Neuregulin 1 genetic variation and anterior cingulum integrity in patients with schizophrenia and healthy controls

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Background: Neuregulin 1 (NRG1) influences the development of white matter connectivity and is implicated in genetic susceptibility to schizophrenia. The cingulum bundle is a white matter structure implicated in schizophrenia. Its anterior component is especially implicated, as it provides reciprocal connections between brain regions with prominent involvement in the disorder. Abnormalities in the structural integrity of the anterior cingulum in patients with schizophrenia have been reported previously. The present study investigated the potential contribution of NRG1 variation to anterior cingulum abnormalities in participants with schizophrenia. Methods: We studied 31 men with schizophrenia and 36 healthy men using diffusion tensor imaging to investigate the association between fractional anisotropy in the anterior cingulum and a single-nucleotide polymorphism (SNP8NRG221533: rs35753505) of NRG1. Results: Consistent with previous reports, fractional anisotropy was significantly reduced in the anterior cingulum in the schizophrenia group. Moreover, the results revealed a significant group (schizophrenia, control) by genotype (C/C, T carriers, including CT and TT) interaction between genetic variation in NRG1 and diagnosis of schizophrenia, such that the patients with the T allele for SNP8NRG221533 had significantly decreased anterior cingulum fractional anisotropy compared with patients homozygous for the C allele and healthy controls who were T carriers. Limitations: Limitations of our study included the small sample size of the TT subgroup and our use of only fractional anisotropy as an index of myelin integrity. In addition, the use of diffusion tensor imaging acquisition methods limited our ability to study other brain regions that may be involved in schizophrenia. Conclusion: Our results suggest that NRG1 variation may play a role in the pathophysiology of anterior cingulum abnormalities in patients with schizophrenia.

Contexte : La neuroréguline-1 (NRG1) influence sur le développement de la connectivité de la substance blanche et participe à la prédisposition génétique à la schizophrénie. Le faisceau cingulaire est une structure de la substance blanche qui interviennent dans la schizophrénie. Sa partie antérieure est particulièrement en cause, puisqu’elle assure les connexions réciproques entre les principales régions du cerveau qui jouent un rôle dans la maladie. Des rapports ont déjà fait mention d’anomalies de l’intégrité structurale du cingulum antérieur chez des patients schizophrènes. La présente étude a analysé la contribution potentielle des fluctuations de NRG1 dans les anomalies affectant le cingulum antérieur chez les participants atteints de schizophrénie. Méthodes : Nous avons étudié 31 hommes atteints de schizophrénie et 36 hommes en bonne santé au moyen de techniques d’imagerie par tenseur de diffusion afin d’explorer le lien entre l’anisotropie fractionnelle au niveau du cingulum antérieur et un polymorphisme de nucléotides simples (PNS8NRG221533: rs35753505) de la NRG1. Résultats : Conformément à des rapports antérieurs, l’anisotropie fractionnelle s’est révélée significativement moindre dans le cingulum antérieur du groupe atteint de schizophrénie. De plus, les résultats ont révélé une interaction significative selon le groupe (schizophrènes c. témoins) en fonction du génotype (porteurs des allèles C/C et T, y compris CT et TT) entre la variation...
Introduction

Convergent findings implicate genes involved in white matter development in the pathophysiology of schizophrenia. Neuregulin 1 (NRG1) has recently been shown to play a role in the development of white matter, as well as in susceptibility to schizophrenia. Increasing evidence suggests that NRG1 affects the regulation of central nervous system (CNS) myelination by inducing the migration and differentiation of oligodendrocytes in the CNS. Moreover, preclinical evidence demonstrates that alterations in NRG1–ErbB signalling lead to abnormalities in oligodendrocyte structure and function, such as reduced myelin thickness and slower conduction velocity in CNS axons. These changes can cause alterations in dopaminergic function and behaviour relevant to neuropsychiatric disorders. In patients with schizophrenia, altered levels of NRG1 type III (SMDF), which is related to myelination, have been found in peripheral leukocytes.

More recently, the NRG1ζ isoform was demonstrated to be significantly reduced in white matter of the frontal cortex in patients with schizophrenia. Magnetic resonance imaging studies provide further evidence of the association between NRG1 and the structural integrity of medial frontal white matter in healthy individuals. These data suggest that NRG1 contributes to the pathophysiology of schizophrenia by exerting an effect on white matter.

Replications of the linkage between the NRG1 gene and schizophrenia in several populations implicate NRG1 as a candidate gene for schizophrenia. Since the deCODE haplotype was linked to schizophrenia by a deCODE group, the single-nucleotide polymorphism (SNP; SNP8NRG221533: rs35753505) from the deCODE haplotype has been reported to be associated with schizophrenia in diverse populations, including the northern Chinese Han population.

Neuregulin 1 does present population differences in allele and haplotype frequencies. Studies of East Asian (mainly Chinese, Japanese and Korean) populations have been inconsistent in finding associations between schizophrenia and SNP8NRG221533 and other polymorphisms. Even within the Chinese population, the results were diverse. In the present study, we examined SNP8NRG221533 as it is associated with schizophrenia, specifically in the northern Chinese Han population from which our sample was drawn. This SNP has also been associated with the structural integrity of medial frontal white matter in healthy individuals.

Diffusion tensor imaging (DTI) provides the opportunity to study white matter architecture. One of the measurements used in DTI is fractional anisotropy, which provides a measure of the organization and coherence of white matter tracts. Decreases in fractional anisotropy have been detected in disorders of CNS myelination, suggesting that fractional anisotropy is a measure sensitive to myelination abnormalities. Convergent evidence has implicated dysmyelination in schizophrenia, and the cingulum bundle is especially implicated as a structure with white matter pathology in schizophrenia. The white matter of the anterior component of the cingulum bundle is particularly implicated in schizophrenia as it provides reciprocal connections between brain regions with prominent involvement in the disorder, including regions that myelinate late and are implicated in the emergence of the disorder in late adolescence/young adulthood such as the dorsolateral prefrontal, the anterior cingulate and the temporal cortices. Indeed, abnormality in structural integrity of the anterior cingulum is a relatively well-replicated finding among patients with schizophrenia in DTI studies. In our first DTI study of patients with schizophrenia, we investigated multiple white matter regions and the only region that demonstrated a significant difference in schizophrenia was the anterior cingulum bundle. In a subsequent study, we optimized acquisition for study of the cingulum and again found decreased fractional anisotropy values in patients with schizophrenia that were localized in the anterior cingulum region.

In the present study, we used DTI to test the hypothesis that variation in SNP8NRG221533 influences the structural integrity of the anterior cingulum in patients with schizophrenia.

Methods

Participants

We recruited 2 relatively homogeneous groups of men of northern Chinese descent (Han): patients with schizophrenia (International Classification of Diseases, version 10, criteria established by the consensus of clinical and structured clinical interview) and healthy controls. Participants provided written informed consent after complete description of the study in accordance with the protocol approved by the Medical Ethics Committee of Peking University.

Diffusion tensor imaging acquisition and processing

We acquired diffusion tensor images according to methods described previously. Briefly, we obtained all scans using a
1.5 T magnetic resonance scanner (GE Medical Systems) equipped with shielded magnetic field gradients of up to 40 mT/m. We acquired diffusion-weighted images with a single-shot echo-planar imaging sequence in alignment with the anterior commissure–posterior commissure plane. We applied the diffusion sensitizing gradients along 25 non-collinear directions with a b value of 1000 s/mm² together with an acquisition without diffusion weighting. The acquisition parameters were repetition time (TR) = 4000 ms, echo time (TE) = 80 ms, matrix = 128 × 128, field of view (FOV) = 24 × 24 cm², number of excitations = 3, slice thickness = 3 mm without gap. We also obtained T₁-weighted sagittal magnetic resonance images (3-dimensional [3-D] spoiled gradient recalled acquisition) for anatomic localization (TR = 8.5 ms, TE = 3.2 ms, matrix = 256 × 256, FOV = 24 × 24 cm, slice thickness = 1.5 mm without gap).

The detailed processing protocol has been described in our previous paper.²⁸ Briefly, we calculated fractional anisotropy values in the anterior cingulum using BioImage Suite (www.bioimagesuite.org). For the cingulum delineation, we reoriented DTI data into the coronal-oblique plane, resliced to cubic voxel dimensions of 1 mm³ and smoothed by a 3-D isotropic Gaussian kernel with sigma 1 mm. We then calculated the diffusion tensor matrices and fractional anisotropy according to the methods of Basser and colleagues.²⁸ The absolute RGB (red-green-blue) colour-encoding scheme defined the directionality of the principal eigenvector:¹⁰ left–right fibres in red, anterior–posterior fibres in green and superior–inferior fibres in blue. The colour-coded diffusion tensor imaging provided excellent distinction between the cingulum and nearby bundles such as the corpus callosum (Fig. 1a and b). An operator blind to diagnosis and genotype delineated the cingulum bundle region of interest (ROI). The ROI included voxels containing fibres travelling in the anterior–posterior direction that also exhibited fractional anisotropy higher than 0.2 to exclude grey matter (Fig. 1c).⁴¹ We defined the coronal-oblique slice passing through the midpoint of the anterior commissure–posterior commissure line as the reference slice after the 3D spoiled gradient recalled acquisition scan was coregistered to the non-diffusion weighted images. We calculated anterior cingulum fractional anisotropy as the average of fractional anisotropy values over 5 slices (5-mm intervals between slices) anterior to the reference slice. The inter-rater reliabilities for manual delineation of the cingulum were satisfactory with intraclass correlation coefficients of 0.91–0.93.

**DNA collection**

We extracted DNA samples from white blood cells. We genotyped SNP8NRG22153 (rs35753505) by direct sequencing from a polymerase chain reaction template. We applied the automated DNA sequencer (ABI PRISM TM377) for sequencing. Primers were as follows: upper 5′GCATTAGAACCTGAACCTGCAGTGA3′ and lower 5′TGGAACCTCTC-CATCTTTCAC3′.

**Statistical analysis**

We performed all statistical analyses using SPSS for Windows software, version 10.0 (SPSS Inc.). We performed independent Student t tests to assess potential differences in demographic and clinical features between participant groups. We investigated genotype frequencies with the Fisher exact test to test for Hardy–Weinberg equilibrium. According to the allele distributions in our large sample,¹⁶ we separated the control and schizophrenia groups into 2 subgroups, respectively: those homozygous for the more frequent allele (C) and carriers of the less frequent allele (T) who were heterozygous and homozygous for that allele. We used a mixed-effects model analysis with diagnosis (control, schizophrenia) and genotype (C/C, T carriers) as between-subject variables to explore the possibility of differences in effects between these 2 genotype subgroups.

![Fig. 1. Sagittal (a) and coronal (b) images from the tensor colour map display the anterior–posterior coursing fibres of the cingulum in green, left–right fibres in red and superior–inferior fibres in blue. Coronal image (c) from the fractional anisotropy map displays the voxels with fractional anisotropy values higher than 0.2. The red and yellow circles in coronal images (b and c) demonstrate the delineations for the right and left cingulum.](image)
antipsychotic medications (risperidone, olanzapine or clozapine). The healthy controls did not have histories of Axis I mental disorders, nor did their first-degree relatives. No participants had histories of major medical disorders, head injuries (loss of consciousness longer than 5 min), neurologic disorders or alcohol or substance abuse or dependence.

Within the control group, 18 participants were homozygous for the C allele (C/C) and 18 were T carriers (30% T/T). Within the schizophrenia group, 15 had the C/C genotype and 16 were T carriers (25% T/T). Genotype distributions in the 2 groups did not differ significantly from each other (p = 0.54) or from Hardy–Weinberg equilibrium (p = 0.68 for the schizophrenia group and p = 0.14 for the control group). The groups did not differ significantly in age (p = 0.75) or education (p = 0.10). Age at onset of schizophrenia and medication dose did not differ significantly between patients with different genotypes (Table 1).

The main effect of diagnosis on anterior cingulum fractional anisotropy values was significant: F1,42 = 7.07, p = 0.010. Fractional anisotropy in the anterior cingulum was lower in the schizophrenia group than in the control group, which is consistent with the results of other studies by our group and studies by other research groups.34–36 The diagnosis by genotype interaction was significant: F1,42 = 6.78, p = 0.012. Among patients with schizophrenia, T carriers had significantly lower fractional anisotropy than C/C homozygotes: F1,21 = 5.27, p = 0.029. Within-genotype comparisons showed that T carriers with schizophrenia had significantly decreased fractional anisotropy values in the anterior cingulum compared with healthy controls who were T carriers: F1,21 = 18.00, p < 0.001 (Fig. 2). There were no other significant 2- or 3-way interactions. In the secondary analysis exploring the potential for different effects between the CT and TT genotypes there was no significant effect of genotype (F1,30 = 0.065, p = 0.80) or diagnosis by genotype interaction (F1,30 = 0.937, p = 0.34).

Discussion

Our results are consistent with those of the most recent studies suggesting that NRG1 contributes to the pathophysiology of schizophrenia by exerting an effect on white matter. For example, Winterer and colleagues37 found that variation in the same NRG1 SNP examined in our study is associated with medial frontal white matter integrity in healthy white people. In that study, voxel-based DTI was used, and the regions that differed significantly in fractional anisotropy between the 2 genotype groups included the anterior cingulum. Another group detected an association between white matter density and integrity in the anterior limb of the internal capsule and NRG1 variant SNP243177 (rs6994992), another SNP from the deCODE haplotype.38 That group also reported that SNP243177 affected frontotemporal brain function and psychotic symptoms in individuals at high risk for schizophrenia.39 Taken together, these findings suggest that although the specific pathophysiological contributions of NRG1 variation to alterations in anterior cingulum structural integrity in patients with schizophrenia cannot be concluded from the present results, it seems feasible that SNPs in the NRG1 gene or related haplotypes may alter NRG1 expressions in the anterior cingulum, which then influence myelin or oligodendrocyte development and possibly plasticity in adulthood. Of note, it is possible that all these SNPs might be markers of the deCODE haplotype and that the disease locus might be in another part of the NRG1 gene.

We recently performed genetic association studies for the SNPs (rs2919390, rs6988339 and rs3735774) located in the region encoding the SMDF isoform, which is associated with myelination.40 We identified an association between rs3735774 (Arg46Gly) and schizophrenia in our case-control and family-based association studies; however, the

Table 1: Characteristics of study participants, by genotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Genotype; group; mean (SD)*</th>
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<tbody>
<tr>
<td></td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>Controls (n = 18)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>28.56 (7.41)</td>
</tr>
<tr>
<td>Education, yr</td>
<td>14.17 (1.75)</td>
</tr>
<tr>
<td>Age at onset, yr</td>
<td>NA</td>
</tr>
<tr>
<td>Dose of medication, mg</td>
<td>NA</td>
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<tr>
<td>FA values, least square mean (SE)</td>
<td>0.413 (0.021)</td>
</tr>
</tbody>
</table>

FA = fractional anisotropy; NA = not applicable; SD = standard deviation; SE = standard error.

*Unless otherwise indicated.
†Mean chlorpromazine equivalents.
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neuroimaging sample contained only 3 patients who were C carriers and 4 healthy controls who were C carriers. Thus there was not sufficient statistical power to investigate effects on our DTI measures. This would be an SNP of interest to explore in future studies. Our pilot results did not show the association of SNPs (rs2919390 and rs6988339) with schizophrenia (unpublished results).

Our study implicates an interaction between the diagnosis of schizophrenia and the NRG1 genetic variation SNP8NRG221533 in influencing the structural integrity of white matter in the anterior cingulum. Interestingly, Law and colleagues observed a significant interaction between the diagnosis of schizophrenia and another NRG1 genetic variation (SNP8NRG221132) on type I mRNA in the hippocampus. Although the 2 studies examined different SNPs and different brain regions, taken together, they suggest that NRG1 variation interacts with the diagnosis of schizophrenia to influence the expression of corticolimbic pathology in the disorder. These studies also raise the possibility that different NRG1 variations may play different roles in the pathophysiology of corticolimbic abnormalities in the disorder.

Although these data provide preliminary indications of how genetic variation in high-risk regions influences susceptibility to corticolimbic abnormalities in patients with schizophrenia, NRG1 is a complex gene, and many unanswered questions about its specific effects in schizophrenia remain. Neuregulin 1 is a large gene that may exert its effects by many isoforms through alternative splicing. The specific variations that confer susceptibility to schizophrenia and that might contribute to myelination abnormalities are not clear.

Postmortem histological studies of people with schizophrenia have revealed abnormalities in the expression patterns of NRG1 mRNA isoforms in the dorsal prefrontal cortex and the hippocampus. One postmortem study that specifically investigated frontal SNP8NRG221533 expression did not detect differences in these expression patterns in patients with schizophrenia. However, the tissue investigated in that study was derived from cortical grey matter. Thus this does not necessarily contradict our current finding, which was derived from a measurement in a white matter fibre bundle where it would be more likely to observe myelination-related differences. Confirmation of the role of SNP8NRG221533 in studies of the cingulum is warranted.

Limitations

Our study had some limitations. One limitation was the use of only fractional anisotropy values as an index of myelin integrity. In future studies, other DTI measurements (e.g., apparent diffusion coefficient, relative anisotropy) may provide complementary information. In addition, we used DTI acquisition methods to optimize study of the cingulum; however, this limited our ability to study other brain regions that may also be involved in schizophrenia. Further studies are warranted to explore other brain regions and other possible genetic risk regions. Finally, owing to the small sample size of the TT subgroup, we did not perform the analyses on the fractional anisotropy values in the anterior cingulum among 3 genotype groups. Therefore, it could not be concluded from the current study whether the effect of the T allele is dominant or codominant.

In summary, a genetic variation in NRG1 is associated with more pronounced abnormalities in the structural integrity of the anterior cingulum in patients with schizophrenia. Further understanding of the contribution of variation in NRG1 may help to elucidate the pathophysiological mechanisms underlying white matter abnormalities in corticolimbic circuitry in the disorder.

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Competing interests: None declared.

Contributors: Drs. Wang, Jiang, Zang and D. Zhang designed the study. Drs. Wang, Sun, Zhu, H. Zhang, Yue, Qu, Lu and Hong acquired data. Drs. Wang, Teng, Luo, Zhu, Zang, Yue, Qu, Hong, Huang and Blumberg analyzed data. Drs. Wang and Blumberg wrote the article. Drs. Wang, Jiang, Sun, Teng, Luo, Zhu, Zang, H. Zhang, Yue, Qu, Lu, Hong, Huang, Blumberg and D. Zang reviewed the article. All authors provided approval for publication.

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