Screening for *FMR1* CGG Expansions in Thai Male Patients with Normal Results for Spinocerebellar Ataxia Diagnostics Testing

การตรวจคัดกรองหาการเพิ่มขยายจำนวนซ้ำซีจีจีของยีนเอฟเอ็มอาร์วันในผู้ป่วยชายไทยที่มีผลการตรวจวินิจฉัยโรคสไปโนซีรีเบลลาร์อะแท็กเซียเป็นปกติ

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ABSTRACT

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder with core features of intention tremor and gait ataxia caused by CGG repeat expansions in premutation range of the *FMR1* gene. This study aimed to ascertain the prevalence of *FMR1* CGG expansions among ataxia Thai males negative for the spinocerebellar ataxia (SCA) genes testing. A total of 447 males negative for SCA 1-3 testing were screened for *FMR1* CGG repeat expansions. Although none of males with *FMR1* premutation allele were found, seven males with intermediate alleles were identified (1.6%), supporting evidence for small *FMR1* CGG repeat expansions at the risk of developing FXTAS. Our study adds support to fragile X screening to include ataxia patients with unknown cause.

บทคัดย่อ

กลุ่มอาการสั่นและเดินเซ เป็นกลุ่มโรคที่เกิดจากการเสื่อมของเซลล์ประสาทในช่วงท้าย มักแสดงอาการสำคัญ คือ อาการสั่นและเดินเซ ซึ่งมีสาเหตุมาจากการเพิ่มขยายจำนวนซ้ำซีจีจีของยีนเอฟเอ็มอาร์วันในช่วงพรีมิวเตชั่น การศึกษานี้จึงมีวัตถุประสงค์ที่จะตรวจหาความชุกของการเพิ่มขยายจำนวนซ้ำซีจีจีของยีนเอฟเอ็มอาร์วันในกลุ่มตัวอย่างผู้ป่วยชายที่มีอาการเดินเซแต่มีผลการตรวจวินิจฉัยโรคสไปโนซีรีเบลลาร์อะแท็กเซียเป็นปกติ โดยจะทำการตรวจคัดกรองหาการเพิ่มขยายจำนวนซ้ำซีจีจีของยีนเอฟเอ็มอาร์วันในกลุ่มตัวอย่างชาย จำนวน 447 คน ที่มีผลการตรวจโรคสไปโนซีรีเบลลาร์อะแท็กเซียชนิด 1-3 เป็นปกติ ผลการทดลองพบว่าไม่มีผู้ป่วยที่มีจำนวนซ้ำในช่วงพรีมิวเตชั่น แต่พบผู้ป่วยที่มีจำนวนซ้ำในช่วงอินเตอร์มีดิอิท จำนวน 7 คน (1.6%) จากผลการทดลองสรุปว่าการเพิ่มขยายจำนวนซ้ำซีจีจีที่น้อยของยีนเอฟเอ็มอาร์วันมีความเสี่ยงที่จะพัฒนาไปเป็นกลุ่มอาการสั่นและเดินเซ จึงสนับสนุนให้ตรวจคัดกรองหาโครโมโซมเอ็กซ์เปราะในผู้ป่วยที่มีอาการเดินเซไม่ทราบสาเหตุ

Keywords: *FMR1*, Fragile X-associated tremor/ataxia syndrome, Spinocerebellar ataxia
คำสำคัญ: ยีนเอฟเอ็มอาร์วัน กลุ่มอาการสั่นและเดินเซ โรคสไปโนซีรีเบลลาร์อะแท็กเซีย

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Introduction

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset X-linked inherited neurodegenerative disorder caused by expansions of CGG repeats in the 5' untranslated region (5'UTR) of the fragile X mental retardation 1 (*FMR1*) gene. The full mutation repeat expansions (>200 CGG repeats) generally lead to transcriptional silencing of the *FMR1* gene and subsequent absence of fragile X mental retardation protein (FMRP) production, resulting in fragile X syndrome (FXS) which is the most common cause of X-linked inherited intellectual disability. In contrast, FXTAS is caused by premutation repeat expansions (55-200 CGG repeats) which cause upregulation in *FMR1* mRNA transcription and overproduction of *FMR1* mRNA. This excess mRNA is believed to cause FXTAS (Hagerman, Hagerman, 2016). FXTAS affects approximately 40-45% of males with premutation and 8-16% of females with premutation after the age of 50 (Jacquemont et al., 2004). Furthermore, FXTAS affects individuals with intermediate repeat expansions (41-54 CGG repeats or 45-54 CGG repeats) have been also reported indicating that intermediate alleles could also cause evaluated *FMR1* mRNA (Hall et al., 2012; Liu et al., 2013).

The main clinical features of FXTAS are intention tremor, cerebellar ataxia, and Parkinsonism. Other clinical manifestations include memory and executive function deficits, autonomic dysfunction, brain atrophy with white matter disease, and cognitive decline which are variable symptoms in some patients (Hagerman, Hagerman, 2016). Currently, diagnosis of FXTAS needs to carry on a molecular evaluation of fragile X mutation consisted of neurological and radiological evaluation (Hall et al., 2014). Individuals over 50 years with symptoms suggesting FXTAS or with cerebellar ataxia of unknown cause are recommended for fragile X mutation testing (Berry-Kravis et al., 2007). Since heterogeneity of FXTAS clinical characteristics overlaps with the clinical characteristics of many neurological and neurodegenerative disorders, patients with these clinical characteristics are often initially diagnosed with other disorders including spinocerebellar ataxia (SCA), essential tremor, Parkinson disease, and Alzheimer disease. Especially, individuals who are over 50 years with cerebellar ataxia of unknown cause are usually assigned initial genetic testing of SCA before a diagnosis of FXTAS.

Cerebellar ataxia, which is one of the main cardinal features of FXTAS, overlaps with clinical features of SCA. SCA is a genetically heterogeneous group of neurodegenerative ataxia diseases and there are more than 40 distinct subtypes including the common SCA types 1, 2, and 3. The identifying clinical symptom of SCA is ataxia which usually begins in middle adulthood of the patients (45-65 years old) (Klockgether et al., 2019). In fact, the significant proportions of *FMR1* CGG repeat expansions in males referred for SCA testing before a diagnosis of FXTAS have been reported in several populations (Adams et al., 2008; Aydin et al., 2018; Biancalana et al., 2005; Faruq et al., 2014; Rodriguez-Revenga et al., 2007; Seixas et al., 2011; Tan et al., 2004; Van Esch et al., 2005). Indeed, patients suggesting FXTAS are erroneously referred for SCA testing and found to be negative. Mostly in Thailand, there are no additional laboratory requirements to screen FXTAS in these patients because most medical professionals do not have firsthand experiences with FXTAS and subsequent may not be fully aware of the pathogenesis and the complicated genetics of FXTAS which make

it difficult to an accurate diagnosis. Therefore, FXTAS should be considered after SCA testing was found to be negative (Aydin et al., 2018; Biancalana et al., 2005; Faruq et al., 2014; Rodriguez-Revenga et al., 2007; Van Esch et al., 2005). To establish a sufficient diagnostic strategy for FXTAS diagnosis, more information about the prevalence of *FMR1* CGG repeat expansions associated with ataxia patients presenting SCA like-phenotype is required in the Thai population.

Objective of the study

The purpose of this study was to determine the prevalence of *FMR1* CGG expansions in Thai males negative for spinocerebellar ataxia types 1-3 (SCA types 1-3) testing.

Methodology

Ethical compliance

This study was approved by the Ethics Committee, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkla, Thailand (REC.62-018-5-2) and the Ethics Committee, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand [190/2562 (EC3)]. Written informed consent was obtained from all participants.

Subjects and procedures

This study was a retrospective study conducted at Songklanagarind Hospital, a central laboratory for fragile X syndrome diagnosis in Thailand. A total of 447 Thai males who were referred for SCA types 1, 2, and 3 testing and found to be negative for CAG expansions in the *ATXN* 1, 2, and 3 genes were included in this study (Klockgether et al., 2019). We excluded males with obvious physical characteristics or symptoms related to other genetic disorders. Also, males who were diagnosed with other disorders like a brain tumor or liver disease, which are the secondary causes, were excluded. All DNA samples of participants were extracted from peripheral blood samples. The DNA samples from leftover clinical blood samples were collected with a volume of 100 µL in a tube and transported from Siriraj Hospital to Songklanagarind Hospital.

The CGG repeat sizes of the *FMR1* gene of all samples were analysed by fragment analysis (fluorescent PCR). If the fragment analysis results were interpreted as premutation alleles or no amplification, methylation-specific PCR was then performed to determine the methylation status of the *FMR1* gene. Subsequently, for premutation and full mutation alleles, Southern blot analysis was performed to confirm the CGG repeat sizes and the methylation status of the *FMR1* gene. The details of each analysis method were described below. The overall workflow of this study is illustrated in Figure 1.

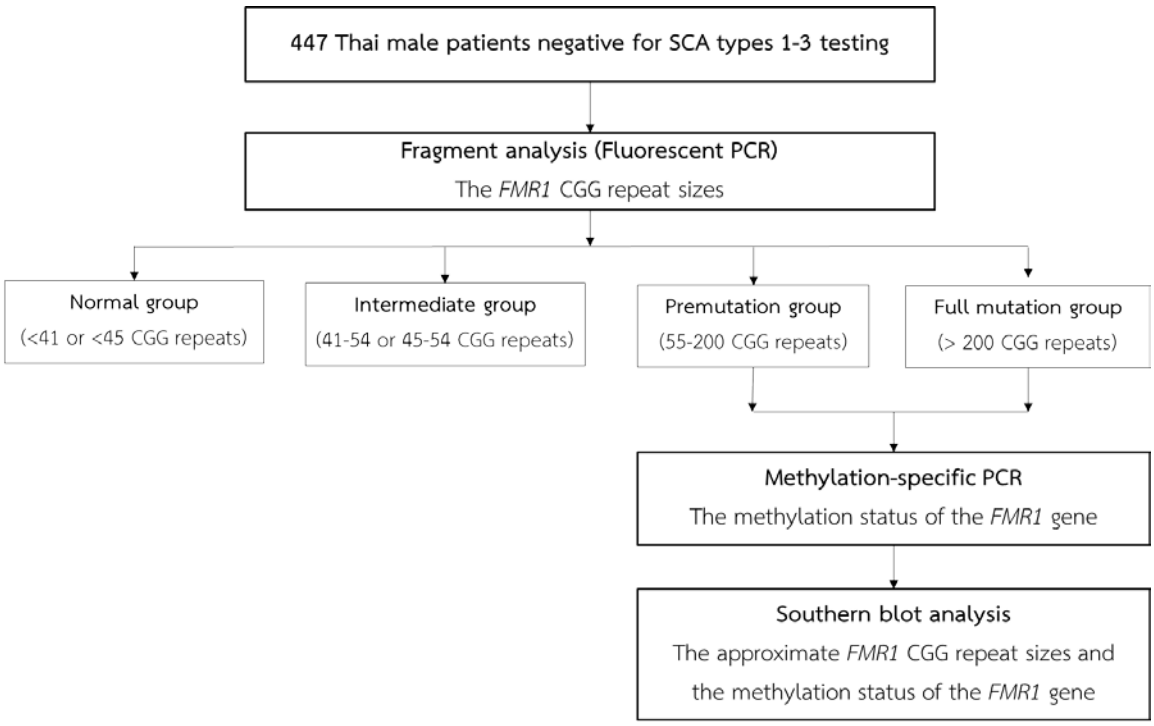

Figure 1 Flow diagram representing procedures of *FMR1* CGG repeat expansions screening among SCA negative Thai male patients

Fragment analysis (Fluorescent polymerase chain reaction) for detection of the *FMR1* CGG repeat sizes

Fragment analysis was implemented to detect the CGG repeat sizes of the *FMR1* gene in each DNA sample of the participants. The number of CGG repeats in each DNA sample was determined by a fluorescent labelled-forward primer (FAM-5'-CAG CGT TGA TCA CGT GAC GTG GTT TCA GTG-3') and a reverse primer (5'-GTG GGC TGC GGG CGC TCG AGG-3'), which annealed across CGG repeats of the *FMR1* gene. Amplification was carried out in 10 µL mixtures containing 0.2 µM each of primers, 1X PCR buffer (Immolase, Bioline), 1.5 mM $MgCl_2$, 0.2mM dNTPs (dGTP replaced with 7-deaza GTP), 2.2 M betaine, 0.5 U Immolase DNA polymerase (Bioline) and 50 ng of genomic DNA samples. Thermal cycle amplification was as follows: denaturation at 95°C for 15 min, 10 cycles of 95°C for 35 s, 64°C for 35 s, and 72°C for 2 min, followed by 25 cycles of 95°C for 35 s, 64°C for 35 s, and 72°C for 2 min with plus a 10 s increment for each cycle. The fluorescent signals of the PCR products were detected by capillary electrophoresis on ABI 3500 genetic analyser (Applied Biosystems, Foster City [CA], USA) to determine the fragment sizes of each sample related to the number of CGG repeats. Briefly, 1 µL of each PCR product was mixed with 0.3 µL of the 600 LIZ-size standard markers and 10.7 µL of HiDi formamide. The samples were denatured at 95°C for 2 min and 4°C for 5 min prior to loading into each well of a flat deck plate. The results were shown on graphs and the CGG repeat sizes of each sample calculated using the Gene Mapper® 5 software of the genetic analyser.

Methylation-specific polymerase chain reaction for determination of the methylation status of the *FMR1* gene

Methylation-specific PCR was performed to detect the methylation status of the *FMR1* gene. In the initial step, sodium bisulphite treatment, each DNA sample of participants was modified using the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine [CA], USA), according to the manufacturer's protocol. The kit contains sodium bisulphite to convert unmethylated cytosine residues to uracil while leaving methylated cytosine residues unconverted. Then, the 2 µL (calculated 100-150 ng) of bisulphite treated DNA were amplified by a PCR master mix with 0.24 µM a pair of *FMR1* promoter unmethylated forward primer (5'-GTG TTT GAT TGG GTT GAA TTT TTG-3') and *FMR1* promoter unmethylated reverse primer (5'-ATT TAA TTT CCC ACA CCA CTA AAT ACA C-3'), 0.24 µM a pair of *FMR1* promoter methylated forward primer (5'-GTT GCG GGT GTA AAT ATT GAA ATT ACG-3') and *FMR1* promoter methylated reverse primer (5'-ATT TAA TTT CCC ACG CCA CTA AAT ACA C-3'), 0.3 a pair of *XIST* promoter methylated forward primer (5'-AAT TAA AGT AGG TAT TCG CGG TTT CG-3') and an *XIST* promoter methylated reverse primer (5'-TTT TTC CTT AAC CCA TCG AAA TAT CG-3'), 0.5 a pair of *XIST* promoter unmethylated forward primer (5'-AAA AGT GGT TGT TAT TTT AGA TTT GTT-3') and an *XIST* promoter unmethylated reverse primer (5'-CTA CCT CCC AAT ACA ACA ATC ACA C-3'), 1X PCR buffer (Qiagen, containing 15 mM $Mgcl_2$), 1.0 mM $MgCl_2$, 0.2 mM dNTPs, and 1.0 U HotStart Taq DNA polymerase (Qaigen). An initial denaturation at 95°C for 10 min was followed by 40 cycles of 95°C for 1 min, 61°C for 45 s, and 72°C for 45 s and followed by a final extension at 72°C for 10 min. The PCR products were separated on 2.5% agarose gel electrophoresis, stained with ethidium bromide, and the results were detected under a UV transilluminator. This method was adapted from the previous study (Charalsawadi et al., 2005).

Southern blot analysis for determination of the approximate CGG repeat sizes and methylation status of the *FMR1* gene

Southern blot analysis is the gold standard method for fragile X testing. This method was performed to determine the approximate CGG repeat sizes and methylation status of the *FMR1* gene to confirm the presence of premutation or full mutation alleles. Southern blot analysis was performed using a protocol previously described (Rousseau et al., 1991). Eight to ten µg of each DNA sample were digested by a pair of restriction enzymes, which were a methylation-insensitive enzyme (EcoR1) and a methylation-sensitive enzyme (EagI) at 37°C for 16-18 hours. The digested DNA were then separated on 0.8% agarose gel electrophoresis and transferred to a nylon membrane. After that, each digested DNA sample on the nylon membrane was hybridized with an StB12.3 probe labelled with alkaline phosphatase, and the results were detected by chemiluminescent (detection system, Amersham Pharmacia Biotech; RPN 3680).

Data analysis

The numbers of CGG repeats of all 447 SCA negative male patients were classified into four groups depending on CGG repeat sizes including normal (<41 or <45 repeats), intermediate (41-54 or 45-54 repeats), premutation (55-200 repeats), and full mutation (>200 repeats). The results were determined the distribution of *FMR1* CGG repeat alleles in Thai males. The prevalence of *FMR1* CGG repeat expansions in Thai males with SCA negative and 95% confidence interval (CI) was analysed using a Poisson distribution.

Results

Distribution of *FMR1* CGG repeat alleles in Thai males

This present study recruited 447 males negative for SCA types 1, 2, and 3 testing in Thailand. The CGG repeat sizes observed in our study ranging from 19-53 CGG repeats which the median of CGG repeat sizes was 29 CGG repeats. Among these, the most frequent *FMR1* alleles was 29 CGG repeats (51.45%), followed by 30 CGG repeats (24.83%) and a minor allele at 36 CGG repeats (7.38%) (Figure 2).

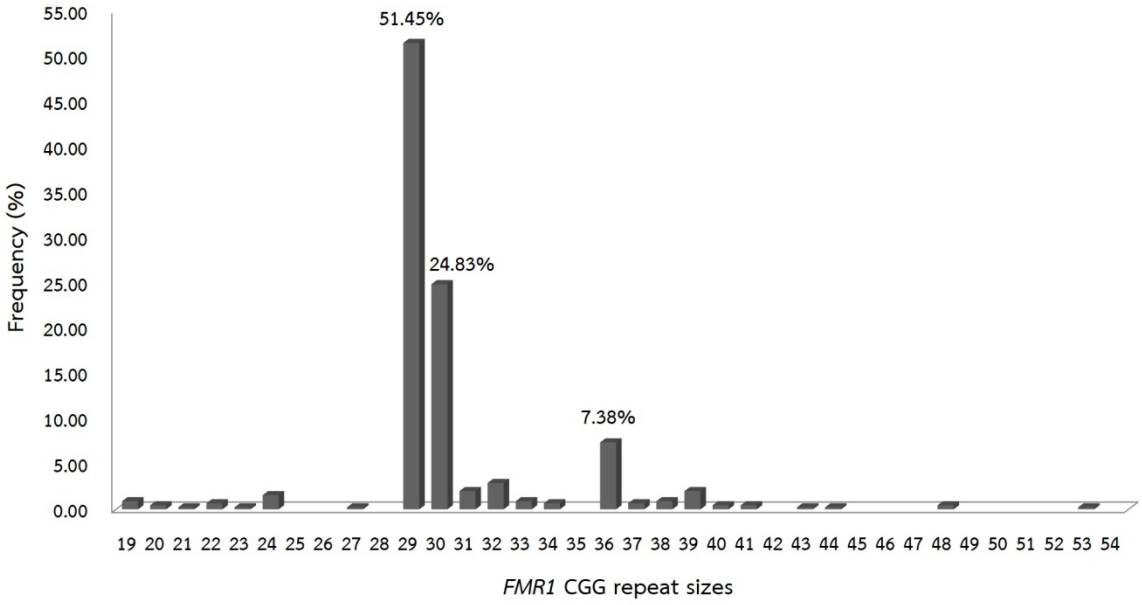

Figure 2 Distribution of *FMR1* CGG repeat size alleles. Histograms show the frequency of alleles seen in 447 Thai males negative for SCA testing.

Prevalence of *FMR1* CGG repeat expansions among Thai males with SCA negative

We performed to analyse CGG repeat expansions of the *FMR1* gene in 447 male patients negative for SCA types 1, 2, and 3 testing in Thailand. We identified 444 patients with normal-sized CGG repeats. Although no male *FMR1* premutation was detected in this study, three males carrying intermediate alleles with 45-54 CGG repeats were identified, giving a prevalence of 1 in 149 males

[0.7% (95% CI: -0.08%-1.4%)] (Figure 3). There were seven males with intermediate alleles that were defined as 41-54 CGG repeats, thus the prevalence of intermediate alleles of this cohort was 1 in 64 males [1.6% (95% CI: 0.4%-2.7%)]. Table 1 showed the prevalence of *FMR1* CGG repeat expansions among Thai males negative for SCA testing in our study compared with the other reported prevalences.

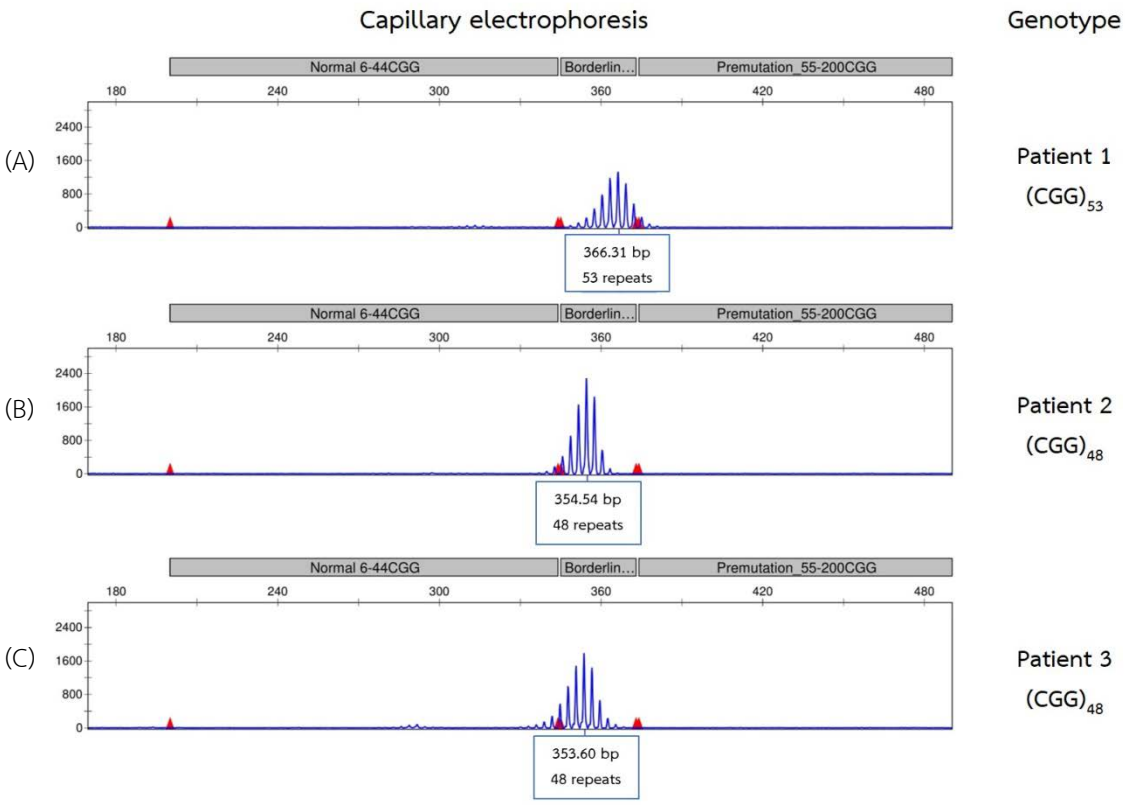

Figure 3 Fragment analysis results of fluorescent PCR for the *FMR1* gene from male patients with intermediate alleles (A) Patient with 53 CGG repeats and (B and C) Patients with 48 CGG repeats.

Table 1 The reported prevalences of *FMR1* CGG expanded alleles in ataxia males with SCA negative

References	Countries	Clinical features	Ascertainments	Prevalences	
				Intermediate	Premutation
(Tan et al., 2004)	Singapore	Movement disorders (Ataxia and tremor)	Negative for the SCA 1, 2, 3, 6, 7, 8, 10, and 12, Friedreich ataxia, and DRPLA	0/30 (0%)[a]	0/30 (0%)
(Biancalana et al., 2005)	France	Cerebellar symptoms	Negative for the SCA 1, 2, FRDA1, and/or SCA 3, 6, and 7	6/95 (6.3%)[b]	1/95 (1.1%)
(Van Esch et al., 2005)	Belgium	Ataxia; Age >50	Negative for the SCA 1, 2, 3, 6, and 7	NA	5/122 (4.1%)

Table 1 The reported prevalences of *FMR1* CGG expanded alleles in ataxia males with SCA negative (Cont.)

References	Countries	Clinical features	Ascertainments	Prevalences	
				Intermediate	Premutation
(Rodriguez-Revenga et al., 2007)	Spain	Ataxia; Age >45	Negative for the SCA 1, 2, 3, 6, 7, 8, and DRPLA	NA	1/87 (1.1%)
(Adams et al., 2008)	USA	Ataxia; Age >50	Negative for the SCA 1, 2, 3, 6, and/or SCA7 and/or DRPLA	16/286 (5.6%)[b]	1/286 (0.3%)
(Seixas et al., 2011)	Portugal	Ataxia; Age > 50	Negative for the SCA, HD, and PD	NA	1/54 (1.9%)
(Faruq et al., 2014)	India	Cerebellar ataxia, tremor, and other neurologic symptoms	Negative for the SCA 1, 2, 3, 6, 7, 8, 12, and 17, LOCA, and MSA	0/23 (0%)[a]	2/23 (8.6%)
(Aydin et al., 2018)	Germany	Cerebellar ataxia, dysarthria, and other neurologic symptoms	Negative for the SCA 1, 2, 3, 6, 7, 12, and 17	7/440 (1.6%)[b]	1/440 (0.2%)
The present study (2020)	Thailand	Ataxia	Negative for the SCA 1, 2, and 3	3/447 (0.7%)[a] 7/447 (1.6%)[b]	0/447 (0%)

SCA, Spinocerebellar ataxia; HD, Huntington disease; PD, Parkinson disease; DRPLA, Dentatorubral-pallidoluysian atrophy; FRDA, Friedreich ataxia; LOCA, Late-onset progressive cerebellar ataxia; MSA, Multiple system atrophy
NA, not available; [a]Intermediate CGG repeat between 45-54; [b]Intermediate CGG repeat between 41-54

Discussion and Conclusions

The present study is the first effort of fragile X mutation screening in Thai group of ataxia males negative for SCA types 1, 2, and 3 testing. We found no *FMR1* CGG expanded alleles in premutation range among this cohort except for patients with *FMR1* intermediate alleles. Up to date, *FMR1* premutation expansions are well-known associated with FXTAS, which is a progressive neurodegenerative disorder. The expansions lead to an increase in *FMR1* mRNA transcription. This mRNA separates and blocks the other RNAs and proteins, resulting in the disruption of normal cell function (Hagerman, Hagerman, 2016). There have been several studies about the prevalences of *FMR1* premutation in ataxia male patients negative for expansions in known SCA gene among the different populations, giving an overall prevalence of 0-4.1% (Table 1). However, almost studies reported the prevalences of *FMR1* premutation in SCA negative males in the European and American populations. There are only two studies in the Asian populations including Singapore and India. Our study explored that the prevalence of *FMR1* premutation alleles among ataxia males negative for SCA types 1, 2, and 3 testing in Thailand was 0% (0/447) which was lower than other

studies in the European and American populations. Interestingly, among the Asian populations, the result of this study indicated that the prevalence of *FMR1* premutation alleles in Thailand was similar to studies in Singapore (Tan et al., 2004), but was lower than in India (Faruq et al., 2014). This might be from the ethnic background of the populations. As well as criteria of sample selection is a difference which Faruq et al. (2014) recruited patients presenting SCA type 12 like-phenotype presenting cerebellar ataxia or tremor along with other neurological features like upper limb incoordination, hand tremor, hyperreflexia, nystagmus, and Parkinsonian signs. These clinical features were high relative with clinical symptoms of FXTAS more than the other two studies (Faruq et al., 2014; Tan et al., 2004).

In recent years, *FMR1* intermediate alleles are also found to be associated with neurological symptoms due to these smaller CGG repeat expansions which could raise in *FMR1* mRNA transcription similar to premutation alleles. The American College of Medical Genetic have defined CGG repeats from 45-54 as intermediate alleles which of their lower risk of CGG repeat expansions into full mutation in the later generation (Monaghan et al., 2013). In fact, the *FMR1* mRNA level increased starting at 39 CGG repeats (Loesch et al., 2007), so there is also another guideline defined intermediate alleles as 41-60 CGG repeats (Sherman et al., 2005). There are two reports about individuals with intermediate alleles presented clinical and neuroradiological findings consistent with FXTAS (Hall et al., 2012; Liu et al., 2013). Table 1 showed several previous studies that they found intermediate alleles ranged from 0-6.3% in their cohort studies although they did not ascertain clinical analysis of founded patients. Our study established the prevalence of *FMR1* intermediate alleles with 45-54 repeats in SCA negative Thai males was 0.7% (3/447, 1 in 149). Moreover, we also classified 41-54 CGG repeats as intermediate alleles, the prevalence of intermediate alleles among ataxia males negative for SCA testing in Thailand was high as 1.6% (7/447, 1 in 64). This result demonstrated that smaller *FMR1* CGG repeat expanded alleles in intermediate range among ataxia patients in Thailand was quite high compared to other two studies in the Asian populations (Faruq et al., 2014; Tan et al., 2004). Unfortunately, we did not have data on clinical and radiological findings. The present study did not represent an exact clinical analysis of whether all patients with intermediate presented actual clinical and radiological findings associated with FXTAS or not, but it could believe that all included patients in this study have ataxia. Consequently, our finding proposed to support the evidence that smaller *FMR1* CGG repeat expansions with 41-54 repeats are associated with neurological disorders.

Despite individuals over 50 years with symptoms of FXTAS or onset of cerebellar ataxia of unknown cause are recommended for fragile X premutation testing at this time, the fact a few patients with FXTAS are referred to and evaluated by physicians (Berry-Kravis et al., 2007). Besides, almost ataxia patients negative for SCA testing were not referred to additional laboratory requirements in Thailand. Our results, the high prevalence of smaller *FMR1* CGG repeat expanded alleles in Thai males with SCA negative, will help to define optimal practical diagnostic procedures for FXTAS and educate physicians that fragile X screening should be offered in patients presenting ataxia or clinical symptoms associated with FXTAS. The accurate diagnosis would provide appropriate treatment and genetic counseling to the patients and their families. For

treatment, the current treatment of patients with FXTAS is based on their symptoms. Patients need to maintain a healthy lifestyle and avoid exposure to neurotoxins, illicit drugs, pesticides, and alcohol consumption which contributed to the increased severity of symptoms. (Muzar et al., 2014; Saldarriaga et al., 2019). Also, there have been promising studies of targeted treatment in ongoing clinical trials that may help to reduce symptoms of FXTAS such as allopregnanolone, and citicoline (Hall et al., 2020; Trivedi et al., 2019). For genetic counseling, physicians should refer to the laboratory request for fragile X mutation testing in their family members as well which they are also at risk of developing FXTAS, and other fragile X-associated disorders. Moreover, their female offspring, who inherited *FMR1* premutation alleles will be obligated carriers and are at risk of having children with fragile X syndrome, should be suggested about family planning.

The strength of this study, we screened *FMR1* CGG repeat expansions in Thailand which is one of the Asian populations. This educates the prevalence of expanded alleles in different areas of Asia that might be profitable to future screening in the other Asian populations because there are very few studies at this time. Nevertheless, there are also has several limitations in this study. First, although the sample size in this study was larger than the two previous studies in the Asian populations, it was still small as compared to other *FMR1* screening studies. Another, the populations of this study may not be representative of the entire Thai populations, as most cases were mainly the Central and Southern Thai populations. As well as we recruited patients with SCA-like phenotype, other clinical features resembling FXTAS symptoms were not specified. Consequently, we suggested that further study should screen in larger samples and select patients with clinical features that more closely resemble FXTAS for help to find new cases and determine the significance of the finding. As well as further studies should screen patients in all regions of Thailand for evaluation of exact the prevalence of *FMR1* CGG repeat expansions in ataxia patients in the Thai populations.

To conclude, the present study proved that the prevalence of smaller *FMR1* CGG repeat expansions with 41-54 repeats in ataxia males negative for SCA common types testing was high as 1 in 64 (1.6%). This finding offered to define a sufficient practical approach for FXTAS diagnosis that fragile X screening should be considered in patients with ataxia or patients who were referred for SCA testing and found to be negative.

Acknowledgements

This study was supported by the Education & Public Welfare Foundation and Faculty of Medicine, Prince of Songkla University for research foundation.

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