schizophrenia: meta-analysis. *Schizophr Res* 2009;109:1-9.
14. Frith CD, Frith U. Interacting minds — a biological basis. *Science* 1999;286:1692-5.
15. Miller CA. Developmental relationships between language and theory of mind. *Am J Speech Lang Pathol* 2006;15:142-54.
16. Ruffman T, Slade L, Crowe E. The relation between children's and mothers' mental state language and theory-of-mind understanding. *Child Dev* 2002;73:734-51.
17. Dahlgren S, Dahlgren Sandberg A, Larsson M. Theory of mind in children with severe speech and physical impairments. *Res Dev Disabil* 2010;31:617-24.
18. Girgenti MJ, Loturco JJ, Maher BJ. ZNF804a regulates expression of the schizophrenia-associated genes PRSS16, COMT, PDE4B, and DRD2. *PLoS ONE* 2012;7:e32404.
19. Geschwind DH, Sowinski J, Lord C, et al. The autism genetic resource exchange: a resource for the study of autism and related neuropsychiatric conditions. *Am J Hum Genet* 2001;69:463-6.
20. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* 1994;24:659-85.
21. Goldberg TE, Iudicello J, Russo C, et al. BDNF Val66Met polymorphism significantly affects d' in verbal recognition memory at short and long delays. *Biol Psychol* 2008;77:20-4.
22. Vernes SC, Newbury DF, Abrahams BS, et al. A functional genetic link between distinct developmental language disorders. *N Engl J Med* 2008;359:2337-45.
23. Palo OM, Antila M, Silander K, et al. Association of distinct allelic haplotypes of Drosoph Inf ServC1 with psychotic and bipolar spectrum disorders and with underlying cognitive impairments. *Hum Mol Genet* 2007;16:2517-28.
24. Beaver KM, Delisi M, Vaughn MG, et al. Association between the A1 allele of the DRD2 gene and reduced verbal abilities in adolescence and early adulthood. *J Neural Transm* 2010;117:827-30.
25. Balter M. Genetics. First gene linked to speech identified. *Science* 2001;294:32.
26. Kircher T, Krug A, Markov V, et al. Genetic variation in the schizophrenia-risk gene neuregulin 1 correlates with brain activation and impaired speech production in a verbal fluency task in healthy individuals. *Hum Brain Mapp* 2009;30:3406-16.
27. Park J, Willmott M, Vetuz G, et al. Evidence that genetic variation in the oxytocin receptor (OXTR) gene influences social cognition in ADHD. *Prog Neuropsychopharmacol Biol Psychiatry* 2010;34:697-702.
28. Waga C, Okamoto N, Ondo Y, et al. Novel variants of the SHANK3 gene in Japanese autistic patients with severe delayed speech development. *Psychiatr Genet* 2011;21:208-11.
29. Cagliani R, Riva S, Marino C, et al. Variants in SNAP25 are targets of natural selection and influence verbal performances in women. *Cell Mol Life Sci* 2012;69:1705-15.
30. Roll P, Vernes SC, Bruneau N, et al. Molecular networks implicated in speech-related disorders: FOXP2 regulates the SRPX2/uPAR complex. *Hum Mol Genet* 2010;19:4848-60.
31. Lennertz L, Rujescu D, Wagner M, et al. Novel schizophrenia risk gene TCF4 influences verbal learning and memory functioning in schizophrenia patients. *Neuropsychobiology* 2011;63:131-6.
32. Steinberg S, Mors O, Borglum AD, et al. Expanding the range of ZNF804A variants conferring risk of psychosis. *Mol Psychiatry* 2011;16:59-66.
33. Turner MA. Generating novel ideas: fluency performance in high-functioning and learning disabled individuals with autism. *J Child Psychol Psychiatry* 1999;40:189-201.
34. Yirmiya N, Gamliel I, Shaked M, et al. Cognitive and verbal abilities of 24- to 36-month-old siblings of children with autism. *J Autism Dev Disord* 2007;37:218-29.
35. Linden DE, Lancaster TM, Wolf C, et al. ZNF804A genotype modulates neural activity during working memory for faces. *Neuropsychobiology* 2013;67:84-92.
36. Balog Z, Kiss I, Keri S. ZNF804A may be associated with executive control of attention. *Genes Brain Behav* 2011;10:223-7.
37. Esslinger C, Kirsch P, Haddad L, et al. Cognitive state and connectivity effects of the genome-wide significant psychosis variant in ZNF804A. *Neuroimage* 2011;54:2514-23.
38. Rasetti R, Sambataro F, Chen Q, et al. Altered cortical network dynamics: a potential intermediate phenotype for schizophrenia and association with ZNF804A. *Arch Gen Psychiatry* 2011;68:1207-17.
39. Takenouchi K, Nishijo H, Uwano T, et al. Emotional and behavioral correlates of the anterior cingulate cortex during associative learning in rats. *Neuroscience* 1999;93:1271-87.
40. Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci* 2000;4:215-22.
41. Frith CD, Corcoran R. Exploring 'theory of mind' in people with schizophrenia. *Psychol Med* 1996;26:521-30.
42. Baron-Cohen S, Wheelwright S, Hill J, et al. The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry* 2001;42:241-51.
43. Couture SM, Penn DL, Losh M, et al. Comparison of social cognitive functioning in schizophrenia and high functioning autism: more convergence than divergence. *Psychol Med* 2010;40:569-79.
44. Muris P, Steerneman P, Meesters C, et al. The ToM test: a new instrument for assessing theory of mind in normal children and children with pervasive developmental disorders. *J Autism Dev Disord* 1999;29:67-80.
45. Couture SM, Penn DL, Roberts DL. The functional significance of social cognition in schizophrenia: a review. *Schizophr Bull* 2006;32(Suppl 1):S44-63.
46. Walters JT, Corvin A, Owen MJ, et al. Psychosis susceptibility gene ZNF804A and cognitive performance in schizophrenia. *Arch Gen Psychiatry* 2010;67:692-700.

47. Chen M, Xu Z, Zhai J, et al. Evidence of IQ-modulated association between ZNF804A gene polymorphism and cognitive function in schizophrenia patients. *Neuropsychopharmacology* 2012;37:1572-8.
48. Hashimoto R, Ohi K, Yasuda Y, et al. The impact of a genome-wide supported psychosis variant in the ZNF804A gene on memory function in schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 2010;153B:1459-64.
49. Umeda-Yano S, Hashimoto R, Yamamori H, et al. The regulation of gene expression involved in TGF-beta signaling by ZNF804A, a risk gene for schizophrenia. *Schizophr Res* 2013;146:273-8.
50. Oyler GA, Higgins GA, Hart RA, et al. The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. *J Cell Biol* 1989;109:3039-52.
51. Pozzi D, Condliffe S, Bozzi Y, et al. Activity-dependent phosphorylation of Ser187 is required for SNAP-25-negative modulation of neuronal voltage-gated calcium channels. *Proc Natl Acad Sci U S A* 2008;105:323-8.
52. Osen-Sand A, Catsicas M, Staple JK, et al. Inhibition of axonal growth by SNAP-25 antisense oligonucleotides in vitro and in vivo. *Nature* 1993;364:445-8.
53. Gosso MF, de Geus EJ, van Belzen MJ, et al. The SNAP-25 gene is associated with cognitive ability: evidence from a family-based study in two independent Dutch cohorts. *Mol Psychiatry* 2006; 11:878-86.
54. Söderqvist S, McNab F, Peyrard-Janvid M, et al. The SNAP25 gene is linked to working memory capacity and maturation of the posterior cingulate cortex during childhood. *Biol Psychiatry* 2010; 68:1120-5.
55. Posthuma D, Luciano M, Geus EJ, et al. A genome-wide scan for intelligence identifies quantitative trait loci on 2q and 6p. *Am J Hum Genet* 2005;77:318-26.
56. Guerini FR, Bolognesi E, Chiappedi M, et al. SNAP-25 single nucleotide polymorphisms are associated with hyperactivity in autism spectrum disorders. *Pharmacol Res* 2011;64:283-8.

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Molecular Autism

RESEARCH Open Access

N-ethylmaleimide-sensitive factor interacts with the serotonin transporter and modulates its trafficking: implications for pathophysiology in autism

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Abstract

Background: Changes in serotonin transporter (SERT) function have been implicated in autism. SERT function is influenced by the number of transporter molecules present at the cell surface, which is regulated by various cellular mechanisms including interactions with other proteins. Thus, we searched for novel SERT-binding proteins and investigated whether the expression of one such protein was affected in subjects with autism.

Methods: Novel SERT-binding proteins were examined by a pull-down system. Alterations of SERT function and membrane expression upon knockdown of the novel SERT-binding protein were studied in HEK293-hSERT cells. Endogenous interaction of SERT with the protein was evaluated in mouse brains. Alterations in the mRNA expression of SERT (SLC6A4) and the SERT-binding protein in the post-mortem brains and the lymphocytes of autism patients were compared to nonclinical controls.

Results: *N*-ethylmaleimide-sensitive factor (NSF) was identified as a novel SERT-binding protein. NSF was co-localized with SERT at the plasma membrane, and NSF knockdown resulted in decreased SERT expression at the cell membranes and decreased SERT uptake function. NSF was endogenously co-localized with SERT and interacted with SERT. While *SLC6A4* expression was not significantly changed, *NSF* expression tended to be reduced in post-mortem brains, and was significantly reduced in lymphocytes of autistic subjects, which correlated with the severity of the clinical symptoms.

Conclusions: These data clearly show that NSF interacts with SERT under physiological conditions and is required for SERT membrane trafficking and uptake function. A possible role for NSF in the pathophysiology of autism through modulation of SERT trafficking, is suggested.

Keywords: Serotonin transporter, NSF, Interaction, Membrane trafficking, Autism, Post-mortem brain, Lymphocyte

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特集

自閉症の分子基盤

自閉症のPET研究について

中村和彦* 鈴木勝昭** 尾内康臣*** 辻井正次§ 森 則夫**

KEY WORDS

・PET
・セロトニン・トランスポーター
・活性型ミクログリア
・末梢マクロファージ

SUMMARY

自閉症は脳内セロトニンの機能異常によるというセロトニン仮説が最も有力視された．われわれはPETにより，自閉症脳内の広汎な脳部位におけるセロトニン・トランスポーター脳内密度の低下を明らかにした．さらにその低下は自閉症の中核症状である社会性の障害やこだわり症状と関係していることを見出した．自閉症に関する免疫系の異常については各種の研究があるが，われわれはミクログリアに注目した．ミクログリアは，感染，出血，虚血での貪食，脳細胞の保護，神経回路形成や神経伝達の恒常性を維持する役割をもつ．PETにより，自閉症脳内の広汎な脳部位における，活性型ミクログリア増加を明らかにした．そして活性型ミクログリアが多いほど，社会性の障害が子どものころも現時点においても強いことを見出した．自閉症は遺伝的要因と環境的要因によって胎生期において骨髄系などに影響を受け血管脳関門が閉じられる前に末梢マクロファージが脳内に移行する量が増え，胎生期から活性型ミクログリアによって脳内のセロトニン系などのシナプス形成障害を起こし自閉症の病因となると推測した．

はじめに
—自閉症発症の遺伝要因と環境要因について—

自閉症は高血圧症，糖尿病などと同様で遺伝要因，胎生期での環境要因他，多くの事柄が関与する多因子疾患であることが定説となった．自閉症は家族性の疾患や遺伝病ではない．自閉症の発症は，約400個の遺伝子が関与し，人によって遺伝子の組み合わせが違い，特徴としては孤発例（家系の中に他に自閉症の人がいない）が多いと報告されている．**図1**で示すように遺伝要因としては，さまざまな遺伝子が報告されている．自閉症では，一卵性双生児の一致率が70～90%であり，従来遺伝的な関与が強いと考えられ，さまざまな自閉症関連遺伝子が報告されている[1]．環境要因としても，各種報告があり，最近は遺伝要因よりは環境要因のほうが強いと報告され，新しい双生児研究よると自閉症の環境要因は55%と報告された[2]．環境要因としては，父親の高年齢，体外受精，出生時低体重，多産，妊娠中母体感染症などが報告され，最近では妊娠中や，生まれてから1年における大気汚染のPM2.5，PM10が自閉症と関連しているとの報告がある[3]．

1．自閉症の画像研究について

脳科学の最近の進歩，検査機器の向上に伴い，自閉症に関する精神医学研究は日々進んでいる．さまざまな研究手法の中で，画像研究は直接脳の様子がわかることより注目されている．自閉症の画像研究に関しては，MRI（magnetic resonance imaging），SPECT（single-pho-

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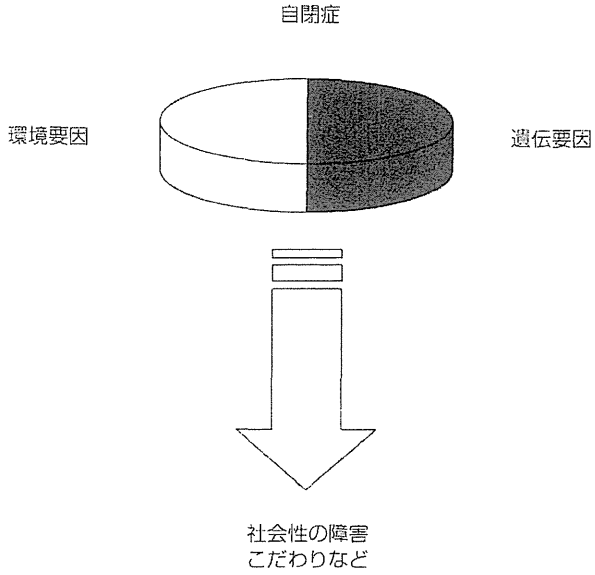

図 1. 自閉症の環境要因と遺伝要因
環境要因：父親の高年齢，体外受精，出生時障害，低体重，多産，妊娠中母体感染症，夏の出産，大気汚染（NO_2，PM2.5，PM10）[3]
＊遺伝的要素が37%，妊娠中・新生児期早期の環境要素が55%のリスクが想定される[2]
遺伝要因：一卵性双生児の一致率，Fragile X mental retardation 1，Neuroligin3，4，Neurexin 1，SHANK3，Contactin-associated protein-like 2，Protocadherin 10 他さまざま（1%程度），CNV（copy number variation）（7～10%），Genome-wide association study（GWAS），OR（odd ratio）1.1～1.3倍，epigenetics.

ton emission computed tomography），PET（positron emission tomography）を用いた数多くの研究が報告されている．また研究内容についても，神経伝達系の異常，脳の構造や大きさの異常，表情認知や追視の異常などさまざまな報告がある．筆者らはその中で，PETを用いた自閉症研究を行っている[1～6]．PETは脳内における神経伝達機能の測定が可能である．ドパミン系，セロトニン系，アセチルコリン系，ベンゾジアゼピン系，ヒスタミン系などさまざまなトレーサー（分子プローブ）があり，それらを用いることで脳内の生化学的過程の画像化や定量的解析ができる．

2．PETについて

PETについて少し解説をする．PETとはpositron emission tomography（陽電子放出型断層撮影）の略称

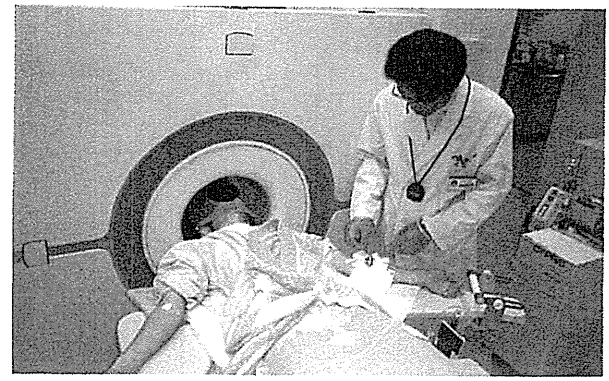

図 2. PETスキャン

で，陽電子（ポジトロン）の体内分布を画像化する撮影法である．ポジトロンは，電子と同じ質量をもち，電子とは正反対のプラスの電荷をもっている．PETはサイクロトロン，薬剤合成標識装置，PET装置という一連のシステムによって支えられた統合技術であるとともに，工学，物理学，化学，薬学，医学知識の産物である．PETでは，生体内の生理的・生化学的情報をとらえることが可能である．さらに，生体内のいろいろな機能を調べることによって，病気の早期診断や治療後の経過を知ることができる．特に，脳疾患，心臓病，腫瘍に対しては，個々の機能的異常を正確かつ事前にみつけられるという点で優れた検査法である．このように，PETは科学および社会に貢献する先端技術である．われわれのPETは頭部専用PETスキャナ（SHR12000，Hamamatsu Photonics KK，Hamamatsu，Japan）を用いた（図2）．最近はPETによるがん検診が日本各地で行われており，PETの機械が普及した．われわれの用いたPETスキャンは，頭を入れるためだけの穴（ガントリー）がある．がん検診用だとこの穴は体が入るぐらい大きさであるが，これは頭部用につくられた特別なPETで，穴が小さいので，ここに頭を入れると，センサーとの距離が近く精度の良い値を取ることができる．サイクロトロンは隣の施設にあり，トレーサーを研究所で化学者が生成の具合をチェックしながら合成する．PETは，イメージングの専門家である浜松医科大学・尾内康臣教授，放射線技師，画像解析のコンピューター専門技師，トレーサー合成担当，浜松医科大学のスタッフなどいろいろな方が共同で行う．1日に2人しか撮影

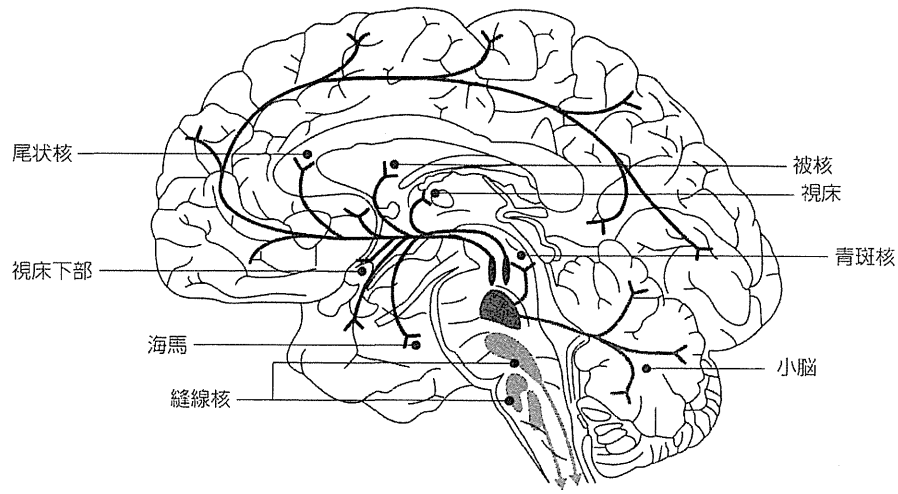

図 3. セロトニン神経系

できず，1回のPETを行うのに約100万円の実費がかかる．そしてPET研究をしていくうえで，脳が薬物の影響を受けていない状態の人を対象にすることが重要である．NPO法人アスペ・エルデの会の自閉症の当事者の人は薬を服用せずに，療育指導によって補っている人が多いので，そういう人に協力して頂いた．

3．セロトニン系に着目したPET研究

神経伝達のプロセスには，神経伝達物質の生合成・貯蔵・放出，受容体への結合・代謝・再取り込みなど多くの機能が関与している．したがって，神経伝達機能を対象としたPETの分子イメージングには，神経伝達物質の神経細胞内の合成酵素やシナプス間隙中に存在する分解酵素などの酵素，シナプス前膜や後膜に存在する受容体，再取り込み部位やシナプス小胞膜に存在するトランスポーターなどが対象となる[7]．自閉症においてはさまざまな神経伝達系の研究が報告されている．自閉症の病態に関する最初の研究は，セロトニン神経系である（**図3**）．1977年から自閉症の血液中や血小板においてセロトニン値の上昇が報告された[8][9]．さらに，セロトニンの前駆体であるトリプトファンの欠乏食を与えるとこだわり症状が強くなり，不安や不幸せ感が上昇すると報告された[10]．そしてうつ病の治療薬である選択的セロトニン再取り込み阻害薬（selective serotonin reuptake inhibitor：SSRI）の使用によって自閉症のこだわり症状や強迫症状に効果があるとの報告がある[11]．さらにセロトニン系の重要な因子であるセロトニン・トランスポーターに関する自閉症研究では，セロトニン・トランスポーター遺伝子の制御部分であるプロモーター領域の機能に関連するポリモルフィズムのshort allele（S）とlong allele（L）と自閉症との相関が報告された[12]．そして，2歳から4歳の44名の男子の自閉症対象群の各脳部位の体積とこのポリモルフィズムとの相関について，short alleleはcorticalやfrontalの部位の灰白質の体積の10～16%に関連していた[13]．さらに自閉症のゲノムスキャン解析を行うと，セロトニン・トランスポーターのある17番染色体の部位に有意差があると報告された[14]．Chuganiらは[15]～[17]，脳におけるセロトニン合成能についてmethyl-L-tryptophanをトレーサーとして用いたPET研究を行った．自閉症の子ども30名（2～15歳）とてんかんの子ども16名（3ヵ月～13歳）を比較した（正常の子どもは倫理的にPETが施行できないゆえ，てんかん群を正常群としている）．正常群では5歳までは，大人の200%以上のセロトニン合成能があり，その後次第に大人のレベルまで減少する．一方自閉症の子ども群においては，セロトニン合成能は2歳から15歳まで次第に上昇し大人の150%までしかならなかった．子どもの早い段階ではセロトニン合成能が正常群では高いが，自閉症群では何らかの障害を受けセロトニン合成能が低いと考えられる．このように自閉症児におい

ては，成長過程においてセロトニン系メカニズムの障害が派生していることが推測される．そしてSPECTによってセロトニン・トランスポーターの脳内分布が報告されている[18]．使用したトレーサーは123I nor-β-CITである．15名の自閉症の子どもと10名の正常対象群を比較したところmedial frontal cortexにおいてセロトニン・トランスポーターが低下していた．medial frontal cortexは心の理論や，他人の考えや意思を理解するための重要な領域である．現在のところセロトニン神経系としてPETで現在測定できるのは，セロトニン・トランスポーター，5-HT_{1A}レセプター，5-HT_{2A}レセプターである．そこでわれわれはPETを用いて，セロトニン神経終末の構成要素であるセロトニン・トランスポーター脳内密度を定量した．そして，自閉症のセロトニン神経系の状態を健常者と比較検討し，同疾患のセロトニン神経系の異常の有無を検索し，臨床症状との関連を研究した[19]．対象は自閉症20名（すべて男性：年齢：18～26歳），および，性別，年齢の合致した健康健常者20名（すべて男性）である．ADI-R（Autism Diagnostic Interview-Revised）で自閉症の診断基準を満たし，Wechsler Adult Intelligence Scale（WAIS）で総合IQが70以上である．自閉症のうち，他の精神疾患，脳の器質的異常を有する者，重篤な身体疾患（甲状腺機能障害，免疫疾患などを含む），および，精神科薬物療法を受けた既往のある者は除外した．これは，Structured Clinical Interview for Diagnostic and Statistical Manual Ⅳ（SCID）に準じた問診を本人およびその家族に施行することにより決定した．

PETには頭部専用PETスキャナ（SHR12000，Hamamatsu Photonics KK，Hamamatsu，Japan）を用いた．トレーサーにはセロトニン・トランスポーターへの選択性の高い［11C］（＋）McN5652を用いた．臨床スコアとの相関について，自閉症に対する臨床症状は，社会性障害についてはFaux Pas Test［fou-pa：］を用いた．これは成人の自閉症の心の理論の障害を計るテストとして考案されたものである（合計20問）[20]．こだわり症状に対しては強迫症状スケールであるYale-Brown Obsessive Compulsive Scale（Y-BOCS）を用いた．それらの臨床スコアとPET画像との相関を検討した．結果は自閉症では健常者と比較して，大脳皮質全般，基底核，中脳，小脳に渡る広範囲の部位でセロトニン・トランスポーターが有意に低下していた（**図4**）．Faux Pas Testで測定した自閉症の心の理論の障害の程度と帯状回におけるセロトニン・トランスポーターの低下は相関していた（**図5**）．われわれは自閉症群が，健常者群にくらべ，大脳皮質全般，基底核，中脳，小脳などの脳部位でセロトニン・トランスポーターが有意に低下していることを見出した．自閉症に関して，重要な所見は，血液におけるセロトニンの上昇である．これは，セロトニン・トランスポーターが形成される，発達段階のときにセロトニン終末の脱落によって引き起こされると考えられている[21,22]．おそらく脳においても，同様であろうと推測されている．それゆえ，脳のさまざまな部位におけるセロトニン・トランスポーターの低下は，発達段階においてセロトニン神経伝達系が変化していることに起因すると推測される．本研究では，Faux Pas Testで測定した自閉症の心の理論の障害の程度と帯状回におけるセロトニン・トランスポーターの低下は相関していた．心の理論と帯状回の関連については，先行研究により支持されているところである．たとえば，rCBF（regional cerebral blood flow）をみるSPECT研究[23]や，18F-deoxyglucoseを用いたPET研究[24]ではCARS（Childhood Autism Rating Scale）やADI-Rでスコア化した社会性の障害，心の理論に関与していると考えられるコミュニケーション障害が，帯状回の血流量や代謝と関与していると報告されている．これらのことから帯状回は心の理論を制御する重要なメカニズムであることが示唆された．次に自閉症のこだわりの指標としての強迫症状とセロトニン神経系の関係については，セロトニン・トランスポーターの低下と強迫症状の強度との有意な相関が認められた．自閉症の主な臨床症状の1つとして強迫的で繰り返される行動があげられ，具体的には，行動，興味および活動が限定され，反復的で常同的で強度で，異常なほど，1つまたはいくつかの興味だけに熱中することや特定の無意味な習慣や儀式にかたくなにこだわることがあげられる．強迫症状の責任部位について，自閉症の各脳部位でのセロトニン・トランスポーターの低下と強迫症状の疾患内相関を検討したところ視床において

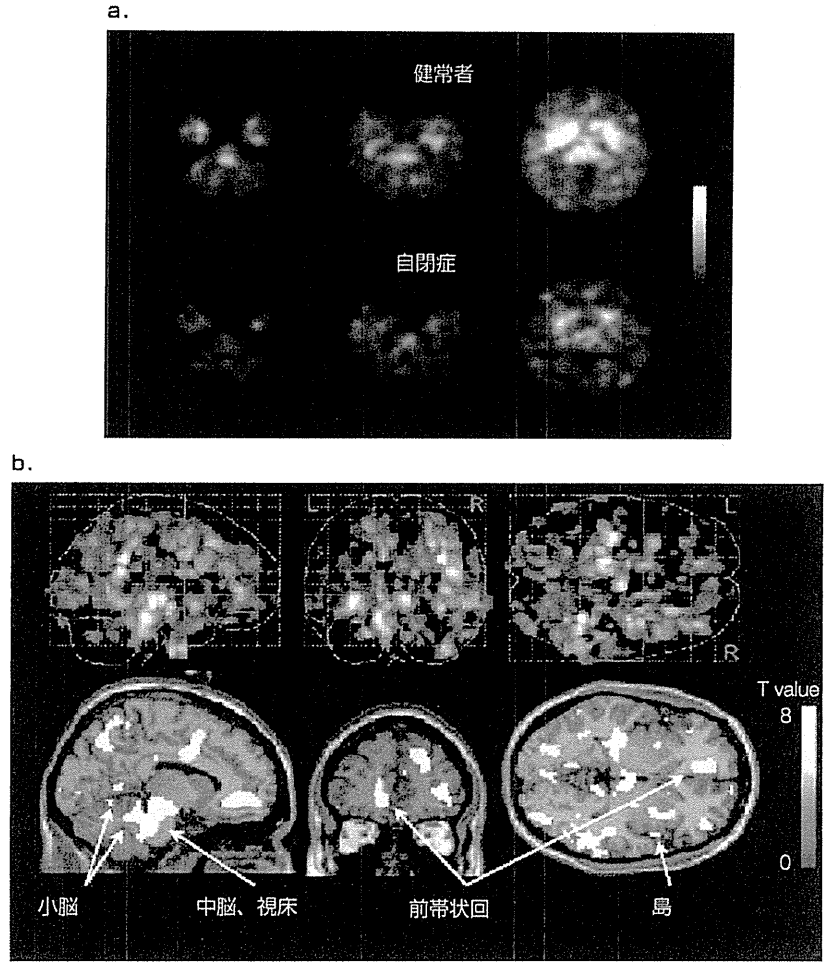

図 4. 自閉症では脳内セロトニン・トランスポーター密度が低下している
a：代表的画像
b：全脳解析

相関が認められ，視床においてセロトニン神経機能が低いほど強迫症状が強いことが明らかになった（**図 5**）．視床は強迫性障害の治療薬である SSRI が集積する重要な部位である．自閉症において強迫症状に対する SSRI の効果については有効な人と有効でない人がある．強迫症状の責任部位が複数あると考えられるが，SSRI は主に視床に分布されるので，強迫症状に対する SSRI の薬物効果には限界があると推察される．

今回の結果により，自閉症のセロトニン機能の障害は，出生後から始まっていると推測される．ゆえに，生後の脳の発達時期にセロトニン神経伝達を正常化するような治療法開発の参考になると考えられる．そして療育的観点からは療育により別の経路，側副路でセロトニン機能障害を補う考え方がある．たとえば，脳梗塞の人がリハビリをするように，自閉症の人は療育を受けながら別の神経経路で能力を補っていく必要がある．療育指導は，指導する側の固定観念にとらわれずに工夫して，個々にあった療育指導の方策を築き上げる必要がある．今後更なる研究や療育方法の開発が必要である．

4．免疫系に着目した PET 研究

自閉症に関する免疫系の異常については各種の研究がある．遺伝学的研究からは *HLA* 遺伝子や *MHC class Ⅲ* 遺伝子が自閉症と関連[25-27]しているとの報告があり，血液学的研究からは tumor necrosis factor-α（TNF-α），インターロイキン（IL）-6，macrophage chemoattrac-

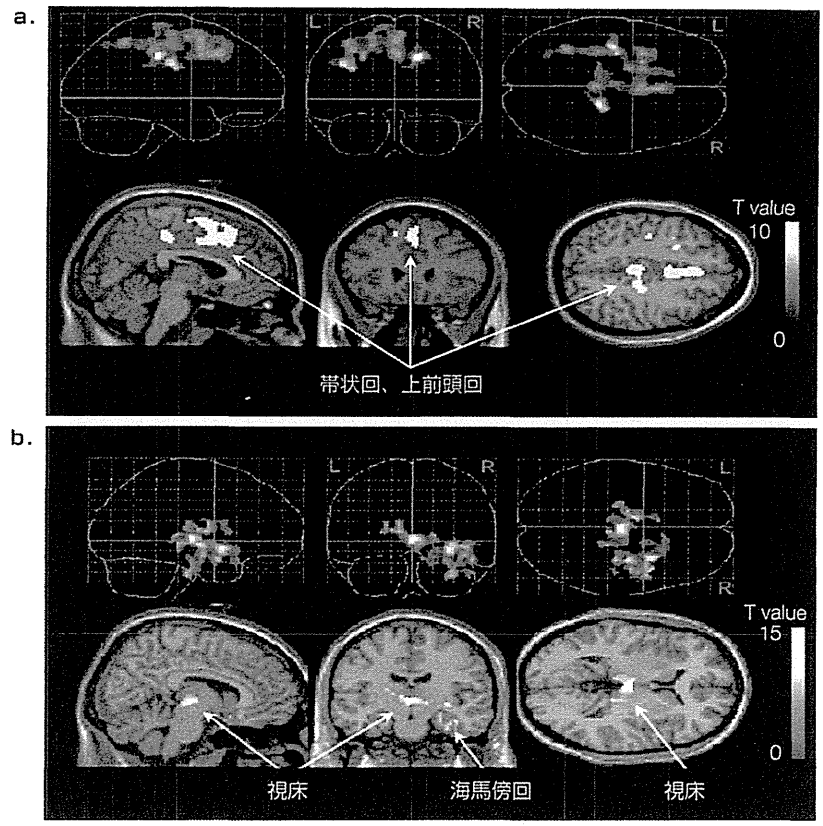

図 5. セロトニン・トランスポーター密度の低下は 2 つの中核症状と関係している

a：「心の理論の獲得障害」は帯状回，上前頭回のセロトニン・トランスポーター密度の低下と関係していた．

b：「強迫症状（こだわり）」は視床，海馬傍回のセロトニン・トランスポーター密度の低下と関係していた．

tant protein-1（MCP-1）の量が自閉症者では増大しているとの報告がある[28)~32)]．また自閉症の子どもをもつ母親は自閉症の脳に対する抗体をもつ[33)~36)]との報告や，自閉症死後脳研究からは脳実質中および脳脊髄液中の TNF-α，IL-6，MCP-1 の増加が報告されている[37)]．

5．ミクログリアについて

われわれはミクログリアに注目した．ミクログリアは脳の中に均一に分布する中胚葉由来の免疫担当細胞である．脳内でのミクログリアの役割は 1 つ目として，感染，出血，虚血で急速に活性化し，活性型ミクログリアになり異物を貪食する．2 つ目として逆の作用として保護作用のある抗炎症性サイトカインを産生し脳細胞を保護する．3 つ目として興味深いことに，脳における神経回路形成や神経伝達の恒常性を維持する役割をもつ．ミクログリアと自閉症の関連については自閉症の死後脳研究で，中前頭回，前帯状回，小脳において活性型ミクログリアやアストログリアの増加が認められている[38)39)]．

この PET 研究はセロトニン系の研究と同様に NPO 法人アスペ・エルデの会の当事者の人で ADI-R（Autism Diagnostic Interview-Revised），ADOS（Autism Diagnostic Observation Schedule）で自閉症スペクトラム（autism spectrum disorder：ASD）と診断し，薬物療法を受けている者，慢性の炎症性疾患を有する者，神経疾患（てんかんを含む）を有する者，IQ が 80 未満の者は除外し，研究の目的と内容について十分説明し，本人および保護者の同意が得られた者のみを対象とし，定型発達の男性を対照とした．

結果は ASD の脳内では，活性型ミクログリアが広汎な部位で増加していた（**図 6**），活性型ミクログリアの

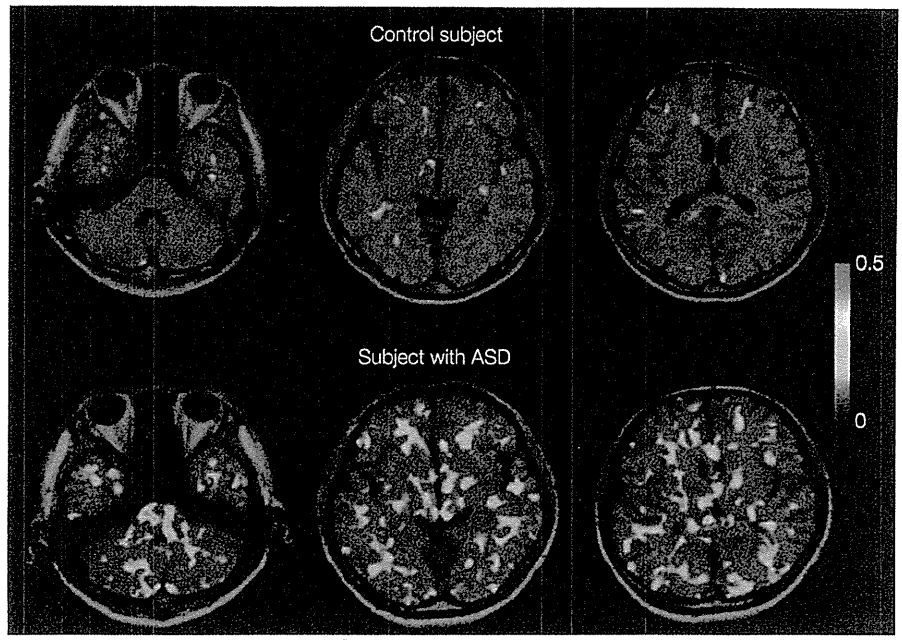

図 6. 活性型ミクログリアの広汎な部位での増加
(Suzuki K *et al*, 2013[1] より引用)

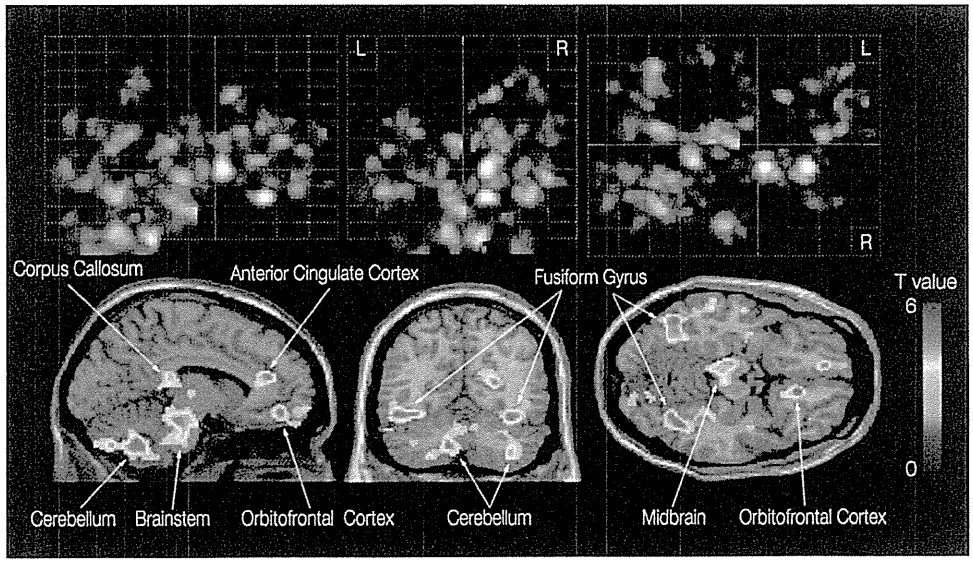

図 7. 活性型ミクログリアの帯状回，眼窩前頭回，紡錘状回などでの増加
(Suzuki K *et al*, 2013[1] より引用)

増加は，小脳と脳幹（中脳，橋）で最も顕著であった．これに加え，ASD の病態との関係が指摘されている脳前部帯状回，眼窩前頭回，紡錘状回にも顕著な増加が認められた（**図 7**）．これらの脳部位における活性型ミクログリアは，ASD 群でも対照群でも，互いに有意に正相関していた（**図 8**）．さらに疾患内比較で，ASD 群の中で活性型ミクログリアが多い群（High-BP）と低い群（Not-High-BP）の 2 群に分けお互いの臨床症状を比較した．多い群については ADI-R による社会性の障害（ADI-R Social Score），これは子どものころの社会性の

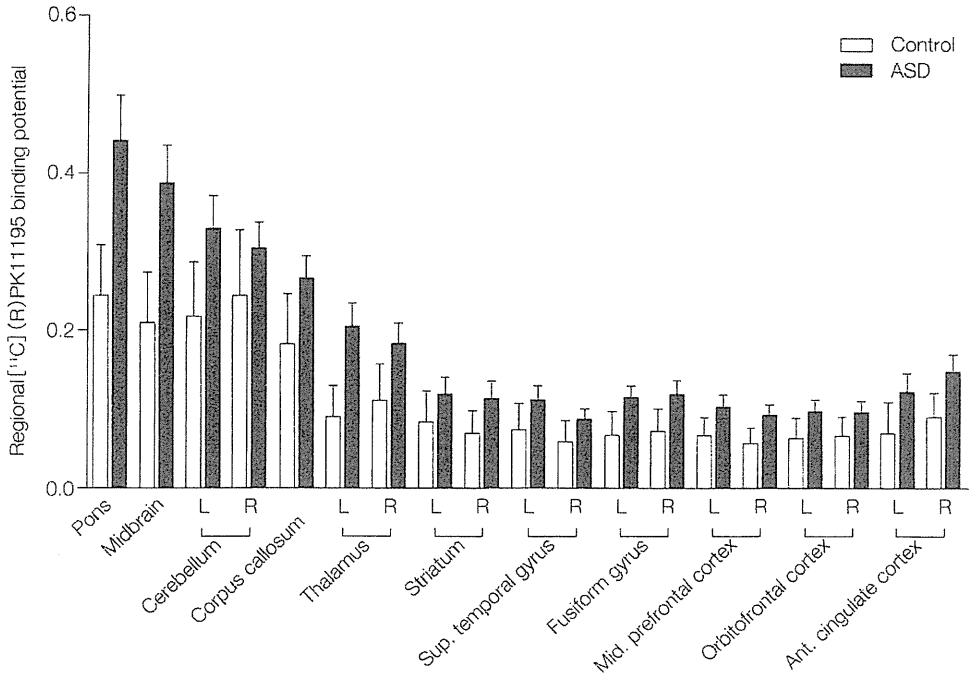

図 8. 活性型ミクログリアのすべての脳部位での増加
(Suzuki K *et al*, 2013[11]より引用)

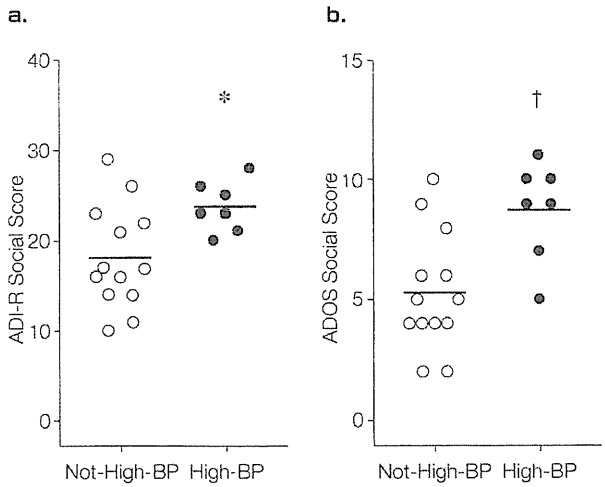

図 9. 活性型ミクログリアの増加と社会性の障害
(Suzuki K *et al*, 2013[11]より引用)

障害の程度を示すがこの指標が高く，ADOSによる社会性の障害（ADOS Social Score）つまり現時点における社会性の障害の程度を示すがこの指標が高いことが明らかになった（**図9**），活性型ミクログリアが多いほど，自閉症の障害の程度が子どものころも現時点においても強いことが明らかになった．以上の結果から，成人ASDの脳内では過剰なミクログリア活性化がおきていることが示唆された．つまり，ミクログリアの活性化は，ASD脳内で小児期から成人期まで継続している現象であると考えられる．ASD群では，対照群と同様に，すべての脳部位のミクログリア活性が互いに正相関していたという所見から（**図6**），脳内のすべてのミクログリアが一様に活性化していることが示唆された．すなわち，局所の炎症や神経傷害を反映したミクログリアの活性化ではなく，ASD脳内のほぼすべてのミクログリアが過剰な反応性を有することを示している．脳のミクログリアの由来は胎生期に血液脳関門が形成される以前に，末梢のマクロファージが脳内に沈着したことによる．ASDでは，ミクログリア活性化により出生前に正常なシナプス形成が阻害される可能性（toxic），またはシナプス形成が不整になり，その結果としてミクログリアが活性化する（protective）考え方があるが，われわれは，過剰なミクログリア活性化によって出生前における正常なシナプス形成が阻害されると考えた．ゆえにわれわれが報告したセロトニン系の異常はセカンダリーと

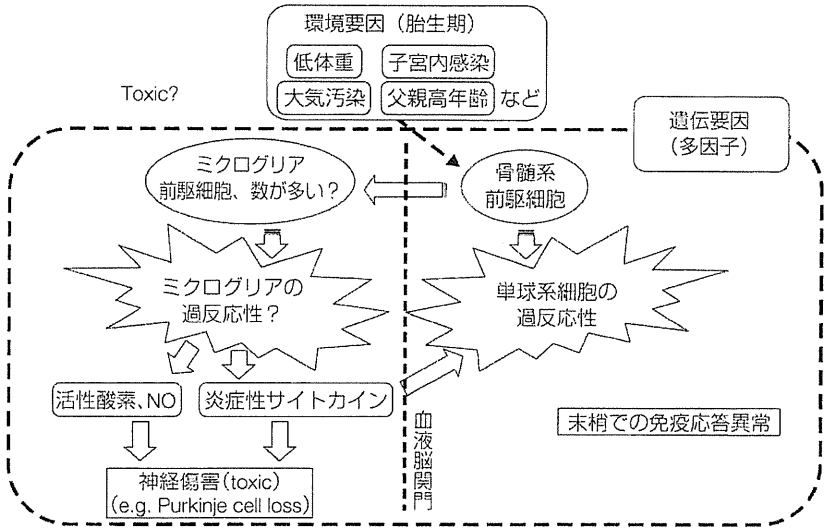

図 10. 自閉症の病態仮説

考えた．**図 10** で自閉症の病態仮説を示した．自閉症は各種の遺伝的要因とさまざまな環境的要因によって胎生期に骨髄系などに影響し末梢のマクロファージが脳内に移行する量が増え，胎生期に増えている活性型ミクログリアによって脳内のシナプス形成障害をおこし ASD の病因となると考えた．

おわりに

われわれは自閉症の PET 研究を各種行い自閉症の脳内での障害を，PET 脳画像研究によって明らかにすることができた．自閉症は近年ではその近縁状態も含めれば 100 人に 1 人とも言われる発現頻度の高い障害であるが，身体障害のような「見える障害」ではないために，社会的な理解が遅れ，今まで，親の育て方が悪い，あるいは本人のわがままだなどといった偏見に満ちた間違った理解をされていたが，今回の研究においても，自閉症が間違いなく脳機能の障害によって生じていることが明らかになり，自閉症の社会的理解を推し進めることにつながると考えられる．なお，この研究は，NPO 法人アスペ・エルデの会の成人当事者が積極的に研究への協力を行い，継続的な自助活動やそれらを背景とする長期に渡る発達支援によって可能になった．

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文　献

1) Folstein SE, Rosen-Sheidley B：Genetics of autism：complex aetiology for a heterogeneous disorder. *Nat Rev Genet* **2**：943-955, 2001
2) Hallmayer J, Cleveland S, Torres A *et al*：Genetic heritability and shared environmental factors among twin pairs with autism. *Arch Gen Psychiatry* **68**：1095-1102, 2011
3) Volk HE, Lurmann F, Penfold B *et al*：Traffic-related air pollution, particulate matter, and autism. *JAMA Psychiatry* **70**：71-77, 2013
4) Suzuki K, Sugihara G, Ouchi Y *et al*：Microglial activation in young adults with autism spectrum disorder. *JAMA Psychiatry* **70**：49-58, 2013
5) Nakamura K, Sekine Y, Ouchi Y *et al*：Brain serotonin and dopamine transporter bindings in adults with high-functioning autism. *Arch Gen Psychiatry* **67**：59-68, 2011
6) Suzuki K, Sugihara G, Ouchi Y *et al*：Reduced acetylcholinesterase activity in the fusiform gyrus in adults with autism spectrum disorders. *Arch Gen Psychiatry* **68**：306-313, 2011
7) 佐治英朗：イメージングプローブの開発と応用．*Drug Delivery System* **23**：24-32，2008
8) Hanley HG, Stahl SM, Freedman DX：Hyperserotonemia and amine metabolites in autistic and retarded children. *Arch Gen Psychiatry* **34**：521-531, 1977
9) Ciaranello RD：Hyperserotonemia and early infantile autism. *N Engl J Med* **307**：181-183, 1982

10) McDougle CJ, Naylor ST, Cohen DJ *et al* : Effects of tryptophan depletion in drug-free adults with autistic disorder. *Arch Gen Psychiatry* **53** : 993-1000, 1996
11) McDougle CJ, Naylor S, Cohen DJ *et al* : A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. *Arch Gen Psychiatry* **53** : 1001-1008, 1996
12) Devlin B, Cook EH Jr, Coon H *et al* : Autism and the serotonin transporter : the long and short of it. *Mol Psychiatry* **10** : 1110-1116, 2005
13) Wassink TH, Hazlett HC, Epping EA *et al* : Cerebral cortical gray matter overgrowth and functional variation of the serotonin transporter gene in autism. *Arch Gen Psychiatry* **64** : 709-717, 2007
14) Yonan AL, Alarcon M, Cheng R *et al* : A genomewide screen of 345 families for autism-susceptibility loci. *Am J Hum Genet* **73** : 886-897, 2003
15) Chugani DC, Muzik O, Behen M *et al* : Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. *Ann Neurol* **5** : 287-295, 1999
16) Chugani DC, Muzik O, Rothermel R *et al* : Altered serotonin synthesis in the dentatothalamocortical pathway in autistic boys. *Ann Neurol* **42** : 666-669, 1997
17) Chandana SR, Behen ME, Juhasz C *et al* : Significance of abnormalities in developmental trajectory and asymmetry of cortical serotonin synthesis in autism. *Int J Dev Neurosci* **23** : 171-182, 2005
18) Makkonen I, Riikonen R, Kokki H *et al* : Serotonin and dopamine transporter binding in children with autism determined by SPECT. *Dev Med Child Neurol* **50** : 593-597, 2008
19) Nakamura K, Sekine Y, Ouchi Y *et al* : Brain serotonin and dopamine transporter bindings an adults with high-functioning autism. *Arch Gen Psychiatry* **67** : 59-68, 2010
20) Baron-Cohen S, O'Riordan M, Stone V *et al* : Recognition of faux pas by normally developing children and children with Asperger syndrome or high-functioning autism. *J Autism Dev Disorder* **29** : 407-418, 1999
21) Cook EH Jr : Brief report : pathophysiology of autism : neurochemistry. *J Autism Dev Disorder* **26** : 221-225, 1996
22) Whitaker-Azmitia PM : Behavioral and cellular consequences of increasing serotonergic activity during brain development : a role in autism? *Int J Dev Neurosci* **23** : 75-83, 2005
23) Ohnishi T, Matsuda H, Hashimoto T *et al* : Abnormal regional cerebral blood flow in childhood autism. *Brain* **123** : 1838-1844, 2000
24) Haznedar MM, Buchsbaum MS, Wei TC *et al* : Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. *Am J Psychiatry* **157** : 1994-2001, 2000
25) Warren RP, Singh VK, Cole P *et al* : Increased frequency of the null allele at the complement C4b locus in autism. *Clin Exp Immunol* **83** : 438-440, 1991
26) Warren RP, Odell JD, Warren WL *et al* : Strong association of the third hypervariable region of HLA-DR beta 1 with autism. *J Neuroimmunol* **67** : 97-102, 1996
27) Odell D, Maciulis A, Cutler A *et al* : Confirmation of the association of the C4B null allelle in autism. *Hum Immunol* **66** : 140-145, 2005
28) Jyonouchi H, Sun S, Le H : Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol* **120** : 170-179, 2001
29) Croonenberghs J, Bosmans E, Deboutte D *et al* : Activation of the inflammatory response system in autism. *Neuropsychobiology* **45** : 1-6, 2002
30) Schwarz E, Guest PC, Rahmoune H *et al* : Sex-specific serum biomarker patterns in adults with Asperger's syndrome. *Mol Psychiatry* **16** : 1213-1220, 2011
31) Ashwood P, Krakowiak P, Hertz-Picciotto I *et al* : Altered T cell responses in children with autism. *Brain Behav Immun* **25** : 840-849, 2001
32) Suzuki K, Matsuzaki H, Iwata K *et al* : Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. *PLoS One* **6** : e20470, 2011
33) Dalton P, Deacon R, Blamire A *et al* : Maternal neuronal antibodies associated with autism and a language disorder. *Ann Neurol* **53** : 533-537, 2003
34) Singer HS, Morris C, Gause C *et al* : Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations : A pregnant dam mouse model. *J Neuroimmunol* **211** : 39-48, 2009
35) Braunschweig D, Ashwood P, Krakowiak P *et al* : Autism : maternally derived antibodies specific for fetal brain proteins. *Neurotoxicology* **29** : 226-231, 2008
36) Croen LA, Braunschweig D, Haapanen L *et al* : Maternal mid-pregnancy autoantibodies to fetal brain protein : the early markers for autism study. *Biol Psychiatry* **64** : 583-588, 2008
37) Zimmerman AW, Jyonouchi H, Comi AM *et al* : Cerebrospinal fluid and serum markers of inflammation in autism. *Pediatr Neurol* **33** : 195-201, 2005
38) Vargas DL, Nascimbene C, Krishnan C *et al* : Neuroglial

activation and neuroinflammation in the brain of patients with autism. *Ann Neurol* **57** : 67-81, 2005
39) Morgan JT, Chana G, Pardo CA *et al* : Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biol Psychiatry* **68** : 368-376, 2010

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Relationship between brain network pattern and cognitive performance of children revealed by MEG signals during free viewing of video

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ABSTRACT

Application of graph theory to analysis of functional networks in the brain is an important research trend. Extensive research on the resting state has shown a "small-world" organization of the brain network as a whole. However, the small-worldness of children's brain networks in a working state has not yet been well characterized. In this paper, we used a custom-made, child-sized magnetoencephalography (MEG) device to collect data from children while they were watching cartoon videos. Network structures were analyzed and compared with scores on the Kaufman Assessment Battery for Children (K-ABC). The results of network analysis showed that (1) the small-world scalar showed a negative correlation with the simultaneous processing raw score, a measure of visual processing (Gv) ability, and (2) the children with higher simultaneous processing raw scores possessed network structures that can be more efficient for local information processing than children with lower scores. These results were compatible with previous studies on the adult working state. Additional results obtained from further analysis of the frontal and occipital lobes indicated that high cognitive performance could represent better local efficiency in task-related sub-networks. Under free viewing of cartoon videos, brain networks were no longer confined to their strongest small-world states; connections became clustered in local areas such as the frontal and occipital lobes, which might be a more useful configuration for handling visual processing tasks.

1. Introduction

The development of neurophysiological imaging devices has enabled us to carry out research on correlation, coherence, coupling, and synchronization between brain regions (Kikuchi et al., 2013; Lowe, Mock, & Sorenson, 1998; Stam, 2004; van den Heuvel & Hulshoff Pol, 2010). By studying the connection index of different regions, we can understand the information transfer pattern and the structure of the brain's functional network in the brain. Several studies have suggested that functional networks in the brain possess an optimal small-world structure (Achard, Salvador, Whitcher, Suckling, & Bullmore, 2006; Alexander-Bloch et al., 2013; Bassett & Bullmore, 2006; Bullmore & Bassett, 2011; Bullmore & Sporns, 2012; Douw et al., 2010; Langer, Pedroni, & Jancke, 2013; Liu et al., 2008; Micheloyannis et al., 2006; Smit, Stam, Posthuma, Boomsma, & de Geus, 2008; Stam, 2004; Stam, Jones, Nolte, Breakspear, & Scheltens, 2007; van den Heuvel, Stam, Boersma, & Hulshoff Pol, 2008; Micheloyannis et al., 2006). Small-worldness is a concept in graph theory, which describes a network with an optimal balance between strong local connections, with high levels of local clustering for efficient information processing, and long-distance connections for efficient global information transfer (Watts & Strogatz, 1998). Research on schizophrenia, Alzheimer's disease, and autism has revealed different brain network small-world indices between patients and control groups (Micheloyannis, Pachou, Stam, Breakspear et al., 2006; Stam, Jones, Nolte, Breakspear, & Scheltens, 2007; Tsiaras et al., 2011). Studies on resting and working state networks have suggested that both intelligence and educational backgrounds may be reflected in various parameters of the small-worldness of brain graphs (Micheloyannis, Pachou, Stam, Breakspear et al., 2006; van den Heuvel, Stam, Kahn, & Hulshoff Pol, 2009). There has also been research on the network structure of children, who possess small-world organization similar to that of young adults (Supekar, Musen, & Menon, 2009). However, the small-worldness of children's brain graphs in a working state has not yet been well characterized. In adults, intellectual performance is reported to be related to the small-worldness of the brain

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(van den Heuvel et al., 2009). Moreover, research in children has shown a correlation between left dominance of coherence and cognitive performance during the task of watching cartoon videos (Kikuchi et al., 2011). We therefore hypothesized an association between network features and cognitive performance when children were watching videos freely.

In this paper, we describe relationship between cognitive performance and graph indices of children during free viewing of a video. Magnetoencephalographic (MEG) data were recorded from children by means of a custom-made child-sized MEG device. The association matrix of the brain network was estimated using the mutual information method (Cover & Thomas, 1991). Brain functional networks were modeled as undirected graphs for further network topology investigation. We used the raw scores of the Kaufman Assessment Battery for Children (K-ABC) (Kaufman & Kaufman, 1983) as a measure of cognitive performance.

2. Method

2.1. Participants

Thirty children (25 boys, 5 girls) without a psychiatric history participated in the study. The participants had a mean age of 63.9 (39–81) months. All participants were native Japanese with normal hearing ability.

2.2. Psychological performance test

The Japanese adaptation of the K-ABC was given to all children (Kaufman & Kaufman, 1983). The K-ABC is an intelligence test developed from neuropsychological theory for assessing cognitive development. Three general raw scores were evaluated by the K-ABC, namely, the sequential processing scale, the simultaneous processing scale, and the achievement scale. The mean and standard deviation of raw scores on each of these were 15.3 ± 5.4, 29.1 ± 6.4 and 41.4 ± 11.3, respectively.

The sequential processing scale was evaluated from two subtests. They were (1) hand movement: the child copied a series of hand gestures the examiner performed, and (2) number recall: the child repeated a string of numbers in the same order as the examiner gave them. The sequential processing scale is categorized as short term memory (Gsm) ability in the Cattell–Horn–Carroll (CHC) theory (Flanagan, Ortiz, & Alfonso, 2013).

The simultaneous processing scale was assessed by (1) magic window: the child identified a picture through a slit, (2) face recognition: the child looked at a photograph of one face and then selected the same face shown in a difference pose, and (3) gestalt closure: the child described or named a partially completed inkblot drawing. The simultaneous processing scale is considered to reflect visual processing (Gv) ability in the CHC model.

The achievement scale was obtained from (1) expressive vocabulary: children named familiar objects in a photograph, (2) arithmetic: children solved arithmetic that was presented in a story, and (3) riddle: children retrieved a word that was based on hints that were associated with the word. These subtests generally evaluate crystallized intelligence (Gc) and quantitative reasoning (Gq) ability in the CHC model.

2.3. MEG recordings

MEG measurements and the K-ABC were taken on two separate days. A custom-made, 151-channel whole-head coaxial gradiometer MEG (PQ 1151R; KIT/Yokogawa Corp, Kanazawa, Japan) for children was used to record MEG data at a sampling rate of 1000 Hz (Kikuchi et al., 2013). The sensor layout is shown in Fig. 1. Each child selected a single cartoon video program with a story of his or her preference before the recording session. Before the recording, one of the authors (YY) escorted each participant into the shielded room (Daido Steel, Nagoya, Japan). The MEG recording sessions required the participants to lie comfortably on a bed with their head inside the helmet of the MEG system and to view the video program projected onto a screen. The sound of the video was delivered to the participants binaurally through a tube. The staff member stayed in the shielded room to take care of the participants when necessary and to confirm that each participant was enjoying the cartoon video. In each session, we acquired 3-min MEG time series data. During the recordings, the children's parent(s) could observe them through a TV monitor. Collection of data was in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Kanazawa University Hospital.

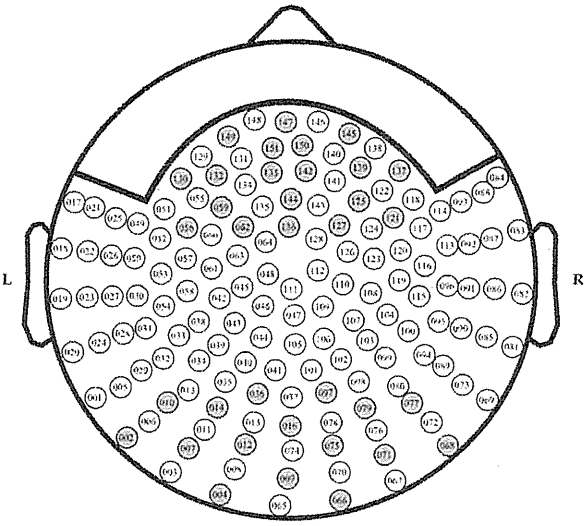

Fig. 1. Spatial layout of all MEG sensors, where the shaded sensors in color indicate the 35 sensors selected for sub-network analysis of the frontal and occipital lobes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.4. Data preprocessing

Data were preprocessed in MATLAB (The MathWorks, Inc.) in conjunction with the FieldTrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011). The recorded signals were filtered by an infinite impulse response Chebyshev Type I filter. The pass band of the filter was set at 0.1–100 Hz.

The limited self-restraint of children made it difficult for them to complete the recording session perfectly. Therefore, we applied independent component analysis (ICA) to isolate and remove artifacts from the MEG data. The ICA was performed by the FastICA algorithm in the FieldTrip toolbox. Parameters of the FastICA were set as follows: stopping residual was 0.0001, nonlinearity function was $g = u^3$, and the maximum number of iterations was 1000.

We automatically removed artifacts with extremely high Kurtosis. Kurtosis, also called the fourth-order cumulant, is a classical measure of non-Gaussianity. For Gaussian data, kurtosis is equal to 1. It has been successfully used in other research to detect artifacts (Escudero, Hornero, Abásolo, Fernández, & López-Coronado, 2007). For most non-Gaussian random variables, kurtosis is not equal to 1.

MEG data were resampled at 250 Hz after artifact removal. In order to avoid the transition of brain function and other effect of

possible fatigue and distraction, periods from 40 s to 60 s at 5000 samples per channel were selected for further analysis. All frequency bands of the processed data were used to estimate the association matrix of the brain network.

2.5. Mutual information

To analyze the networks of the children, the association matrix of each child was estimated from the MEG data using the concept of Mutual Information (MI), which is a measure of the interdependence between two time series. The most significant feature of MI is that it is sensitive to both linear and nonlinear interactions (Bullmore & Bassett, 2011). We used the MI of each pair of channels as the element of the association matrix. The MI of two discrete random variables X and Y is defined as

$$I(X;Y) = \sum_{y\in Y}\sum_{x\in X} p(x,y)\log\left(\frac{p(x,y)}{p_x(x)p_y(y)}\right), \quad (1)$$

where $p(x, y)$ is the joint probability distribution function of X and Y, and $p_x(x)$ and $p_y(y)$ are the marginal probability distribution functions of X and Y, respectively.

We estimated MI by separating MEG data into sets of bins. As a result $p_x(x)$ and $p_y(y)$ could be obtained by counting the number of points in each bin. In this study, during the estimation of density functions, the sampling points (x, y) were eliminated as outliers for which either x or y held a value of bilateral 0.5%.

2.6. Network construction and evaluation

We used the association matrix to construct the graph of the brain network (referred to as the "brain graph" in what follows). To construct a brain graph, a threshold value needs to be chosen for determining which channels are connected or not. In fact, there is no universal criterion for determining the threshold of an association matrix. According to the two rules of comparing brain graphs (Bullmore & Bassett, 2011), in this study, the value of threshold was set using the criteria to ensure that all brain graphs possessed the same number of edges for an easy comparison with one another.

In this study, we modeled brain networks as undirected and unweighted graphs. The characteristic path length, the clustering coefficient and the small-world scalar are popular indices for undirected unweighted graphs in small-world network theory (Watts & Strogatz, 1998). These indices can be used to compute information transfer efficiency in the network. According to the Watts–Strogatz (WS) model of a network with N vertices and K edges, a small-world graph should have a short characteristic path length (L) compared with a regular lattice, a large clustering coefficient (C) similar to that of a regular lattice, and a small-world scalar σ greater than 1. The indices are defined as follow:

$$L = \frac{2}{N(N-1)}\sum_{i}\sum_{j=i+1}^{N} d_{ij}, \quad (2)$$

$$C = \frac{2}{N}\sum_{i}\frac{\sum_j\sum_{m\,j+1}^{N} a_{ij}a_{jm}a_{mi}}{k_i(k_i-1)}, \quad (3)$$

$$\sigma = \frac{CL_{\text{regular}}}{LC_{\text{regular}}}, \quad (4)$$

where a_{ij} is equal to 1 when vertices i and j are connected, index k_i is the number of edges incident with i, and d_{ij} is the shortest path length between vertices i and j. L is the average of the shortest path connecting any two vertices on the graph. C is the average probability that two edges of a vertex of the graph are the edges of a triangle. L_{regular} and C_{regular} are L and C, respectively, of a regular lattice graph with N vertices and K edges.

3. Results

3.1. Mean association matrix

Fig. 2 shows the mean association matrix, which was calculated by averaging the association matrices of all the participants.

3.2. Network-theoretical analysis of patterns of the brain data and its relation to performance

3.2.1. Entire brain

To understand the network structure of the entire brain, we calculated the correlation coefficients between the small-world scalar of each child's whole brain network and the raw scores of the global scales in the K-ABC test. Fig. 3 shows the correlation coefficient as a function of the average degree of the network. The range of the degree that allowed prominent topological properties of the network was chosen. The results showed that all correlation coefficients between K-ABC raw scores and small-world scalars were negative. Fig. 3(b) shows that the small-world scalars were negatively correlated with the raw score of the simultaneous processing scale with strong statistical significance; when the average degree of the networks was around 20, the negative correlation was statistically significant ($p < 0.05$).

Next, the brain network and the graph indices were examined under the condition that the average degree of the networks was set at 21.5. Two typical brain networks are shown in Fig. 4: (a) the brain network of a participant with a relatively low simultaneous processing raw score, and (b) a high score participant's brain network. Fig. 5(a) shows the regression line between the small-world scalars and the simultaneous processing raw scores. It showed a strong ($p = 0.00007$) negative correlation with a correlation coefficient of −0.65978. Fig. 5(b) and (c) depict the regression lines for L and C, respectively. L was positively correlated with the simultaneous processing raw score ($\rho = 0.63786$, $p = 0.00015$). There was no significant correlation between C and the simultaneous processing raw score ($\rho = 0.34927$, $p = 0.05852$). Based on these results, we separated the participants into two groups by their simultaneous processing raw score. Comparisons between

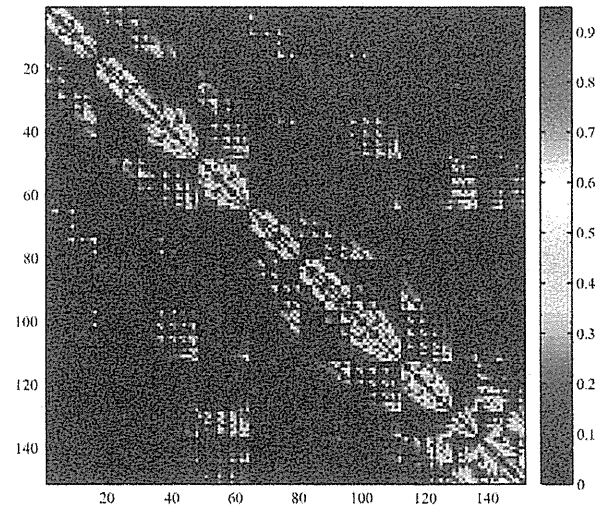

Fig. 2. The mean association matrix (the average association matrix over all participants).

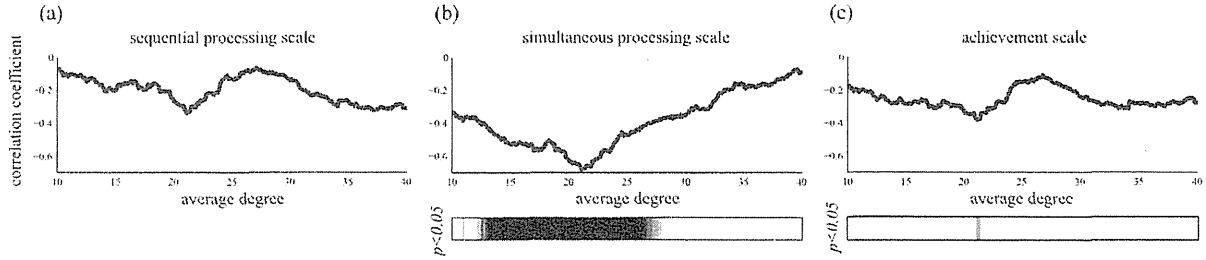

Fig. 3. Correlation coefficient between the small-world scalar and the raw scores of K-ABC. (a–c) For the sequential processing scale, simultaneous processing scale and achievement scale, respectively. The shaded bars indicate regions where the *p*-values were smaller than 0.05, flagging statistically significant effects. The small-world scalar was negatively correlated with the simultaneous processing raw score when the average degree was around 20.

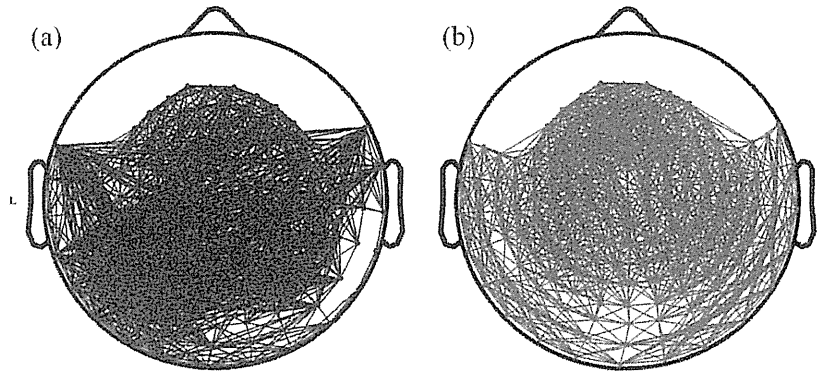

Fig. 4. The brain networks of typical participants are drawn. The average degree across vertices was 21.5. (a) The network of a typical participants with a low simultaneous processing raw score (18) and (b) the network of a typical participants with a high simultaneous processing raw score (33).

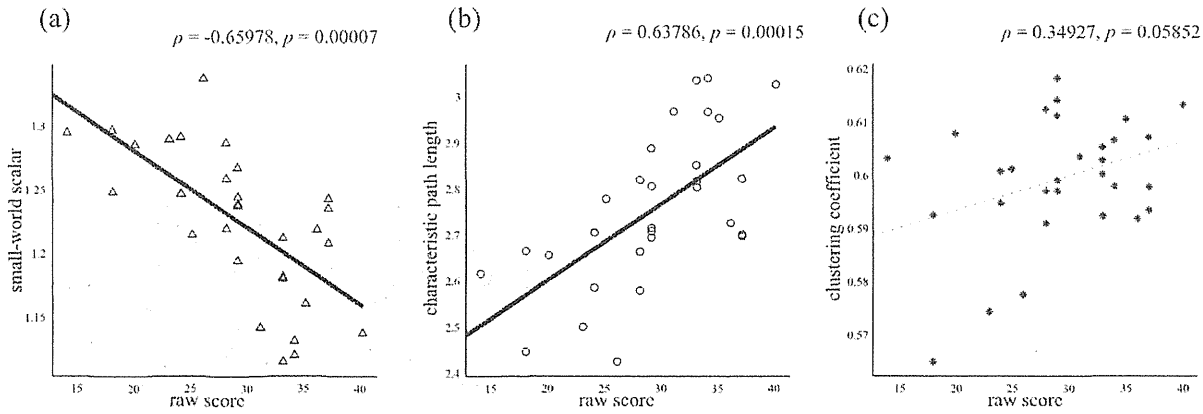

Fig. 5. (a) The regression line of the small-world scalar against the simultaneous processing raw score ($\rho = -0.65978$, $p = 0.00007$), (b) that between *L* and the raw score ($\rho = 0.63786$, $p = 0.00015$), (c) that between *C* and the raw score. The figures show the graph indices that are obtained when the average degree across vertices is standardized at 21.5. The solid lines show correlations having statistical significance. The dashed line indicates failure to reach such significance ($p > 0.05$).

the high-score group and low-score group were made on the *L* and *C* values. The result was shown in Table 1. For further comparison, the indices of regular and random graphs of average degree of 21.5 are also shown. Both indices of the high-score group were closer to those of the regular network than were the indices of the low-score group.

3.2.2. Task-related sub-networks

The small-world scalar showed strong relationship with the simultaneous processing raw score, i.e., the index of Gv ability. Therefore, we explored the sub-network features of the frontal and occipital lobes, which are the brain regions responsible for the high-level cognitive and visual processing. To further explore the local network patterns of the frontal and occipital lobes, *L*, *C*, and small-world scalars of the participants were re-correlated with the raw scores using data from selected MEG sensors over the frontal and occipital lobes (Fig. 1). A pilot study provided the network degree at which significant effects emerge. Fig. 6 shows the correlation coefficient between the small-world scalar and cognitive performance as a function of the average degree. It was observed

Table 1
L and *C* values of the networks found in high and low score groups, as well as the *p*-values between groups. The corresponding values for regular and random networks are shown for comparison.

Network	*L*		*C*	
	M ± Std.	*p*-Value	M ± Std.	*p*-Value
Regular	3.997		0.714	
High score group	2.862 ± 0.127	0.00004*	0.602 ± 0.007	0.17836
Low score group	2.640 ± 0.121		0.596 ± 0.015	
Random	1.899		0.148	

M, mean; Std, standard deviation.
* *p*-Value < 0.05.

that all of the global raw scores were positively correlated with the small-world scalar when the average degree was around 8.

The sub-networks calculated with an average degree of 8 are the focus of this part. Fig. 7 shows (a) the sub-network of a participant with a relatively low K-ABC score, and (b) a high-scoring participant's sub-network. Fig. 8 shows the regression lines of the sub-network small-world scalar, *L*, and *C* values against the simultaneous processing raw scores. The correlation coefficient of the small-world scalars was 0.41367 ($p = 0.02306$). *L* was negatively correlated with the raw scores ($\rho = -0.37470$, $p = 0.04018$), and *C* was positively correlated with the raw scores ($\rho = 0.37044$, $p = 0.04389$).

4. Discussion

4.1. K-ABC scores and small-worldness

Compared with other global raw scores, the simultaneous processing raw score showed the strongest correlation with the whole-brain graph index. A possible reason is that the simultaneous processing scale is also the scale describing the Gv ability: the ability to generate, store, retrieve and transform visual images and sensations (McGrew, 2009). In the present study, the MEG data were obtained while children were watching videos freely. The free viewing of video is a task that mainly exercises the Gv ability. Therefore, the brain network activation may have been strongly related to Gv, also known as simultaneous processing. Thus, the simultaneous processing raw score would show stronger correlation with the graph index of the brain network. Our results showed that the brain graphs of participants with better cognitive performance possessed a lower small-world scalar. Therefore, the networks of children with a high score in simultaneous processing may possess weaker small-worldness during the free viewing of video. We found in Fig. 5(b) that *L* was positively correlated with the simultaneous processing raw score and the effect was statistically significant ($p = 0.00015$). Fig. 5(c) indicates that *C* did not correlate significantly with the simultaneous processing raw score. In Fig. 3(c), we showed that the achievement raw score had a weaker correlation with the small-world scalar than the simultaneous processing raw score. The achievement scale was evaluated by the expressive vocabulary test and the arithmetic test. The expressive vocabulary test evaluated Gq; however, the test also exercised Gv ability because of the task stimulus modality, naming the objects in a photograph.

4.2. Network features of well-functioning brains

For descriptive convenience, we categorize the brains of participants with relatively better cognitive performance (e.g., participants with high IQ or well-educated participants in studies of healthy people), and the brains of control groups without

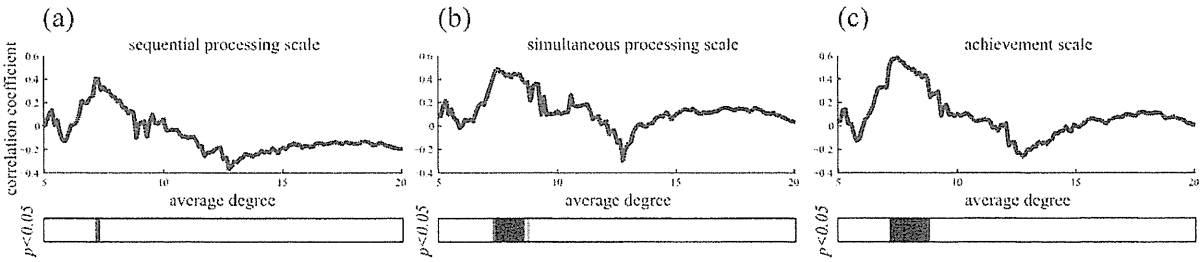

Fig. 6. Correlation coefficients between the small-world scalar of the sub-network and the raw scores of the K-ABC. (a–c) Show the sequential processing scale, the simultaneous processing scale, and the achievement scale, respectively. The shaded bars indicate *p*-values smaller than 0.05. The small-world scalar was positively correlated with all the global raw scores when the average degree was around 8.

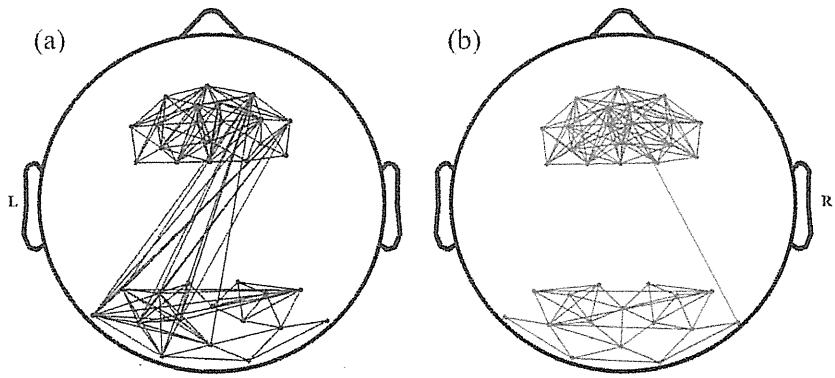

Fig. 7. (a) The sub-network of a typical participant with a low K-ABC total score (37), and (b) the sub-network of a typical participant with a high K-ABC total score (81). The average degree was 8.