Letters to the Editor

[Title]

Spinocerebellar ataxia type 4 is not detected in a cohort from Hokkaido, the northernmost island of Japan.

[Short running title]

SCA4 is not detected in Hokkaido

[Authors]

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Author contributions

SS, KM, YS, MM, II, HY, and IY contributed to study conception and design. SS, KM, YS, MM, II, HY, and IY contributed to data acquisition and analysis. SS, KM, YS, MM, II, HY, and IY drafted the manuscript.

Dear Editor,

Since April 1982, we have been conducting genetic analysis of autosomal dominant spinocerebellar degeneration in Hokkaido[1-3], the northernmost island of Japan, with a population of over 5 million people. Hokkaido is comparable to countries such as Denmark, Finland, and Norway. It is an island separated from the rest of Japan by the sea on all sides. Many of its inhabitants are descendants of settlers from various parts of Japan who arrived after the country's political reformation in 1868 (the Meiji Restoration). This has resulted in a unique genetic composition that differs from other regions of Japan, making Hokkaido a microcosm of the country. The distribution of autosomal dominant spinocerebellar ataxia (SCA) in Japan has been reported to vary across different regions [4]. In contrast, Hokkaido, an island located in the northernmost part of Japan, has been considered to reflect the genetic background of the Japanese population to a certain extent due to its history of accepting immigrants from various regions of the country.

SCA4 was initially described in a Scandinavian-American kindred from Utah/Wyoming and found to be linked to 16q22.1. Its clinical symptoms consist of adult-onset ataxia and axonal sensory neuropathy[5]. In December 2023, it was reported that SCA4 is caused by a GGC repeat expansion in the zinc finger homeobox 3 (*ZFHX3*) gene[6].

We believe that there is significance in confirming whether there are SCA4 patients

within this large-scale cohort in Hokkaido.

So, we investigated SCA4 in subjects with no known pathogenic variants, such as SCA6 or Machado-Joseph disease (MJD)/SCA3 in our Hokkaido Cohort. The GGC repeat count in *ZFHX3* was determined using primers described previously, with pathogenic expansions in the range of 42-72 repeats[6]. According to the size of the allele for which the number of GGC repeats has been determined by direct nucleotide sequencing analysis, we calculated the repeat numbers using the values obtained from fragment analysis and a calculation formula. We calculated the repeat numbers using the values obtained from fragment analysis and a calculation formula. The repeat numbers were determined using the formula (S-105)/3 and sequencing data. To correct for the discrepancy between the repeat numbers obtained from fragment analysis and sequencing, we calculated the average repeat number at the top and bottom of the band. The correction value was determined by multiplying the average difference in the average repeat numbers by 3 bases. The repeat number was then calculated using the formula {(S+4.74)-105}/3. (*S: band length, 105: primer length 168bp-21repeats = 0 repeat value, 3: 3 bases.)

In the cohort from April 1982 to March 1999[1] , out of 349 cases, there were 83 undiagnosed cases. Among these, 65 cases had available samples, and the GGC repeat in ZFHX3 ranged from 19-24, with a median of 20/23. In the cohort from April 1999 to

March 2007[2] , out of 186 cases, there were 62 undiagnosed cases. Among these, 52 cases were available for analysis, and the GGC repeat in ZFHX3 ranged from 14-24, with a median of 20/22. In the cohort from April 2007 to December 2020[3], out of 312 cases, there were 111 diagnosed cases. Among the 110 cases with available samples for analysis, the GGC repeat in ZFHX3 ranged from 14-24, with a median of 20/22.

Therefore, from April 1982 to December 2020, we performed genetic analyses on 847 cases. Among these, 256 cases were not diagnosed with known SCAs such as MJD/SCA3 or SCA6. Out of these 256 cases, DNA samples were available for 227 cases, and none of them were found to have SCA4. Furthermore, from January 2020 to December 2023, among the 31 undiagnosed cases with available DNA samples, the GGC repeat in ZFHX3 ranged from 18-24, with a median of 20/22, and similarly, no SCA4 patients were identified.

In a previous study of this cohort, patients presenting with symptoms such as sensory neuropathy and cerebellar ataxia, which are typical phenotypes of SCA4, were genetically diagnosed with MJD, SCA2, or SCA27B. To the extent that clinical symptoms could be ascertained, there were no patients in this cohort who were strongly suspected of having SCA4 based on clinical presentation alone, among those without a confirmed genetic diagnosis.

In conclusion, these findings suggest that SCA4 is not present or is extremely rare in Hokkaido, the northernmost island of Japan.

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