

Supplementary material

Prevalence and Implications of Fragile X Premutation Screening in Thailand

Areerat Hnoonual^{1,2}, Sunita Kaewfai³, Chanin Limwongse^{4,5} & Pornprot Limprasert^{1,2*}

¹Division of Molecular Pathology, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

²Genomic Medicine Center, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

³Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

⁴Division of Medical Genetics, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

⁵Siriraj Genomics, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

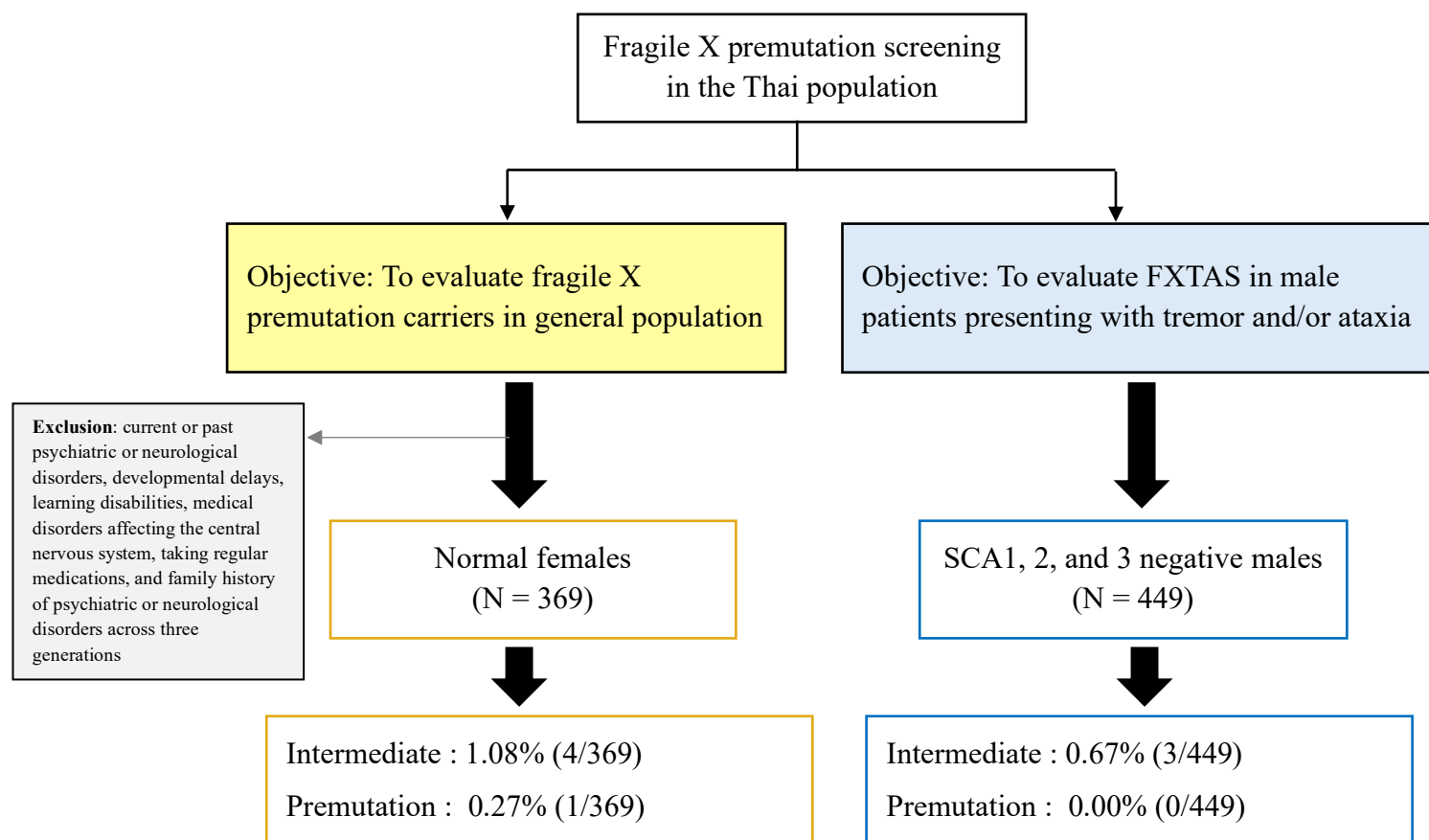


Figure S1. Study schema flow diagram illustrating study design and patient sample cohorts.

FXTAS, fragile X-associated tremor/ataxia syndrome; SCA, spinocerebellar ataxia

kb) and methylated normal band (~5.2 kb) of the *FMR1* gene. **(B)** Fluorescent PCR of the female with a premutation allele (30 and 89 repeats). **(C)** The female carrier with 2 AGG interruptions in the normal allele (30 CGG repeats: 10A9A9) and 1 AGG interruption in the premutation allele (89 CGG repeats: 9A79). **(D)** Sanger DNA sequencing of the unmethylated *FMR1* premutation allele amplified from the bisulfite-treated DNA of the female with a fragile X premutation. The sequence confirms that the female carried a premutation allele of 89 repeats interrupted with one AGG triplet in the pattern of 9A79. Following sodium bisulfite modification of non-methylated *FMR1* alleles, all C residues in the CGG repeat are converted to U, which are then replaced with T during a subsequent PCR. Thus, the sequence of CAA repeats is denoted as CGG repeats, and the number of CAA repeats is represented in the blue boxes. The sequence of AAA repeats is indicated as AGG interruptions, which are represented in the red box.

*The Southern blot results for this female showed a low signal for the normal allele (indicated by the arrowhead). This could be due to non-random X-inactivation. This observation was also noted in the predominant normal methylation allele, although this band could not clearly distinguish between the separated normal and premutation alleles. However, the normal allele with 30 CGG repeats in this individual was primarily evaluated using fluorescent PCR and TP-PCR.

Table S1. *FMRI* expanded allele prevalence data among general population of several countries

Location (Ethnicity)	Targeted population	<i>FMRI</i> CGG repeats	Number of cases with expanded allele	Total number of cases	Prevalence		References
					Prevalence ratio (95% CI)	%	
Asian							
Japan	Females without ID	Premutation	0	227	0/227 (0.0000, 0.0161)	0.00%	Arinami et al., 1993 ¹
Japan	Normal males and females	Intermediate	5	513 males	5/513 (0.0032, 0.0226)	0.97%	Otsuka et al., 2010 ²
		Premutation	0		0/513 (0.0000, 0.0072)	0.00%	
		Intermediate	1	324 females	1/324 (0.0001, 0.0171)	0.31%	
		Premutation	0		0/324 (0.0000, 0.0113)	0.00%	
Taiwan	Pregnant women	Intermediate (40-52)	22	1,002	22/1,002 (0.0138, 0.0331)	2.20%	Huang et al., 2003 ³
		Premutation (>52)	0		0/1,002 (0.0000, 0.0037)	0.00%	
Taiwan	Newborn males	Intermediate	90	4,843	90/4,843 (0.0150, 0.0228)	1.86%	Chow et al., 2003 ⁴
		Premutation	2		2/4,843 (0.0001, 0.0015)	0.04%	
Taiwan	Newborn males	Intermediate	70	10,046	70/10,046 (0.0054, 0.0088)	0.70%	Tzeng et al., 2005 ⁵
		Premutation	6		6/10,046 (0.0002, 0.0013)	0.06%	
Taiwan	Pregnant women	Intermediate	178	20,188	178/20,188 (0.0076, 0.0102)	0.88%	Hung et al., 2019 ⁶
		Premutation	26		26/20,188 (0.0008, 0.0019)	0.13%	
		Full mutation	1		1/20,188 (0.0000, 0.0003)	0.00%	
Taiwan	General women population regardless of FHx of FXS	Premutation	68	39,458	68/39,458 (0.0013, 0.0022)	0.17%	Guo et al., 2021 ⁷
		Full mutation	2		2/39,458 (0.0000, 0.0002)	0.01%	

Location (Ethnicity)	Targeted population	FMR1 CGG repeats	Number of cases with expanded allele	Total number of cases	Prevalence		References
					Prevalence ratio (95% CI)	%	
Asian (Cont.)							
Korea	Women of reproductive age with low-risk group	Intermediate	38	5,425	38/5,425 (0.0050, 0.0096)	0.70%	Kim et al., 2013 ⁸
		Premutation	7		7/5,425 (0.0005, 0.0027)	0.13%	
Korea	Pre-conceptional or pregnant women regarding FHx	Intermediate	75	10,241	75/10,241 (0.0058, 0.0092)	0.73%	Jang et al., 2014 ⁹
		Premutation	13		13/10,241 (0.0007, 0.0022)	0.13%	
Hong Kong	Pregnant women	Intermediate	30	2,650	30/2,650 (0.0077, 0.0161)	1.13%	Cheng et al., 2017 ¹⁰
		Premutation	2		2/2,650 (0.0001, 0.0027)	0.08%	
		Full mutation	1		1/2,650 (0.0000, 0.0021)	0.04%	
China	Unaffected males and females	Premutation	0	534 males	0/534 (0.0000, 0.0069)	0.00%	Huang et al., 2015 ¹¹
		Premutation	1	579 females	1/579 (0.0000, 0.0096)	0.17%	
China	Pre-conceptional or pregnant women	Intermediate	76	11,891	76/11,891 (0.0050, 0.0080)	0.64%	Ma et al., 2019 ¹²
		Premutation	29		29/11,891 (0.0016, 0.0035)	0.24%	
		Full mutation	3		3/11,891 (0.0001, 0.0007)	0.03%	
China	Women of childbearing age	Intermediate	78	10,145	78/10,145 (0.0061, 0.0096)	0.77%	Gao et al., 2020 ¹³
		Premutation	16		16/10,145 (0.0009, 0.0026)	0.16%	
		Full mutation	2		2/10,145 (0.0000, 0.0007)	0.02%	
USA (Asian)	Newborn males and females	Intermediate	0	428 males	0/428 (0.0000, 0.0086)	0.00%	Tassone et al., 2012 ¹⁴
		Premutation	1	1/428 (0.0001, 0.0129)	0.23%		
		Intermediate	5	368 females	5/368 (0.0044, 0.0314)	1.36%	
		Premutation	3	3/368 (0.0017, 0.0236)	0.82%		

Location (Ethnicity)	Targeted population	FMR1 CGG repeats	Number of cases with expanded allele	Total number of cases	Prevalence		References
					Prevalence ratio (95% CI)	%	
Asian (Cont.)							
USA (Asian)	Women referred for carrier screening	Intermediate	85	7,961	85/7,961 (0.0085, 0.0132)	1.07%	Owens et al., 2018 ¹⁵
		Premutation	19		19/7,961 (0.0014, 0.0037)	0.24%	
		Full mutation	1		1/7,961 (0.0000, 0.0007)	0.01%	
Thailand (central region)	Women of reproductive age	Intermediate	3	1,250	3/1,250 (0.0005, 0.0070)	0.24%	Ain et al., 2024 ¹⁶
		Premutation	2		2/1,250 (0.0002, 0.0058)	0.16%	
		Full mutation	0		0/1,250 (0.0000, 0.0024)	0.00%	
Thailand (southern and central regions)	General women population without FHx	Intermediate	4	369	4/369 (0.0030, 0.0275)	1.08%	This study
		Premutation	1		1/369 (0.0001, 0.0150)	0.27%	
		Full mutation	0		0/369 (0.0000, 0.0100)	0.00%	
Western (FXS testing in women, N>5,000)							
Canada	Unselected women regarding FHx	Premutation	41	10,624	41/10,624 (0.0028, 0.0052)	0.39%	Rousseau et al., 1995 ¹⁷
Canada	Unselected women regarding FHx	Intermediate	250	21,411	250/21,411 (0.0103, 0.0132)	1.17%	Lévesque et al., 2009 ¹⁸
		Premutation	39		39/21,411 (0.0013, 0.0025)	0.18%	
USA	Women from cystic fibrosis carrier screening	Premutation	48	11,759	48/11,759 (0.0030, 0.0054)	0.41%	Hantash et al., 2011 ¹⁹
USA (All ethnic)	Newborn males and females	Intermediate	65	7,312 males	65/7,312 (0.0069, 0.0113)	0.89%	Tassone et al., 2012 ¹⁴
		Premutation	17		17/7,312 (0.0014, 0.0037)	0.23%	
		Intermediate	105	6,895 females	105/6,895 (0.0125, 0.0184)	1.52%	
		Premutation	33		33/6,895 (0.0033, 0.0067)	0.48%	

Location (Ethnicity)	Targeted population	FMR1 CGG repeats	Number of cases with expanded allele	Total number of cases	Prevalence		References
					Prevalence ratio (95% CI)	%	
Western (FXS testing in women, N>5,000) (Cont.)							
USA	Carrier screening in general population	Premutation	1,356	290,745	1,356/290,745 (0.0044, 0.0049)	0.47%	Westemeyer et al., 2020 ²⁰
		Full mutation	33		33/290,745 (0.0001, 0.0002)	0.01%	
USA	General obstetric population	Premutation	1,536	354,859	1,536/354,859 (0.0041, 0.0046)	0.43%	Owens et al., 2022 ²¹
Israel	Women of reproductive age	Premutation	58	8,426 (low risk group)	58/8,426 (0.0052, 0.0089)	0.69%	Pesso et al., 2000 ²²
		Full mutation	1		1/8,426 (0.0000, 0.0007)	0.01%	
Israel	Healthy women without FHx of ID	Intermediate/ Premutation (51-200)	138	10,587	138/10,587 (0.0110, 0.0154)	1.30%	Drasinover et al., 2000 ²³
Israel	Women without FHx of FXS	Premutation (>50)	85	9,660	85/9,660 (0.0070, 0.0109)	0.88%	Geva et al., 2000 ²⁴
Israel	Women of childbearing age without FHx of ID	Premutation	124	14,334	124/14,334 (0.0072, 0.0103)	0.87%	Toledano-Alhadeef et al., 2001 ²⁵
		Full mutation	3		3/14,334 (0.0000, 0.0006)	0.02%	
Israel	Women without FHx of FXS, ID, developmental problems or autism	Premutation	231	36,483	231/36,483 (0.0055, 0.0072)	0.63%	Berkenstadt et al., 2007 ²⁶
		Full mutation	1		1/36,483 (0.0000, 0.0002)	0.00%	

Note: Multiple comparisons using the Bonferroni P-value adjustment method were used to determine the differences in frequency between the present study and previous studies. No significant differences were observed, and all pairwise comparisons between our study and other studies resulted in P-values of 1.

FHx, family history; FXS, fragile X syndrome; ID, intellectual disability

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