Potential diagnostic efficacy of Cas9-mediated long-read sequencing in *NOTCH2NLC*-associated neuronal intranuclear inclusion disease

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Introduction

- **Neuronal intranuclear inclusion disease (NIID)** is a neurodegenerative disorder characterized by eosinophilic intranuclear inclusions in neuronal and glial cells.
- The wide range of clinical manifestation in NIID makes the diagnosis difficult.
- It can be suspected by a characteristic MRI feature which is the hyperintensity along the corticomedullary junction on diffusion-weighted imaging (**zigzag edging sign**).
- Recent studies have demonstrated that NIID is caused by a **GGC repeat expansion of the *NOTCH2NLC*** gene and accounts for a substantial portion of undiagnosed adult-onset leukoencephalopathies.

Methods

- We enrolled **107 adult-onset leukoencephalopathy** patients with unknown etiologies for this study.
- We reviewed their brain MRI images and further selected **NIID-suspected patients showing zigzag edging signs**.
- We conducted **Nanopore long-read sequencing** with **Cas9-mediated enrichment** to screen the *NOTCH2NLC* GGC repeat expansion.

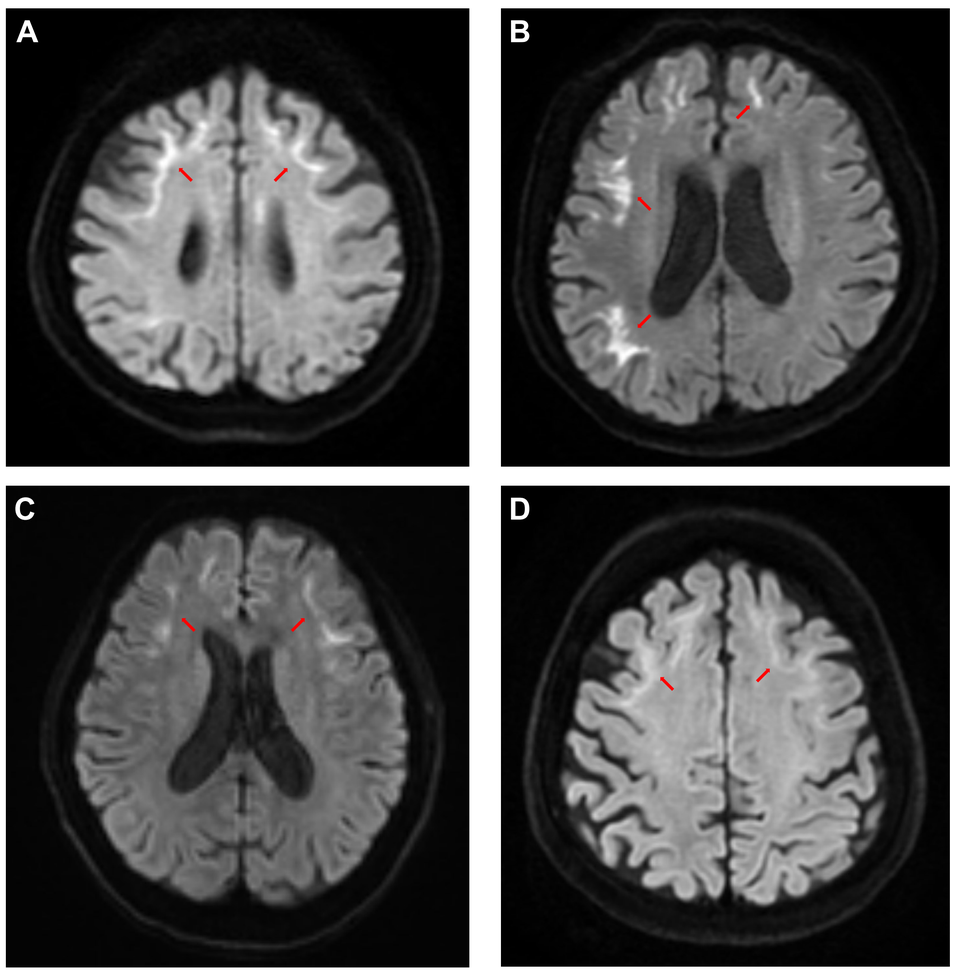

Figure 1. Zigzag edging signs. We showed the diffusion-weighted imaging of four different patients, Patients 1 (A), 3 (B), 5 (C), and 7 (D). Red arrows indicate the zigzag edging signs of each patient.

Results

- There were **19 patients (17.8%) showing zigzag edging signs** in their brain MRI.
- **Two patients** were diagnosed as **fragile X-associated tremor/ataxia syndrome** through *FMR1* genetic testing.
- Cas9-mediated long-read sequencing was applied to a total of **15 patients** whose DNA were available. As a result, **all of them were confirmed to have heterozygous *NOTCH2NLC* repeat expansions**.
- The size of pathogenic alleles range from **87 to 217 repeats** in length.
- The age of NIID patients ranges from 36 to 76 years.

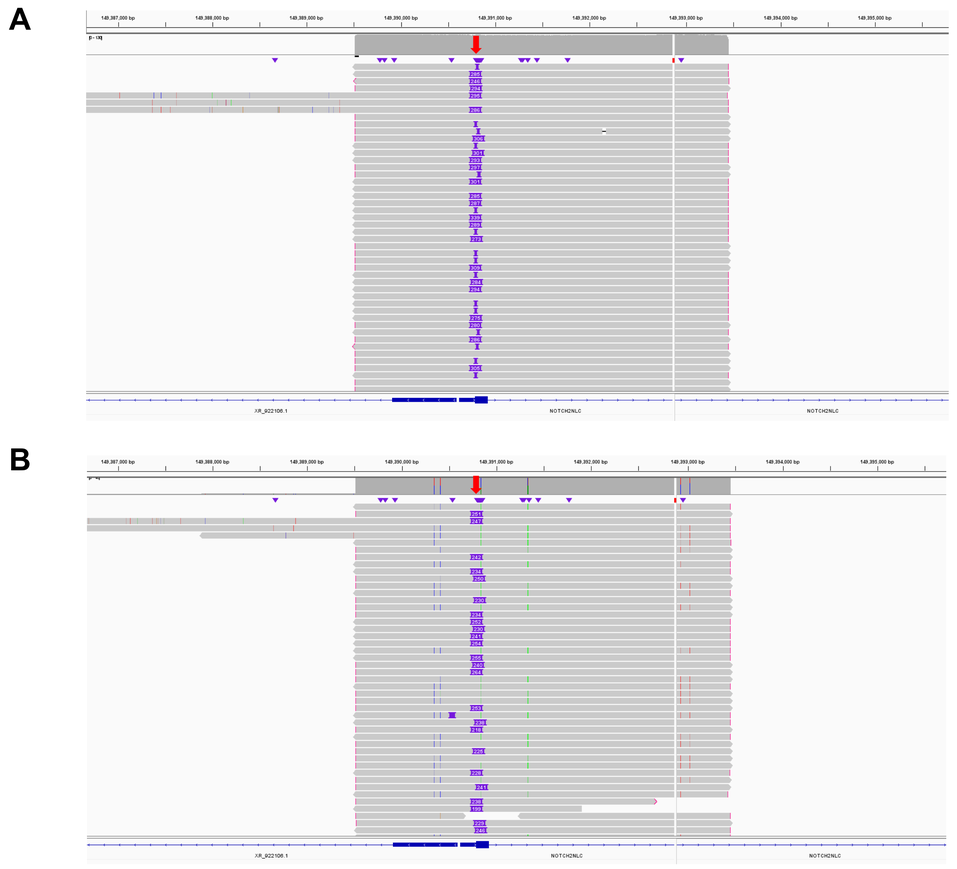

Figure 2. Cas9-mediated long read sequencing. We visualized the aligned reads of Patient 1 (A) and Patient 7 (B) through the Integrative Genomics Viewer program. The numbers within the reads (red arrows) represent the size of repeat expansion.

Conclusion

- We have shown that **a high proportion of patients with leukoencephalopathies, who show zigzag edging signs** on diffusion-weighted imaging, has ***NOTCH2NLC* repeat expansions**.
- **Cas9-mediated long-read sequencing** could be an **efficient approach to screen repeat expansions**, especially for more than several hundreds of base pairs in length.

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