News Release

Title

Rare genetic variants in *CX3CR1* and their contribution to the increased risk of schizophrenia and autism spectrum disorders

Key Points

- **We present the genetic association of a variant in *CX3CR1*, a G protein coupled receptor solely expressed by microglia in the brain, with SCZ and ASD, followed by *in silico* 3D structural modeling of the predicted conformational change of the variant receptor and its *in vivo* disruption of Akt signaling.**
- **Our findings provide the first genetic evidence in a microglia-specific gene for association with neurodevelopmental disorders.**
- **Our results strengthen the association between microglia-specific genes and neurodevelopmental disorders.**

Summary

Prof. Norio Ozaki, Dr. Branko Aleksic (corresponding author), Dr. Kanako Ishizuka (first author) at Department of Psychiatry, Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu, M.D., Ph.D.); Prof. Toshihide Yamashita at Department of Molecular Neuroscience, Osaka University Graduate School of Medicine and Prof. Haruki Nakamura at Institute for Protein Research, Osaka University demonstrated that rare genetic variants in *CX3CR1*, which encodes a G protein coupled receptor solely expressed by microglia in the brain, contributed to the increased risk of schizophrenia (SCZ) and autism spectrum disorders (ASD). This was achieved through collaborative research work with RIKEN Brain Science Institute, Okayama University Graduate School of Medicine, Niigata University Graduate School of Medical and Dental Sciences, Kohnodai Hospital, National Center for Global Health and Medicine, and Fujita Health University School of Medicine.

CX3CR1 has been repeatedly reported to be associated with neurodevelopmental disorders including SCZ and ASD in transcriptomic and animal studies but not in genetic studies. To address the impacts of variants in *CX3CR1* on neurodevelopmental disorders, we conducted coding exon-targeted resequencing of *CX3CR1* in 370 Japanese SCZ and 192 ASD patients using next-generation sequencing technology, followed by a genetic association study in a sample comprising 7,054 unrelated individuals (2,653 SCZ, 574 ASD, and 3,827 controls).

We then performed *in silico* three-dimensional structural modeling and *in vivo* disruption of Akt phosphorylation to determine the impact of the detected variant on CX3CR1 dependent signal transduction. We detected a statistically significant association between the variant Ala55Thr in *CX3CR1* with SCZ and ASD phenotypes (OR = 8.3, P = 0.020). A three-dimensional structural model indicated that Ala55Thr could destabilize the conformation of the CX3CR1 helix 8 and affect its interaction with a heterotrimeric G protein. *In vitro* functional analysis showed that the CX3CR1-Ala55Thr mutation inhibited cell signaling induced by fractalkine, the ligand for CX3CR1. The combined data suggested that the variant Ala55Thr in CX3CR1 might result in the disruption of CX3CR1 signaling. Our results strengthen the association between microglia-specific genes and neurodevelopmental disorders.

Research Background

CX3CR1, a G protein coupled receptor solely expressed by microglia in the brain, has been repeatedly reported to be associated with neurodevelopmental disorders including SCZ and ASD in transcriptomic and animal studies but not in genetic studies.

Research Results

To address the impacts of variants in *CX3CR1* on neurodevelopmental disorders, we conducted coding exon-targeted resequencing of *CX3CR1* in 370 Japanese SCZ and 192 ASD patients using next-generation sequencing technology, followed by a genetic association study in a sample comprising 7,054 unrelated individuals (2,653 SCZ, 574 ASD, and 3,827 controls). We then performed *in silico* three-dimensional structural modeling and *in vivo* disruption of Akt phosphorylation to determine the impact of the detected variant on CX3CR1 dependent signal transduction. We detected a statistically significant association between the variant Ala55Thr in *CX3CR1* with SCZ and ASD phenotypes (OR = 8.3, P = 0.020). A three-dimensional structural model indicated that Ala55Thr could destabilize the conformation of the CX3CR1 helix 8 and affect its interaction with a heterotrimeric G protein. *In vitro* functional analysis showed that the CX3CR1-Ala55Thr mutation inhibited cell signaling induced by fractalkine, the ligand for CX3CR1. The combined data suggested that the variant Ala55Thr in CX3CR1 might result in the disruption of CX3CR1 signaling.

Research Summary and Future Perspective

Our results of the genetic association of the Ala55Thr variant in *CX3CR1* with both SCZ and ASD; the conformational change in the CX3CR1-Ala55Thr mutant compared to CX3CR1 WT that was predicted by *in silico* 3D structural modeling; and the down-regulation of fractalkine-CX3CR1 signaling by the Ala55Thr mutant *in vivo* support the hypothesis that this variant in *CX3CR1* might be a plausible candidate causal variant in SCZ and ASD etiopathologies. Our findings provide the first genetic evidence in a microglia-specific gene for association with neurodevelopmental disorders. A deeper understanding of genetic risk factors and disease pathobiology may lead to major health benefits through the development of methods for the prevention, diagnoses and treatment of such diseases.

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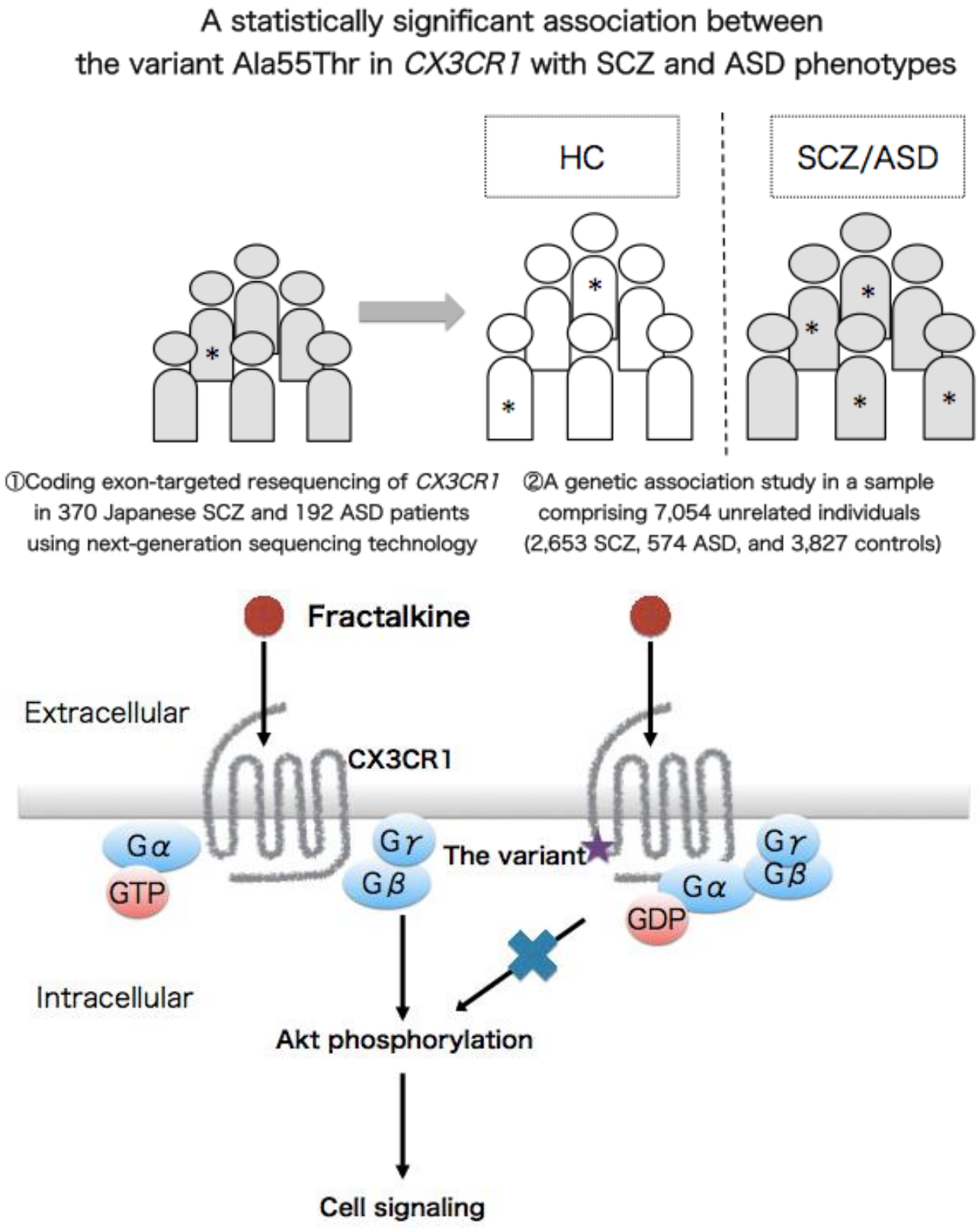

Publication

Kanako Ishizuka, Yuki Fujita, Takeshi Kawabata, Hiroki Kimura, Yoshimi Iwayama, Toshiya Inada, Yuko Okahisa, Jun Egawa, Masahide Usami, Itaru Kushima, Yota Uno, Takashi Okada, Masashi Ikeda, Branko Aleksic, Daisuke Mori, Toshiyuki Someya, Takeo Yoshikawa, Nakao Iwata, Haruki Nakamura, Toshihide Yamashita, Norio Ozaki. Rare genetic variants in

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