博士論文の要旨及び審査結果の要旨

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博士論文名	Heterogenous Genetic, Clinical, and Imaging Features in Patients with Neuronal Intranuclear Inclusion Disease Carrying NOTCH2NLC Repeat Expansion （NOTCH2NLCリピート拡張を有する神経核内封入体症患者における遺伝的、臨床的、画像的特徴の異質性）
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博士論文の要旨

Background and purpose:

Neuronal intranuclear inclusion disease (NIID) is a neurodegenerative disorder which is caused by abnormal expansion of GGC repeats in NOTCH2NLC. Clinical diagnosis of NIID has been difficult because of substantial heterogenous clinical presentations. Brain MRI typically shows extensive white matter lesions on fluid attenuated inversion recovery (FLAIR) and T2-weighed (T2-WI) images and a high-intensity lesion along the U-fiber on diffusion-weighed images (DWI). These MRI signs often alert clinicians to a possible diagnosis of NIID. A GGC repeat expansion in the 5' untranslated region (UTR) of NOTCH2NLC has been identified as a causative mutation in patients with NIID.

Despite the increased number of reported patients with genetically-confirmed NIID, the relationship between repeat length and sequence in NOTCH2NLC and the diverse phenotypes of NIID remains unclear. In this study, the applicants report heterogenous genetic, clinical, and imaging features in Japanese patients with NIID carrying a NOTCH2NLC repeat expansion.

Methods:

Fifteen patients of Japanese origin with adult-onset NIID who had characteristic clinical and neuroimaging findings were genetically analyzed. The trinucleotide repeats expansions in the 5' untranslated region of NOTCH2NLC was examined by repeat-primed and amplicon-length PCR. Long read sequencing was performed to determine repeat size and sequence. The correlations between expanded repeat lengths and clinical/imaging features were analyzed

Results:

Repeat-primed PCR analysis revealed a sawtooth pattern in 15 patients clinically suspected as

having NIID, suggesting the presence of expanded repeats in NOTCH2NLC and the expanded GGC repeats ranging from 94 to 361 in NOTCH2NLC. One patient carried pure GGC repeat expansion, and remaining patients carried others trinucleotide repeat including GGA, AGC, and GAC. Most frequent trinucleotide insertion was GGA at the end of GGC repeat, which is commonly observed in normal allele. Two patients carried biallelic repeat expansions. There were marked heterogenous clinical and imaging features in NIID patients. Patients presenting with cerebellar ataxia or urinary dysfunction had a significantly larger GGC repeat size than those without. This significant association disappeared when these parameters were compared with the total trinucleotide repeat number. ARWMC score was significantly higher in patients who had non-glycine type trinucleotide interruption within expanded poly-glycine motif than those with pure poly-glycine expansion.

Considerations:

Using repeat-primed and amplicon-length PCR followed by Nanopore long-read sequencing, the applicants identified GGC repeat expansion of NOTCH2NLC in 15 Japanese patients clinically suspected as having NIID. The repeat sequence and size varied among patients with NIID. the applicants identified two NIID patients (Patient 7 and Patient 14) as compound heterozygotes for the repeat expansions. The age at onset of patients carrying biallelic expansions was 50 years for Patient 7 and 60 years for Patient 14. Both exhibited a paroxysmal symptom-dominant phenotype characterized by conscious-ness disturbance, encephalitis episodes, and cognitive decline. Previous studies argued that biallelic repeat expansions likely show a dominant effect on the phenotype of NIID. Applicants' findings on compound heterozygous patients support their notion because the phenotypes of patients with biallelic expansions were comparable to those of patients heterozygous for the expansion.

In addition, the applicants showed that patients presenting with ataxia, or urinary dysfunction had a significantly larger GGC repeat length than those without. These correlations disappeared if these parameters were compared with total repeat size of any trinucleotide. This finding suggests that GGC or poly-glycine repeat size may have a stronger impact on some clinical features in NIID than the total trinucleotide repeat number. This may be supported by a previous report in which translation of GGC repeat expansions into a toxic poly-glycine stretch plays a pathological role in NIID. Moreover, previous research has reported that GGA disruptions may be associated with a muscular weakness-dominant subtype and a younger age at onset. The applicants showed that the presence of non-glycine coding type trinucleotide sequence interruption may modify the severity of the white matter changes as determined by ARWMC. These results suggest that repeat length and sequence in NOTCH2NLC may partly modify the phenotype of NIID

Heterogenous genetic, clinical and neuroimaging features were observed in patients with NIID. Repeat length and sequence in NOTCH2NLC may partly modify some clinical and imaging features of NIID. The present findings demonstrated the potential of repeat-primed and amplicon-length PCR followed by long-read sequence for the accurate genetic diagnosis of NIID.

審査結果の要旨

申請者は、NOTCH2NLC の GGC リピートの異常伸長によって引き起こされ、多様な臨床像を呈する神経変性

疾患である神経核内封入体症（NIID）を対象として、その遺伝的、臨床的、画像的特徴について検討する一連の研究を行った。その結果、Repeat-primed PCR 解析の結果、臨床的に NIID が疑われた 15 人の患者において、NOTCH2NLC における 94 から 361 までの GGC 反復の伸長を認めたこと、1 人の患者は純粋な GGC リピートの伸長を有しており、残りの患者は GGA、AGC、GAC を含む他のトリヌクレオチドリピートを有していたこと、最も頻度の高い三塩基挿入は、正常対立遺伝子でよく観察される GGC リピートの末端にある GGA であったこと、2 人の患者は 2 塩基反復の伸長を有していることなどを明らかにした。さらに。NIID 患者の臨床所見と画像所見は著しく不均一であり、小脳失調症や排尿機能障害を呈する患者では GGC リピートサイズが有意に大きいことなどを明らかにした。

本研究は NOTCH2NLC の反復長および配列が NIID の臨床的ならびに画像的特徴を部分的に修飾する可能性を示唆するものであり、さらに repeat-primed PCR、amplicon-length PCR、ついで long-read sequence が NIID の正確な診断のために有用性を示すものである。NIID の病態理解を深化させ、より精緻な臨床診法の確立に寄与する意義深い研究である。よって博士論文として価値あるものと認められる。