［課程－２］

審査の結果の要旨

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1: *FMR1* genetic study in Japanese population

We recruited a total of 1095 Japanese patients with neurological movement disorders and 384 healthy control subjects (215 males/ 169 females) were screened for the *FMR1* gene. The selected patients were categorized into groups based on their initial clinical diagnosis; 348 patients with cerebellar ataxia (190 males/ 158 females), 484 patients with multiple system atrophy-cerebellar, MSA-C (279 males/ 205 females), 218 patients with multiple system atrophy-parkinsonism, MSA-P (106 males/ 112 females), and 45 patients with neuronal intranuclear inclusion disease (NIID) (23 males, 20 females, and 2 are gender unidentified). No *FMR1* full mutation allele carrier was detected in any subjects. We identified three male patients with the *FMR1* premutation. Two carriers were under evaluation for cerebellar ataxia, giving an estimated FXTAS prevalence 1% of total male patients with cerebellar ataxia (190 patients) and 2.7% among male patients with late-onset cerebellar ataxia (76 patients), which is a high rate considering the low prevalence of the *FMR1* premutation in Japan. The *FMR1* gene test for the FXTAS patients showed 84 CGG repeats including one AGG interruption within the repeats for one patient, while another one had pure 103 CGG repeats. The third patient was under evaluation for multiple system atrophy-parkinsonism (62 male patients), and his genetic analysis showed 60 CGG repeats in the *FMR1* gene disrupted with four AGG interruptions. The patients with cerebellar ataxia showed the remarkable radiological sign, MCP sign in the brain MRI. Both patients met the diagnostic criteria for FXTAS definite, while the patient with MSA-P has not demonstrated the MCP sign. He met the diagnostic criteria for possible MSA based on the Gilman's criteria 2008, and he met the diagnostic criteria for FXTAS probable. His final diagnosis was difficult to identify whether it was FXTAS, MSA, or a combination of both. However, it is noteworthy that the patient had an earlier age at onset compared to the mean age at onset of MSA (58.7 years of age) and FXTAS (typically ≥ 50 years of age). The present study revealed that the *FMR1* premutation alleles are not commonly underlying these presentations in females in Japan. We also identified *FMR1* intermediate in MSA patients and healthy controls. The *FMR1* intermediate alleles were found in two male patients under evaluation for multiple system atrophy-parkinsonism subtype (MSA-P), and one female was under evaluation for

multiple system atrophy-cerebellar subtype (MSA-C), in addition to two healthy male controls and three healthy female controls. However, the prevalence of the *FMR1* intermediate alleles is not significantly higher than in healthy population control (p=0.6012, Fisher exact test).

2: *FMR1* premutation genotype-phenotype correlation study

The age at onset and disease progression are variant between the patients. Therefore, we studied the correlation between the genotype and the phenotype of FXTAS patients and compared them with 188 previously reported FXTAS cases in the literature. We found that tremor is the first recognizable symptom of FXTAS in the majority of patients (57.3%), and ataxia is the second most common presentation (25.2%), followed by parkinsonism and cognitive impairment (3.1%), separately, and memory impairment and fatigue in (1.6%) of the patients, separately. Age of onset of ataxia in males showed inverse linkage to the CGG repeat length in line with previous studies, while there is no correlation between age of onset of ataxia and CGG repeat length in females was elucidated. However, the CGG repeats showed no correlation with age at onset of FXTAS in both genders. The correlation between CGG repeat length and age at onset and age of onset of ataxia has not been studied yet in females. However, the number of recruited females in the present study were extremely low, making these analysis studies limited to elucidate.

3: Characterizing CGG repeat motifs in the *FMR1* premutation

We successfully obtained long read data for the target molecules. We could obtain high resolution of the unique sequences by CCS analysis but characterizing the repeat motifs still face technical limitation.

Such a high throughput and cost-effective technology is needed for studies of the molecular pathogenesis of an increasing number of repeat expansion-associated diseases — especially those alleles which are unable to be analyzed by conventional genetic test methods. However, further investigation is needed for more enrichment which would decrease the loading amount of genomic DNA for isolation and demonstrates higher-throughput target reads in SMRTbell sequencing.

4: *FMR1* normal repeat length distribution and repeat motifs characterization in Japanese population

Also, we characterized CGG repeats in *FMR1* normal alleles in a group of 348 Japanese patients with cerebellar ataxia and 384 healthy control. The modal number of

CGG repeats was 29, followed by 30, and 36 CGG repeats, representing 38.9%, 30.5%, and 11%, respectively, in the general population. The most common CGG repeat pattern were [(CGG)9 (AGG) (CGG)9 (AGG) (CGG)9] followed by [(CGG)10 (AGG) (CGG)9 (AGG) (CGG)9] and [(CGG)9 (AGG) (CGG)9 (AGG) (CGG)6 (AGG) (CGG)9]. representing 29, 30, and 36 CGG repeats respectively. Twenty-nine and 30 are the most common CGG repeat length among world populations and ethnic groups with some exceptions which can be seen in some populations. While the CGG repeat length 36 and the [(CGG)6AGG] pattern is common among Asian people.

This study adds to the literature and supports further evaluation into specific population-wide screening recommendations for FXTAS in Japan. The present study provides the largest specific population-wide screening study for the *FMR1* gene in Japan and establishes the comprehensive for the *FMR1* alleles distribution and prevalence in the country.

よって本論文は博士(医学)の学位請求論文として合格と認められる。