別記様式第 6 号（第 16 条第 3 項，第 25 条第 3 項関係）

論 文 審 査 の 結 果 の 要 旨

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学 位 授 与 の 条 件	学位規則第 4 条第 ①・2 項該当		

論　文　題　目
Altered microbiota composition reflects enhanced communication in 15q11-13 CNV mice
(15q11-13 CNV マウスにおいて腸内細菌叢の変化がコミュニケーションを改善する)

論文審査担当者

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〔論文審査の結果の要旨〕

Autism spectrum disorders (ASD) is a complex and heterogeneous neurodevelopmental disorder. In addition to the core symptoms of ASD, many patients with ASD also show comorbid gastrointestinal problems and gut dysbiosis. Intriguingly, there is evidence that gut microbiota communicate with the central nervous system to modulate behavioral output through the gut-brain axis. Although many studies have reported differences in gut microbiota composition between patients with ASD and control subjects, no particular gut bacteria species is currently implicated in ASD. Based on the emerging link between gut microbiota and ASD and the lack of consensus on specific causative gut bacteria, this study aimed to investigate the microbiota composition in a model mouse of ASD and to identify the specific microbiota community involved in ASD-related behavioral symptoms. This study focused on 15q11-13 duplication (*15q dup*) mouse which is one of ASD model mice. This mouse has 6.3Mb duplication which corresponds to human 15q11-13 duplication, one of the frequently reported chromosomal aberration or copy number variation (CNV) in patient with ASD.

Metagenomic analysis of 16s rRNA gene by Next Generation Sequencing was performed. Metagenomic analysis from fecal sample revealed that species richness and evenness in gut microbiota were significantly lower in *15q dup* mice. No differences were found in relative abundances of gut microbiota at the phylum and genus levels between WT and *15q dup* mice. However, in Operational Taxonomic Units (OTUs)/species level, 13 OTUs were more abundant in WT mice, among which 7 OTUs were not detected in *15q dup* mice.

To determine whether these 13 differential OTUs involve in the behavior observed in 15q dup, a second metagenomic analysis was performed. In the second analysis, combination of 4 weeks antibiotics treatment of ampicillin or neomycin followed by behavior tests of open field and Ultrasonic Vocalization (USV) tests and metagenomic analysis of fecal sample was performed. The USV test showed that neomycin improved USV call number in *15q dup* mice. However, the antibiotics treatment didn't affect locomotor activity and anxiety-like behavior in any mice. Metagenomic analysis after antibiotics treatment showed increased species richness in *15q dup* mice treated with neomycin but not in WT treated with neomycin, as shown by OTUs number, Chao1, and Abundance-based Coverage Estimator (ACE) index. Analysis using UniFrac-PCoA (Unique Fraction metric-Principal Coordinate Analysis) also suggested differences in microbiota composition between WT and *15q dup* mice after neomycin treatment. In *15q dup* mice, neomycin increased the relative abundance of *Bacteroides* and *Parabacteroides* and decreased that of *Parasutterella*. But in WT, neomycin increased the relative abundance of *Roseburia* and *Oscillibacter* but decreased that of Lactobacillus. In OTUs/species level, 7 OTUs which included *Barnesiella viscericola,*

Clostridium hathewayi, Oscillibacter valericigenes, Prevotella sp. Smarlab 121567, Clostridium clostridioforme, Hydrogenoanaerobacterium saccharovorans, and *Roseburia inulinivorans* were more abundant in *15q dup* mice treated with neomycin. In addition, these 7 OTUs are included in the 13 OTUs that were more abundant in WT. These findings suggested that neomycin treatment in *15q dup* mice altered the microbiota composition in favors of the growth of bacteria with beneficial effect on USV call number. Therefore, this study proposed these 7 OTUs as the candidates of beneficial OTUs for USV call number.

Most of these candidates of beneficial OTUs belong to Clostridium clusters XIVa and IV. *Clostridium hathewayi*, *Clostridium clostridioforme*, and *Roseburia inulinivorans* belong to Clostridium cluster XIVa, while *Oscillibacter valericigenes* and *Hydrogenoanaerobacterium saccharovorans* belong to Clostridium cluster IV. Clostridium clusters XIVa and IV are known for their anti-inflammatory properties which includes stimulating Treg cells differentiation and IL-10 production. They also abundantly produce short chain fatty acids (SCFAs), including butyrate, propionate, and acetate. SCFAs may regulate brain function through many pathways, including inhibition of histone deacetylase and modulation of serotonin (5-HT). 5-HT signaling itself has been associated with USV. Given that the candidates of beneficial OTUs identified in this study are SCFA-producing species with the described properties, it is possible that these bacteria are capable of modulating 5-HT levels, thereby affecting USV. Although further studies using human samples or germ-free mice conventionalized with the candidate of beneficial OTUs are required to confirm these findings, this study suggest that beneficial OTUs which produce SCFAs, may enhance USV in the *15q dup* mouse model of ASD.

This paper demonstrates that altered microbiota composition enhances social communication in mice. Thus, all members of the review committee recognized that the paper was of sufficient value to grant PhD (medicine) to Dian Eurike Septyaningtrias.