News Release

Title

A novel rare variant R292H in RTN4R affects growth cone formation and possibly contributes to Schizophrenia susceptibility

Key Points

○We revealed the single nucleotide variants (RTN4R-R292H) within Reticulon 4 Receptor (RTN4R), located within chromosome 22q11.2 (a region that is known to be a hot-spot for psychiatric disorders), increases the susceptibility to developing schizophrenia.

○In silico 3D structural protein assays predicted that RTN4R-R292H change the interaction between RTN4R and LINGO1.

○This study strengthens the evidence for association between rare variants within *RTN4R* and SCZ, and may shed light on the molecular mechanisms underlying the neurodevelopmental disorder.

Summary

RTN4R (Reticulon 4 receptor) plays an essential role in regulating axonal regeneration and plasticity in the central nervous system through the activation of rho kinase, and is located within chromosome 22q11.2, a region that is known to be a hot-spot for schizophrenia (SCZ) and Autism Spectrum Disorder (ASD). Recently, rare variants such as copy number variants (CNV) and single nucleotide variants (SNV) have been a focus of research because of their large effect size associated with increased susceptibility to SCZ and ASD and the possibility of elucidating the pathophysiology of mental disorder through functional analysis of the discovered rare variants. To discover rare variants with large effect size and to evaluate their role in the etiopathophysiology of SCZ and ASD, we sequenced the *RTN4R* coding exons with a sample comprised of 370 SCZ and 192 ASD patients, and association analysis using a large number of unrelated individuals (1716 SCZ, 382 ASD and 4009 controls). Through this mutation screening, we discovered four rare (minor allele frequency < 1%) missense mutations (R68H, D259N, R292H, and V363M) of *RTN4R*. Among these discovered rare mutations, R292H was found to be significantly associated with SCZ ($p = 0.048$). Furthermore, *in vitro* functional assays showed that the R292H mutation affected the formation of growth cones. This study strengthens the evidence for association between rare variants within *RTN4R* and SCZ, and may shed light on the molecular mechanisms underlying the neurodevelopmental disorder.

Research Background

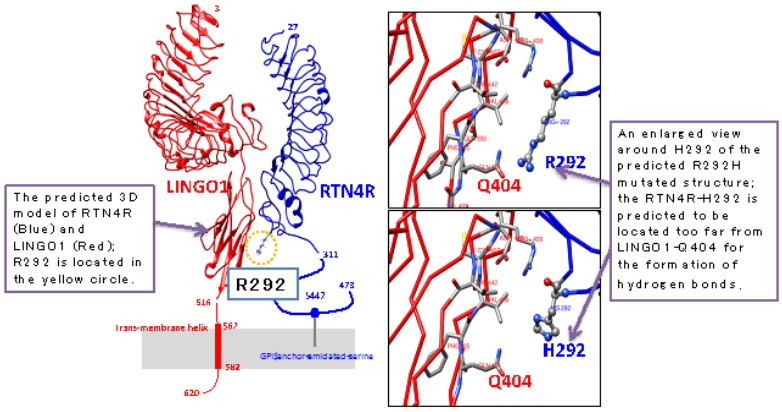
Schizophrenia (SCZ) is a devastating psychiatric disorder that is characterized by hallucinations, delusions, and cognitive deficits, and which causes tremendous societal burdens. The lifetime prevalence of SCZ is approximately 1% in the general population. Recently rare single nucleotide variants SNVs, discovered from sequencing of susceptibility genes, may have large effect sizes and account for a part of the heritability of SCZ, and could contribute to an understanding of the

etiopathophisiology of neurodevelopmental disease through further functional assays.
The Reticulon 4 receptor (*RTN4R*) encodes the RTN4 receptor, which is a known receptor subunit for RTN4 that is one of the most potent myelin-associated inhibitor of axon regeneration and structural plasticity in the central nervous system. *RTN4R* is considered to be a promising candidate gene for SCZ and ASD because *RTN4R* is located at chr22q11.2, which is a hotspot locus for SCZ and ASD. We hypothesized that variants in *RTN4R* possibly contribute to ASD and SCZ susceptibility. Although there have been studies that have focused on SNVs of *RTN4R*, there have been no study that has detected SNVs with a large effect size on SCZ and that focused on SNVs in *RTN4R* in a Japanese SCZ population. Furthermore, it has reported that the common variants of the *RTN4R* had association with Japanese SCZ. Therefore, in this study, in order to discover novel rare *RTN4R* mutations with large effect size and to evaluate the pathogenesis of the discovered mutations, we sequenced the *RTN4R* coding exons using SCZ and ASD samples, and performed the association analysis and in vitro functional assays of the variants which could have large effects.

Research Results

Through this sequencing, we identified four rare missense mutations (MAF < 1%) in *RTN4R*. We then performed genetic association analysis using large independent samples and detected a marginally significant association between SCZ and R292H (OR = 3.9, $P = .048$). Furthermore, we found a possible biological effect of RTN4R-R292H by investigating growth cone collapse of chick dissociated retinal neurons induced by a myelin ligand (RTN4). The possible mechanisms by which the RTN4R-R292H mutation induced reduced growth cone collapse may involve changes in co-receptor association with LINGO1, which is also an SCZ candidate gene and one of the components of RTN4R signaling complex, predicted that R292 could be located at the site of interactions between RTN4R and LINGO1, based on *in silico* 3D protein structure analysis（Figure 1）. We discovered a reduced interaction of LINGO1 with RTN4R-R292H compared to WT by the GST binding analysis as our *in silico* structural analysis predicted. Through these genetic and functional studies, considering that *RTN4R* is located at chr22q11.2, which is a hotspot locus for SCZ and ASD, RTN4R-R292H could be related to the etiopathological role of SCZ, because the pathophysiology of SCZ is believed to be that of aberrant conditions of neurodevelopment.

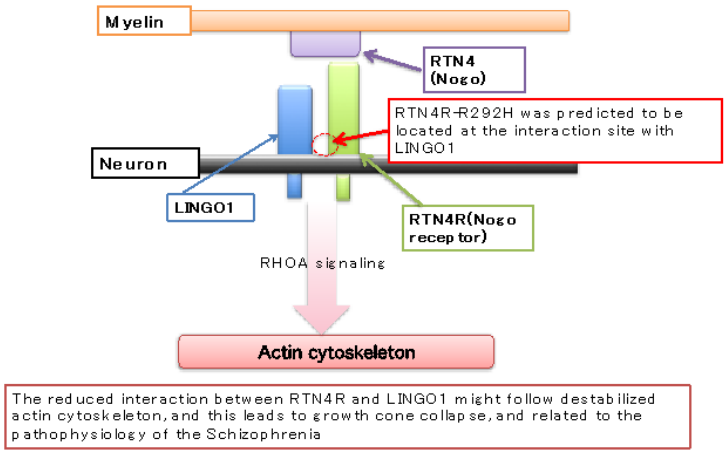
Fig 1. RTN4R-R292H change the interaction with LINGO1 (in silico 3D structual analysis)



Research Summary and Future Perspective

Screening for mutations in RTN4R, a candidate risk gene for SCZ, revealed novel missense mutations that might increase susceptibility to SCZ. Importantly, we detected a significant association between Schizophrenia and RTN4R-R292H, which was found to have the biological effects of changing the interaction between RTN4R and LINGO1 and affecting the growth cone collapse (Figure 2). This study therefore strengthens the evidence for the role of rare variants in RTN4R in the etiopathological role of SCZ. In future research involving a more comprehensive evaluation of *RTN4R*, a study with a much larger sample size, and that examines *RTN4R*-related pathways genes (for example; *LINGO1*, *RTN4*, *RTN4R, and MAG*) will be needed. In addition, examining effects of rare single nucleotide variants using novel techniques such as iPS cells may be promising approach in future genetic studies.

Fig 2. The R292H effet on the RTN4R function



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Publication

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