News Release

Title

Identification of causative gene of neuronal intranuclear inclusion disease (NIID).

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Key Points

○ We found that the gene responsible for the neuronal intranuclear inclusion disease (NIID), whose number has been increasing rapidly in recent years, is the GGC repeat expansion in the human-specific gene *NOTCH2NLC*.

○ We demonstrate the usefulness in elucidating the gene responsible for genetic diseases using a long-read next generation sequencer.

○ We developed a simple detection method for the identified repeat elongation and are expected to be used for NIID diagnosis.

Summary

The collaborative research groups of Jun Sone (Department of Neurology, National hospital organization Suzuka National Hospital, Suzuka, Japan), Gen Sobue (Nagoya University Graduate School of Medicine, Nagoya, Japan and Aichi Medical University, Nagakute, Aichi, Japan) and Naomichi Matsumoto (Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan) et al have identified the causative gene of Neuronal intranuclear inclusion disease (NIID) whose number of cases has been rapidly increasing recently because diagnosis by skin biopsy has become possible.

Research Background

Neuronal intranuclear inclusion disease (NIID) is an incurable disease in which the presence of a abnormal substance called “intranuclear inclusion body” is widely found in the cell nucleus of nervous tissues such as the cerebrum, spinal cord, and peripheral nerves, and in the general organs. Symptoms of NIID include higher brain dysfunction such as dementia, and muscle weakness in the limbs. The inheritance form of NIID is suspected to be autosomal dominant inheritance. NIID could be diagnosed only by pathological autopsy after death for a long time, and the number of cases diagnosed with NIID had been very small. So, NIID was the disease with many unknown points including pathological background, but on 2011, Sone et al found and reported that NIID can be diagnosed by skin biopsy, then the number of NIID patients has increased significantly. In particular, it is assumed that NIID patients account for a certain percentage of patients with dementia, because recently there are many reports of elderly NIID cases developing dementia.

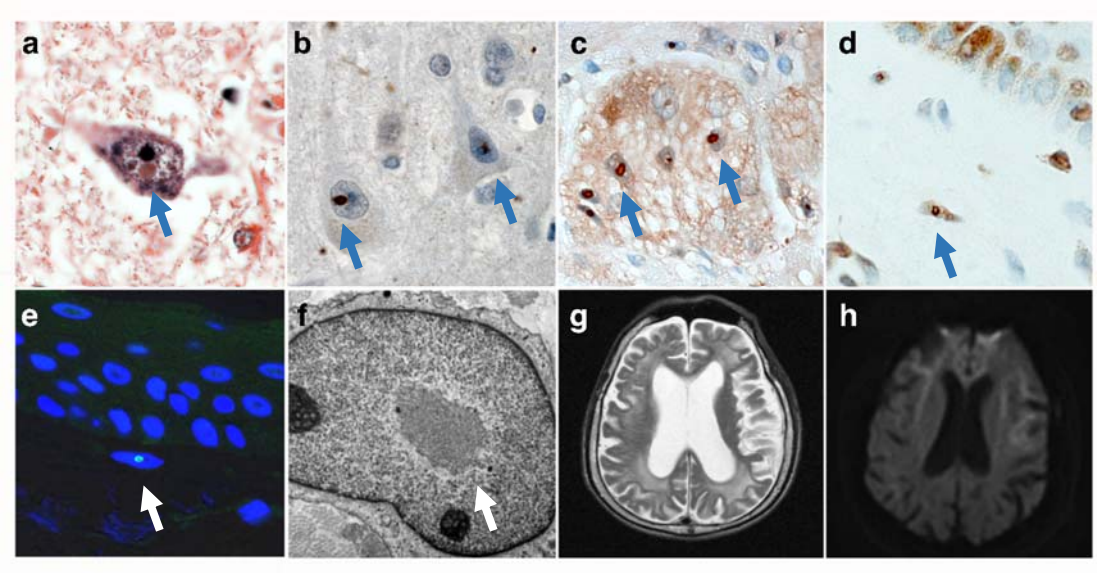


Figure 1：Histopathological findings and MRI findings of NIID

Figure 1 : In NIID, nuclear inclusions are observed in various cells. Head MRI shows leukoencephalopathy in T2 image, DWI image shows abnormal high intensity signal in cortico-medullary junction. a. Spinal cord anterior horn neuron (H&E staining) b. Cerebral cortex neuron (anti-ubiquitin immunostaining) c. Intestinal plexus neurons (anti-ubiquitin immunostaining) d. Skin fibroblasts (anti-ubiquitin immunostaining) e. Skin fibroblasts (anti-ubiquitin immunofluorescence staining) f. skin fibroblasts (electron microscope) g. Head MRI image (T2) h. Head MRI image (DWI).

Research Results

Our group started research in 2005 to search for the causative gene of NIID. As a result of linkage analysis using microsatellite markers in NIID large families with onset of weakness in limbs, we succeeded in narrowing the responsible region to chromosome 1 1p22.1-q21.3 immediately. To investigate this region further, we performed whole exome analysis and whole genome analysis using a short-read next-generation sequencer, but we could not identify the causal gene mutation. However, re-examination of linkage analysis using single nucleotide polymorphism (SNV) information obtained by this whole genome analysis gave almost the same results as linkage analysis using microsatellite markers, It was presumed that the causative gene for NIID is surely present in the region of chromosome 1 1p22.1-q21.3, and gene mutations that are difficult to analyze in the short-read next-generation sequencers, such as extension of repetitive sequences and large-scale gene deletion or insertion, may be the cause of NIID (Figure 2).

a. Family tree of NIID

b. The result of linkage analysis

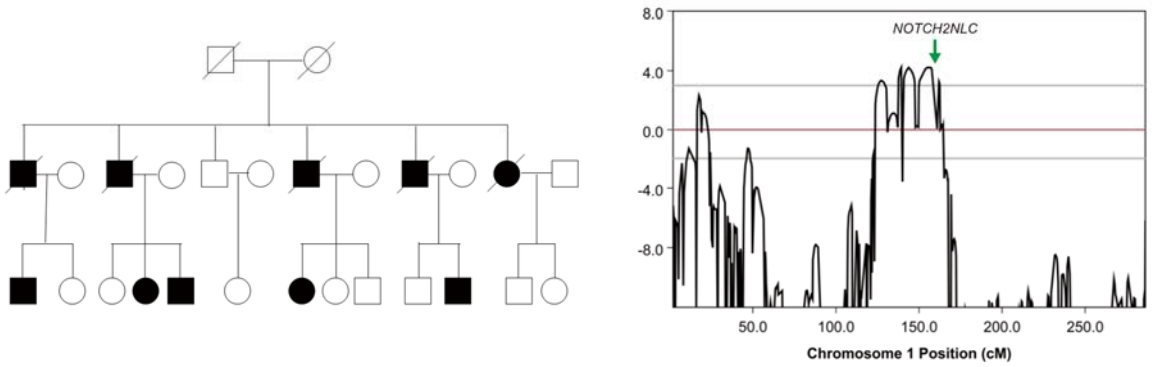
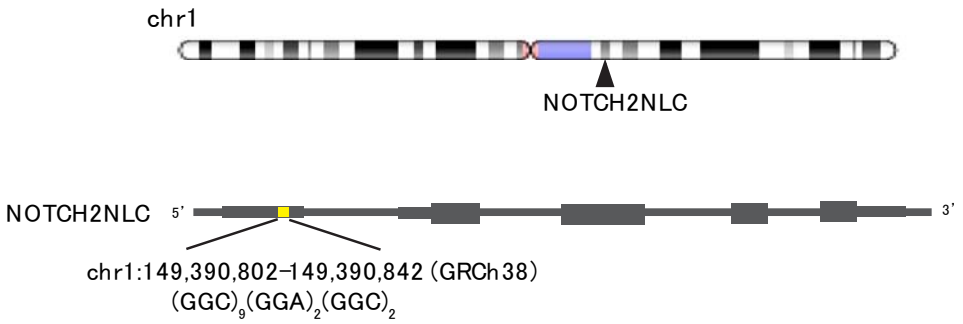


Figure 2：Family tree of NIID and the result of linkage analysis

After that, the long-read type next-generation sequencer appeared. This sequencer is eqiped with a new technology that can read long DNA sequences longer than 10,000 bases. It also exerts power in the determination of repetitive sequences, and in the decoding of sequences with high ratios of G (guanine) and C (cytosine), which are not good at conventional sequencers. We performed whole genome analysis using this long read sequencer, it was observed that the repetitive extension of the DNA sequence "GGC" in the *NOTCH2NLC* gene, which is a human-specific gene that does not exist in other organisms, was abnormally extended. It was recognized in 24 NIID patients from 9 families, including large families that were analyzed with satellite markers and 40 sporadic NIID patients (Figure 3). This study is an important achievement that demonstrates that using the long-read sequencer can decipher the entire genome of a case and lead to elucidation. It is expected to be a major step towards the resolution of many unexplained genetic diseases.

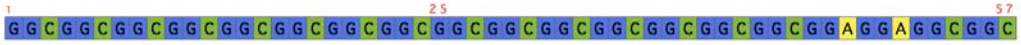
Figure 3：Genetic analysis using a long-read sequencer revealed that in NIID patients, the GGC repeat within the *NOTCH2NLC* gene is elongated.

a：GGC repeat in *NOTCH2NLC* gene

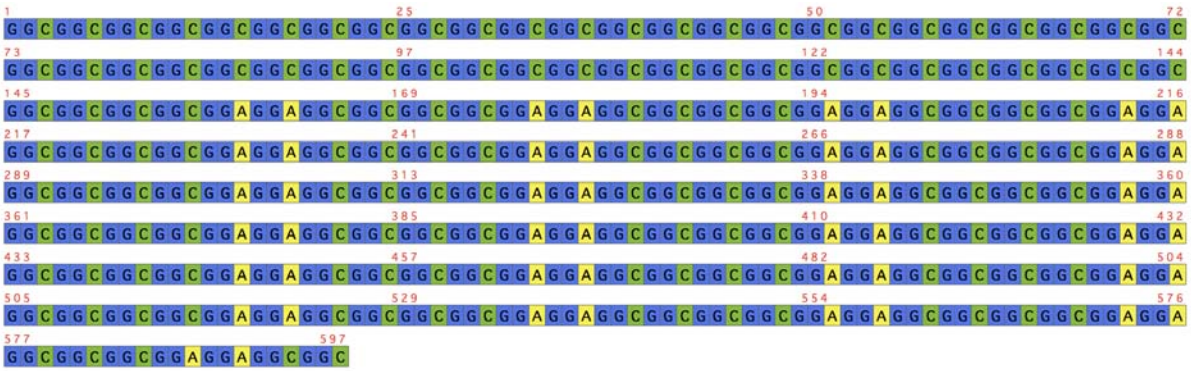


b：GGC repeat analysis with Nanopore sequence

Normal



Expanded repeat sequence



Research Summary and Future Perspective

In this study, we clarified the genetic pathogenesis of NIID for the first time in the world. It is expected that this discovery will lead to more accurate diagnosis of NIID, elucidation of pathological condition and treatment development. In addition, this research is the result of demonstrating that we can analyze the patient's entire genome directly using the new technology, long-read sequencer, and identify the responsible gene of disease. In the future, this technology will open the way to the elucidation of the genetic causes of unresolved genetic diseases.

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Title：Long-read sequencing identifies GGC repeat expansions in *NOTCH2NLC* associated with neuronal intranuclear inclusion disease

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