Table 3.2 Summary of simulated models for FXS screening

Models	Total scores	Threshold	Sensitivity	Specificity
FH + F + E + AH	8	> 1	96.15%	74.72%
2FH + F + E + 2AH	12	>2	100%	65.73%
FH + F + E + AH + T	10	> 2	100%	68.39%
2FH + F + 2AH + T	12	> 3	100%	69.67%
1.5FH + F + E + 1.5AH +T	12	> 3.5	100%	76.13%
2FH + F + E + 2AH +T	14	> 4	100%	76.77%
2FH + F + 0.5E + 2AH +T	13	> 4	100%	78.71%

Table 3.3 Comparison among clinical checklists for fragile X syndrome

Studies (population, country)	Screening methods	Number of items (total score)	Scores (sensitivity,specificity)
Hagerman et al (1991) (males, USA)	Cytogenetics	13 (26)	> 15 (86.7%, 84.8%)*
Laing et al (1991) (males and females, Australia)	Cytogenetics	5 (10)	8-10 (67%, NA)
Butler et al (1991) (males, USA)	Cytogenetics	15 (30)	> 7 (100%, 43.2%)*
Giangreco et al (1996) (males and females, USA)	Molecular	6 (12)	> 4 (100%, 60%)
Arvivo et al (1997) (males age > 16 years, Finland)	Molecular	17 (NA)	> 5 (100%, NA)
Hecimovic et al (1997) (males and females, Croatia)	Molecular	6 (12)	> 4 (96%, 57%)*
This study (males**, Thailand)	Molecular	5 (13)	> 4 (100%, 78.7%)

* calculated from data in the reports, NA = not available

** age $\leq$ 15 years

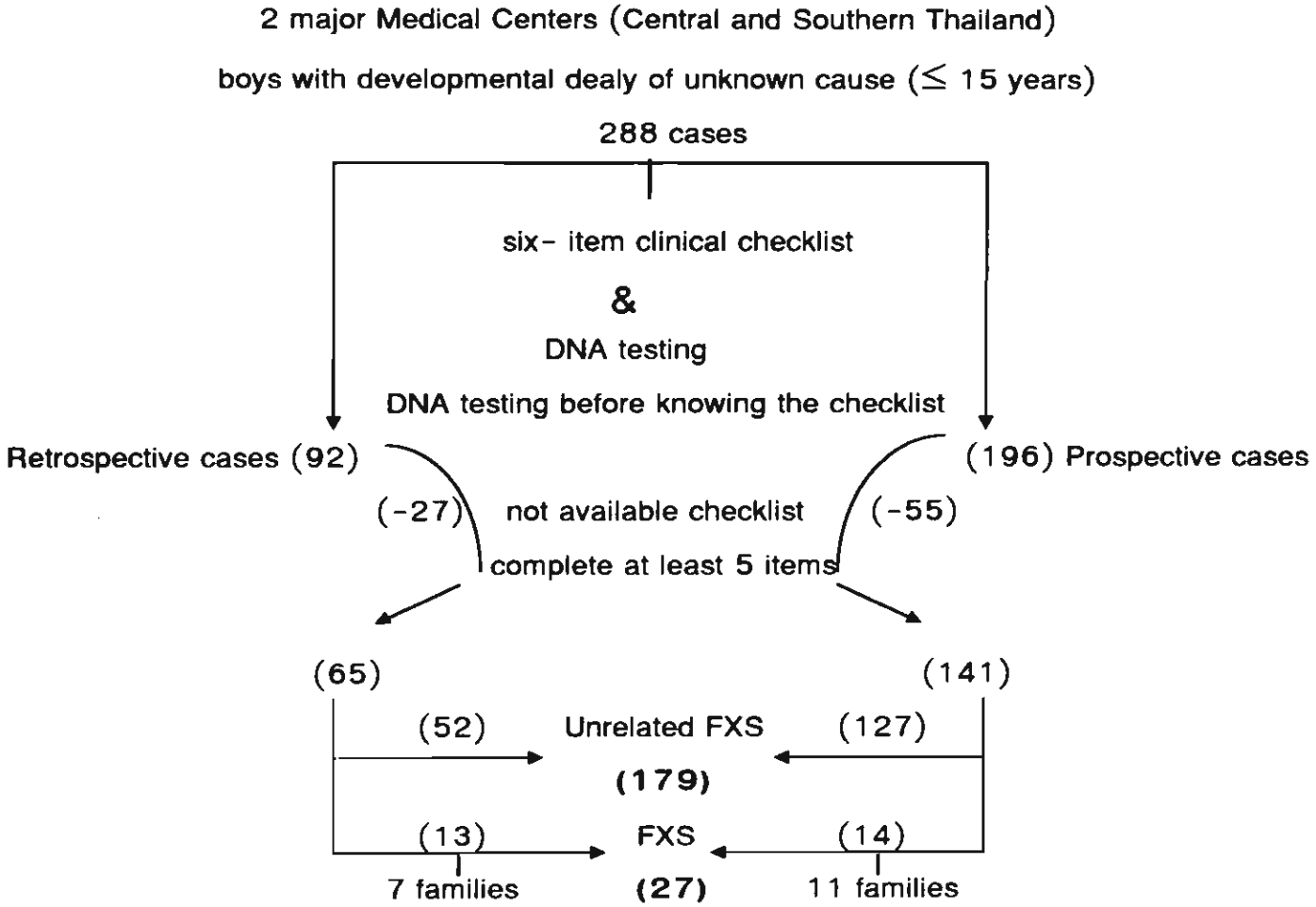


Figure 3.1 Schematic of the study

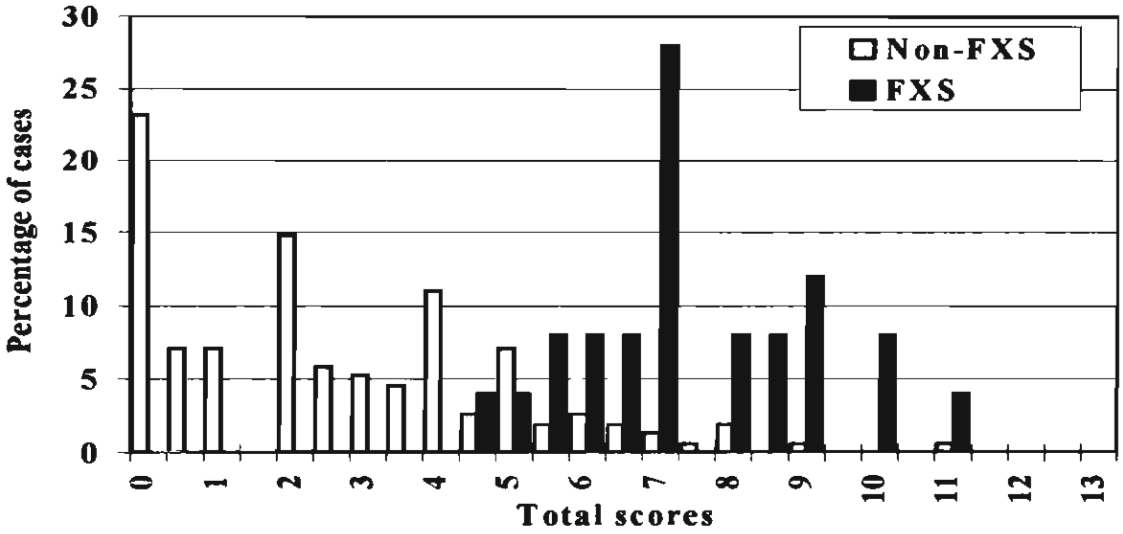


Figure 3.2 Comparison between non-FXS and FXS groups using model, 2 FH + F + 0.5E + 2AH + T = total score. All FXS patients had total scores of more than 4. Approximately 79 % of the non-FXS group had total scores of 4 or less. The total score of 4 is the threshold score for FXS screening in this model.

Chapter IV Haplotype analysis at the CGG-*FMR1* gene

Introduction

Some studies have investigated haplotypes using microsatellites near the *FMR1* gene in various populations: Caucasian (Richards et al, 1992; Jacobs et al, 1993; Macpherson et al; Zhong et al, 1994a, 1999), Belgian-Dutch (Buyle et al, 1993, Hirst et al, 1993), Finn (Haataja et al, 1994; Zhong et al, 1996a), Swedish (reviewed by Chiurazzi et al, 1996a), Italian (Chiurazzi et al , 1996b), Jewish (Pesso, et al 1997; Falik-Zaccai et al, 1997), Sub-Saharah African (Chiurazzi et al 1996c), Greek-Cyprian (Patsalis et al, 1999), Czech (Pekarik et al, 1999), Argentine (Bonaventure et al, 1998), Brazilian (Mingroni-Netto et al, 1999), Japanese (Ricahrds, et al 1994) and Chinese (Zhong et al, 1994b, 1999). In all cases it is apparent that although FXS patients can display several haplotypes, only a few types account for most of the total cases, with a distribution significantly different from that of normal controls. Furthermore, isolated populations showed even more founder effects, with one single dominant haplotype shared by most patients (Zhong et al, 1996a). The relationships between the CGG-*FMR1* repeats and single-nucleotide polymorphisms (SNPs), *ATL1* (A or G) in the intron 1 of the *FMR1* (Gunter et al,1998) and *IVS10+14C/T*, (C or T, our study designated as *IVS10*) in the intron 10 of the *FMR1* (Xu et al,1999) were reported.

A recent conference on FXS has suggested that investigations of different ethnic groups would provide important fundamental information, i.e. relationships of the genotype-phenotype, evaluation of the influence of culture on patient outcome and evolution of the CGG repeat expansion (McCabe ERB et al, 1999). Since molecular analysis of FXS is just beginning in Thailand, no study has been reported regarding the Thai population. Therefore, it would be very interesting to study CGG repeats and haplotype patterns from the Thai population. In addition, analysis of haplotype patterns might provide insight into the evolution of FXS in the Thai population.

We report on the haplotype analysis using three microsatellites, *DXS548*, *FRAXAC1* and *FRAXE*, and two SNPs, *ATL1* and *IVS10*. We found no specific haplotype association between normal control and FXS groups suggesting no founder effect in the Thai population. However, we found specific SNPs haplotype associations in the normal CGG repeats.

Materials and Methods

Subjects

We randomly selected 125 unrelated normal chromosomes with 20-50 CGG repeats and 25 unrelated FXS chromosomes. Of 125 normal chromosomes, we divided into 6 categories regarding rare CGG repeats (20-28 and 31-35), common CGG repeats (29,30 and 36), intermediate CGG repeat (37-50) as shown.

CGG Groups	Number of chromosomes	Percentage
20-28	8	6.4
29	34	27.2
30	32	25.6
31-35	10	8.0
36	25	20.0
37-50	16	12.8
Total	125	100

Microsatellites and SNPs near the CGG-*FMR1*

We chose 3 microsatellites (*DXS548, FRAXAC1 and FRAXE*) and 2 SNPs (*ATL1* and *IVS10*) for this study. The location of these markers is shown in figure 4.1. The sequence of primers and probes, annealing temperature (Ta) and magnesium concentration [Mg^{2+}] are shown in table 4.1.

PCR conditions

The PCR condition for each maker was done according to the Ta and magnesium concentration in table 4.1. The PCR cycles for the FRAXE were as same as the conditions of CGG-FMR1 (chapter II).

The PCR conditions of the *DXS548, FRAXAC1, ATL1* and *IVS10* were as follows: initial denaturation at 95 ^{o}C for 4 minutes, 35 cycles of denature at 95 ^{o}C for 30 seconds, annealing temperature (Table 4.1) for 30 seconds, and extension at 72 ^{o}C for 30 seconds, final extension at 72 ^{o}C for 10 minutes. The PCR reactions were done in a PCR thermal cycler (Perkin Elmer 480).

The detection of microsatellites

The detection of microsatellites used a (CA)n probe for [the] *DXS548* and *FRAXAC1* , and a (CGC)n probe for [the] *FRAXE*. These probes were obtained from the Lifecode company (Stanford, CN, USA). The method of detection was as same as CGG-*FMR1* alleles (chapter II), except for the CA probe which was hybridized and washed at 52 °C. The alleles were designated as CA or CGG repeats according to Zhong et al 1996a, 1999). The other allele systems are shown in table 4.1-4.2 (reviewed by Chiurazzi et al, 1999).

Dot blot hybridization

ATL1 alleles were detected by using dot blot hybridization with ATL1-A and ATL1-G probes. Five microliters of the PCR product was denatured by heating in a boiling water and quick chilling on ice. The neutral nylon membrane was rinsed in distilled water and absorbed excessive water by 3MM chromatography paper. The PCR product was splotted on the moist nylon membrane by hand. If the PCR product was splotted by using dot blot machine, it was denatured with 0.4 N NaOH. The DNA was binded to the membrane by baking in UV or baking at 80 °C for 1 hour. The probes were labeled by DIG-oligonuclotide 3'-end labeling kit from Boehringer Mannheim. The membraned was pre-hybridized at 36 °C for 10 minutes. and hybridized at 36 °C for 1 hour. The membrane was washed at 38 °C for the ATL1-G probe and 41 °C for the ATL1-A probe (15 minutes). The washing buffer compose of 2X SSPE and 0.1%SDS. The membrane was rinsed in buffer 1 at room temperature for 2 minutes. The membrane was rinsed in buffer 2 for 20 minutes. The membrane was incubated in 1:5,000 anti-DIG alkaline phosphatase diluted in buffer 2, at room temperature for 30 minutes. The membrane was rinsed in 0.3% Tween 20 diluted in buffer 1 at room temperature for 20 minutes. The membrane was washed in buffer 3 at room temperature for 5 minutes. The membrane was incubated in 2 ml buffer 3 + 8.8 µl NBT + 6.6 µl BCIP at room temperature until the purple/blue color was seen. Figure 4.2 show the dot blot hybridization for ATL1-A and ATL1-G probes.

Hybridization buffer (10 ml)

Water	7.5	ml
100 X Denhart	500	µl
20 X SSPE	1.5	ml
10% SDS	500	µl

Buffer 1: 100 mM Tris pH 7.5 + 150 mM NaCl

Buffer 2: Buffer 1 + 1% w/v blocking reagent

Buffer 3 : 100 mM Tris pH 9.5 + 100 mM NaCl + 50 mM $MgCl_2$

BStUI digestion of the *IVS10*

The detection of IVS10 polymorphism was determined by BstUI digestion. The reaction was comprised of PCR product (8 µl), 10 X NEB (New England Biolab) buffer II 1 µl (50 M $NaCl_2$, 10 mM Tris HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol) and BstUI 10 units. Add mineral oil to protect vaporization. The reaction incubated at 60 ^{o}C for 3 hours. The digested PCR product was run on 4% Nuseive agarose gel in 1X TBE. The gel was stained with ethidium bromide visualized under an UV-transluminator. The undigested PCR product was 187 bp indicated "T" allele, whereas digested PCR product was 167 and 20 bp indcated "C" allele (Xu et al, 1999, Chen SH, personnel communication).

Results

Allele frequencies of the microsatellites and SNPs

The allele frequencies of the five markers are shown in table 4.2-4.6. The *DXS548* was less polymorphic with heterozygosity of 16.5% in normal chromosomes. The *FRAXAC1* and two SNPs had similar heterozygosities (~42-45%). The *FRAXE* had 17 alleles with heterozygosity of 82.9 % in normal chromosomes. Interestingly, we found that FXS chromosomes had more heterozygosities than normal chromosomes in all markers. However, there were no statistically significant differences between normal and FXS chromosomes in all markers (Chi-square test, $P > 0.05$). Heterozygosities was calculated as $1-\Sigma q^2$ (q=all allele frequencies).

Haplotypes analysis

We analyzed haplotypes from the five markers (*DXS548-FRAXAC1-ATL1 -IVS10-FRAXE*) shown in table 4.7. We found 54 total haplotypes including 40 haplotypes in the normal group, 6 haplotypes in the FXS group and 8 haplotypes in both groups. We observed that most diverse haplotypes came from different *FRAXE* alleles. This may reflect that recombination or mutation involving the *FRAXE* has occurred. This similar observation was also reported in proximal microsatellites nearby the GCC-*FRAXE* (Limprasert et al, 1999). For this reason, we analyzed haplotypes from the remaining four markers. We found 2 major haplotypes

(20-18-G-T and 20-19-A-C) shown in table 4.8. For analysis, we divided the haplotypes into 3 groups; 20-18-G-T, 20-19-A-C and others (Table 4.9). We found no statistically significant haplotype differences between normal and FXS groups.

Although we observed that the G allele of *ATL1* (68.7%) and the T allele of *IVS10* (69.6%) were commonly found in the normal Thai subjects, the A allele of *ATL1* and the C allele of *IVS10* accounted for 71.8% (28/39) and 57.9% (22/38) of the 30 CGG repeat group, respectively (Table 4.10-4.11). Table 4.12 shows *ATL1-IVS10* haplotypes in the CGG repeat groups of the Thai subjects. We found that there is a specific haplotype (A-C) association with the 30 CGG repeat group. Of 28 normal chromosomes with A-C haplotype, 30 CGG repeats accounted for 22 chromosomes (78.5%). In contrast, the G-T haplotype was commonly found in most of the CGG repeat groups except for the 30 CGG repeat group. These findings suggest linkage disequilibrium in the normal Thai chromosomes.

Discussion

We chose the two commonly investigated microsatellites, *DXS548 and FRAXAC1*, in order to compare haplotypes among ethnic groups. However, we did not choose the other commonly investigated microsateliite, *FRAXAC2*, since this marker contains complex (GT)x-C-(TA)y-(T)z. Also, a mutation rate of 3.3 % was observed in this marker (Zhong et al, 1993). We added *FRAXE* in our study because it is very polymorphic. We included two previously reported SNPs, *ATL1* in the intron 1 of *FMR1* (Gunter et al, 1998) and *IVS10* in the intron 10 of *FMR1* (reviewed by Vincent et al, 1998; Wang et al, 1997; Xu et al, 1999). SNPs analysis is now commonly employed as it is considered to be useful for phenotype-genotype associations, particularly complex diseases (Cargill et al, 1999; Halushka et al, 1999). *DXS548* in our study has less polymorhism than other reports (reviewed by Chiurazzi et al, 1996a, Poon et al, 1999). Therefore, *DXS548* might not be useful for linkage study in the Thai population. However, the other markers showed similar heterozygosities of normal chromosomes between Thai and other ethnic groups (reviewed by Chiurazzi et al, 1996a, Zhong et al 1996b, Gunter et al, 1998; Xu et al, 1999). Higher heterozygosities of microsatellites in FXS chromosomes compared to normal chromosomes were found in our study. Zhong et al (1994b) hypothesized that chromosomes with large CGG repeats may be associated with nearby microsatellite instability. In addition, positive allele sizes assoications of the CGG-*FMR1* repeats and nearby microsatellites were reported (Zhong et al, 1995; Brown

et al, 1996c). This may be applicable for our study since we did not find a founder FXS chromosome in the Thai population.

We found no significant association of a specific haplotype in either the normal control or FXS groups. Interestingly, of 14 haplotypes in the FXS group, 6 haplotypes were not found in the control group possibly suggesting new mutations or admixture of immigrant haplotypes. This suggests that no founder chromosome is associated with Thai FXS. These findings contrast with most other reports on FXS founder effects in different ethnic groups (Richards et al, 1992, 1994; Jacobs et al, 1993; Macpherson et al; Buyle et al, 1993; Hirst et al, 1993; Zhong et al, 1994a, 1994b, 1996, 1999; Haataja et al, 1994; Chiurazzi et al, 1996a,1996b,1996c; Falik-Zaccai et al, 1997; Bonaventure et al, 1998; Patsalis et al, 1999; Pekarik et al, 1999; Mingroni-Netto et al, 1999). There was only one report of Pesso et al (1997) showing no founder effect in Ashkenazic Jews. However, Falik-Zaccai et al (1997) showed that Tunisian Jews has a rare founder haplotype. Our data imply that the Thai FXS chromosomes may originate independently in unrelated individuals. Alternatively, FXS mutation in the Thai population may occur in common haploytpes.

We analyzed the most investigated halotype (*DXS548-FRAXAC1*) from our study and 9 previous reports (Table 4.13). Although, 21-18 was the most common founder FXS chromosome (7 from 9 studies) including Chinese FXS chromosomes, there was no 21-18 haplotype in the Thai FXS chromosome. Thai FXS chromosomes contained 4 haplotypes, 20-18, 20-19, 21-19 and 25-22. 20-19 haplotype was commonly found in normal chromosomes but it was found less percentage in FXS chromosomes of the 9 comparative studies. However, we found similar percentages of 20-19 haplotype in both groups. This finding is very intriguing and strongly suggestive that the evolution of Thai FXS mutation might be different from other ethnic groups. Likewise, the rare haplotype, 20-21, was commonly found in Argentine founder FXS chromosomes instead of 21-18 haplotype (Bonaventure et al, 1998). When we analyzed the SNPs (*ATL1-IVS10*) haplotypes, the A-C haplotype was excessively seen in the 30 CGG repeats group. However, the G-T haplotype was commonly found in the other normal CGG repeats groups. This implies that A and C alleles may occur in chromosomes with 30 CGG repeats and could have been conserved for centuries.

The A allele was the most common allele (60.3%) in Caucasians, but it was less common in the Thai subjects (31.2%). The allele distributions of *ATL1* between normal Thai subjects and normal Caucasians had statistically significant differences (Table 4.4). The T allele of *IVS10* was found 64% and 69.6% in normal mixed-Asian and Thai chromosomes, respectively, whereas 8-10% was found in normal Caucasian and African American

chromosomes (Xu et al, 1999). The allele distributions of *IVS10* between normal Thai subjects and normal Caucasians or normal African Americans had statistically significant differences (Table 4.5). The G allele of the *ATL1* was found in 40% of normal chromosome, in contrast to 83% of FXS chromosomes. Gunter et al (1998) suggested that the G allele of *ATL1* may be useful as a predictor of high risk to CGG repeat expansion. This is not applicable for the Thai population since we did not find linkage disequlibrium in this marker. Although linkage disequilibrium was found in the *ATL1* marker and FXS chromosomes in Caucasians, this was not seen in the *IVS10* (Xu et al, 1999). The other factors among different circumstances should be considered.

In addtion to haplotype (*DXS548-FRAXAC1*) being one of the risk factors predisposing to CGG repeat expansion, we know that AGG interruption patterns relate to the CGG repeat instability (Kunst and Warren, 1994; Snow et al, 1994; Hirst et al, 1994; Zhong et al, 1995; Eichler et al, 1994, 1995,1996). Futhermore, the $(CGG)_6AGG$ pattern is exclusively found in Asians (Chen et al, 1997; Hirst et al, 1997; Larsen et al, 1999). Therefore, further investigation of AGG interruptions and two SNPs (*ATL1 and IVS10*) in different populations would be very interesting since such a study has not been reported. It may provide insight into an alternative predisposing factor of the CGG repeat expansion.

Table 4.1 The sequence of primers and probes with annealing temperature (Ta) and magnesium concentration [Mg^{2+}] in our study

Primers/probe	Sequences (5'-->3')	Reference of sequence	Ta (°C)	[Mg^{2+}] (mM)
FRAXAC1 (F)	GAT CTA ATC AAC ATC TAT AGA CTT TAT T	Richards et al (1991)	52	2.5
FRAXAC1 (R)	GAT GAG AGT CAC TTG AAG CTG G	Zhong et al (1993)		
DXS548 (F)	AGA GCT TCA CTA TGC AAT GGA ATC	Riggins et al (1992)	52	2.5
DXS548 (R)	GTA CAT TAG AGT CAC CTG TGG TGC			
Primer 589	GCG AGG AAG CGG CGG CAG TGG CAC TGG G	Knight et al (1993)	65	1.5
Primer 603	CCT GTG AGT GTG TAA GTG TGT GAT GCT GCC G			
ATL1 (F)	CCC TGA TGA AGA ACT TGT ATC TC	Gunter et al (1998)	65	2.5
ATL1 (R)	GAA ATT ACA CAC ATA GGT GGC ACT			
ATL1-G probe	AAA TGC TTT TGC ATT TG	Gunter et al (1998)	-	-
ATL1-A probe	AAA TGT TTT TGC ATT TG		-	-
IVS10 (F)	AGA AGA GGT ATG TTA CAG CG	Xu et al (1999)*	55	1.5
IVS10 (R)	ACT GCA TTA GAG GAC AGA GA			

* *IVS10*+14 C/T was named. We designed *IVS10*+14 C/T as *IVS10* for abbreviated name.

Table 4.2 Allele frequencies of the *DXS548* in normal and FXS Thai chromosomes

Alleles (bps)*	Alleles (CA)n	Number of normal chromosomes (%)	Number of FXS chromosomes (%)
190 (9)	18	1 (0.8)	0
194 (7)	20	114 (91.2)	22 (88.0)
196 (6)	21	7 (5.6)	2 (8.0)
198 (5)	22	1 (0.8)	0
204 (2)	25	2 (1.6)	1 (4.0)
	Total	125	25
	Heterozygosity	16.5%	21.8 %

Normal VS FXS: χ^2 = 1.22 , df = 4, P = 0.88 (Non-significance)

* The other allele system (reviewed by Chiurazzi et al. 1999)

Table 4.3 Allele frequencies of the *FRAXAC1* in normal and FXS Thai chromosomes

Alleles (bps)*	Alleles (CA)n	Number of normal chromosomes (%)	Number of FXS chromosomes (%)
152 (4.D)	18	83 (66.4)	16 (64.0)
154 (3.C)	19	40 (32.0)	8 (32.0)
156 (2.B)	20	2 (1.6)	0
160 (0.Z)	22	0	1 (4.0)
	Total	125	25
	Heterozygosity	45.6 %	48.6 %

Normal VS FXS χ^2 = 5.42 , df = 3, P = 0.14 (Non-significance)

* Other allele systems (reviewed by Chiurazzi et al. 1999)

Table 4.4 Allele frequencies of the *ATL 1* in Thai subjects comparing to Caucasian subjects (Gunter et al , 1998)

	Number of normal chromosomes (%)		Number of FEB chromosomes (%)	
Alleles	**Thai ***	**Caucasian****	**Thai ***	**Caucasian****
A	39 (31.2)	340 (60.3)	8 (32.0)	26 (17.1)
G	86 (68.8)	224 (39.7)	17 (68.0)	126 (82.9)
Total	**125**	**564**	**25**	**152**
Heterozygosity	**42.9 %**	**47.9 %**	**43.5 %**	**28.4 %**

* χ^2 = 0.00, df = 1, P = 1.00 (Non-significance)

** χ^2 = 87.62, df = 1, P < 0.000001 (Significance)

Normal Thai VS Normal Caucasian: χ^2 = 33.81, df = 1, P < 0.000001 (Significance)

Table 4.5 Allele frequencies of the *IVS10* in Thai subjects comparing to the report of Xu et al (1999)

				Number of normal chromosomes (%)		Number of FEB chromosomes (%)
Alleles	**Thai ***	**Mixed Asian**	**Caucasian ****	**African American**	**Thai ***	**Caucasian****
C	38 (30.4)	113 (36.2)	189 (90.9)	142 (89.3)	9 (36.)	46 (97.0)
T	87 (69.6)	199 (63.8)	19 (9.1)	17 (10.7)	16 (64.0)	4 (8.0)
Total	**125**	**312**	**208**	**159**	**25**	**50**
Heterozygosity	**42.3 %**	**46.2 %**	**16.8 %**	**19.1 %**	**46.1 %**	**14.7%**

* χ^2 = 0.10, df = 1, P = 0.75 (Non significance)

** χ^2 = 0.00, df = 1, P = 1.00 (Non significance)

Normal Thai VS Normal Caucasian χ^2 = 128.78, df = 1, P = 0.000001 (Significance)

Normal Thai VS Mixed Asian χ^2 = 1.09, df = 1, P = 0.29 (Non significance)

Normal Thai VS African American χ^2 = 102.11, df = 1, P = 0.000001 (Significance)

Normal Caucasian VS African American χ^2 = 0.10, df = 1, P = 0.75 (Non significance)

Table 4.6 Allele frequencies of the *FRAXAE* in Thai subjects

Alleles (bps)	Allele (GCC)n	Number of normal chromosomes (%)	Number of FXS chromosomes (%)
318	10	3 (2.4)	0
321	11	3 (2.4)	2 (8.0)
324	12	4 (3.2)	1 (4.0)
327	13	2 (3.2)	3 (12.0)
330	14	2 (1.6)	0
333	15	9 (7.2)	2 (8.0)
336	16	2 (1.6)	3 (12.0)
339	17	19 (15.2)	1 (4.0)
342	18	49 (39.2)	4 (8.0)
345	19	12 (9.6)	4 (16.0)
348	20	9 (7.2)	2 (8.0)
351	21	4 (3.2)	0
354	22	1 (0.8)	0
357	23	2 (1.6)	0
361	24	0	0
364	25	0	1 (4.0)
367	26	0	0
370	27	0	1(4.0)
373	28	2 (1.6)	0
376	29	0	0
379	30	0	0
382	31	0	0
385	32	1 (0.8)	1 (4.0)
388	33	0	0
391	34	0	0
393	35	1 (0.8)	0
	Total	**125**	**25**
	Heterozygosity	**82.9%**	**89.6%**

Normal VS FXS: $\chi^2 = 36.1$, df = 18, $P = 0.007$ (Significance)

Table 4.7 Haplotypes from five markers (*DXS548-FRAXAC1-ATL1-IVS10*)

Haplotypes/CGG	20-28	29	30	31-35	36	37-50	FXS
18 18 G T 17	1	0	0	0	0	0	0
20 18 G T 10	0	1	0	0	1	1	0
20-18-G-T 11	0	0	0	0	0	0	1
20 18 G C 11	0	0	0	1	0	0	0
20 18 G T 12	0	2	0	0	0	0	0
20 18 G T 13	0	1	0	0	0	0	0
20 18 G T 15	0	1	0	0	2	1	0
20 18 G C 15	0	1	0	0	1	0	0
20 18 G T 16	0	0	0	0	1	0	3
20 18 G C 16	0	1	0	0	0	0	0
20 18 G T 17	0	2	1	5	2	0	1
20 18 G T 18	2	10	2	1	6	6	4
20 18 G C 18	1	0	0	0	1	0	0
20 18 G T 19	0	5	0	0	0	2	4
20 18 G T 20	0	0	1	0	5	1	0
20 18 G T 21	0	1	0	0	1	0	0
20 18 G C 21	0	1	0	0	1	0	0
20-18-G-T-25	0	0	0	0	0	0	1
20-18-G-T 27	0	0	0	0	0	0	1
20 18 G T 28	0	1	0	0	0	0	0
20 18 G T 32	0	1	0	0	0	0	1
20 18 G T 35	0	0	0	0	1	0	0
21 18 G T 17	0	0	0	0	1	0	0
21 18 G T 18	0	0	0	0	0	1	0
21 18 G T 20	0	0	0	0	1	0	0
21 18 G T 23	0	1	0	0	0	0	0
21 18 A T 18	0	1	0	0	0	0	0
20 19 A C 11	0	0	2	0	0	0	0
20 19 A T 12	1	0	1	0	0	0	0
20 19 A C 13	0	0	1	0	0	0	1
20 19 A C 14	0	0	0	0	0	1	1
20 19 A T 15	0	0	0	1	0	0	0
20 19 A C 15	0	0	1	0	0	0	2
20 19 A T 17	1	0	1	0	0	1	0
20 19 A C 17	0	0	1	0	0	0	0
20-19-A-T-18	0	0	2	0	0	0	0
20-19-A-C-18	2	0	10	1	0	0	0
20-19-A-T-19	0	0	1	0	0	0	0
20-19-A-C-19	0	0	3	0	0	0	0
20-19-A-C-20	0	1	0	0	0	0	2
20-19-A-C-23	0	0	0	0	0	1	0
20-19-A-C-28	0	0	1	0	0	0	0
20-19-G-T-14	0	0	0	0	0	1	0
20-19-G-C-15	0	1	0	0	0	0	0
20-19-G-T-17	0	1	0	0	0	0	0
20-20-A-T-22	0	0	1	0	0	0	0
20-20-G-C-16	0	1	0	0	0	0	0
21-19-A-C-11	0	0	0	0	0	0	1
21-19-A-C-15	0	0	0	0	0	0	1
21-19-A-C-17	0	0	1	0	0	0	0
21-19-A-C-19	0	0	1	0	0	0	0
22-19-A-C-17	0	0	1	0	0	0	0
25-18-G-T-18	0	0	0	1	1	0	0
25-22-G-C-19	0	0	0	0	0	0	1
Total	8	34	32	10	25	16	25

Table 4.8 Haplotypes of four markers (*DXS548-FRAXAC1-ATL1-IVS10*)

Haplotypes/CGG	20-28	29	30	31-35	36	37-50	Total	FXS
18-18-G-T	1	0	0	0	0	0	1	0
20-18-G-T	**2**	**25**	**4**	**6**	**19**	**11**	**67**	**16**
20-18-G-C	1	3	0	1	3	0	8	0
20-19-G-T	0	1	0	0	0	1	2	0
20-19-A-T	2	0	5	1	0	1	9	0
20-19-G-C	0	1	0	0	0	0	1	0
20-19-A-C	**2**	**1**	**19**	**1**	**0**	**2**	**25**	**6**
20-20-A-T	0	0	1	0	0	0	1	0
20-20-G-C	0	1	0	0	0	0	1	0
21-18-G-T	0	1	0	0	2	1	4	0
21-18-A-T	0	1	0	0	0	0	1	0
21-19-A-C	0	0	2	0	0	0	2	2
22-19-A-C	0	0	1	0	0	0	1	0
25-18-G-T	0	0	0	1	1	0	2	0
25-22-G-C	0	0	0	0	0	0	0	1
Total	**8**	**34**	**32**	**10**	**25**	**16**	**125**	**25**

Table 4.9 Haplotypes of four markers (*DXS548-FRAXAC1-ATL1-IVS10*) found in the control and FXS groups

Haplotypes	Control	FXS
20-18-G-T	67	16
20-19-A-C	25	6
others	33	3
Total	**125**	**25**

Normal VS FXS: $\chi^2 = 2.37$, df = 2, $P = 0.31$ (Non-significance)

Table 4.10 The distribution of A and G alleles in CGG repeat groups

Allele/CGGs	20-28	29	30	31-35	36	37-50	FXS
A	4	2	**28**	2	0	3	8
G	4	**32**	4	8	**25**	13	17
Total	8	**34**	32	10	**25**	16	25

Table 4.11 The distribution of C and T alleles in CGG repeat groups

Allele/CGGs	20-28	29	30	31-35	36	37-50	FXS
C	3	6	**22**	2	3	2	9
T	5	**28**	10	8	**22**	14	16
Total	8	**34**	32	10	**25**	16	25

Table 4.12 The distribution of the *ATL1-IVS10* in CGG repeat groups

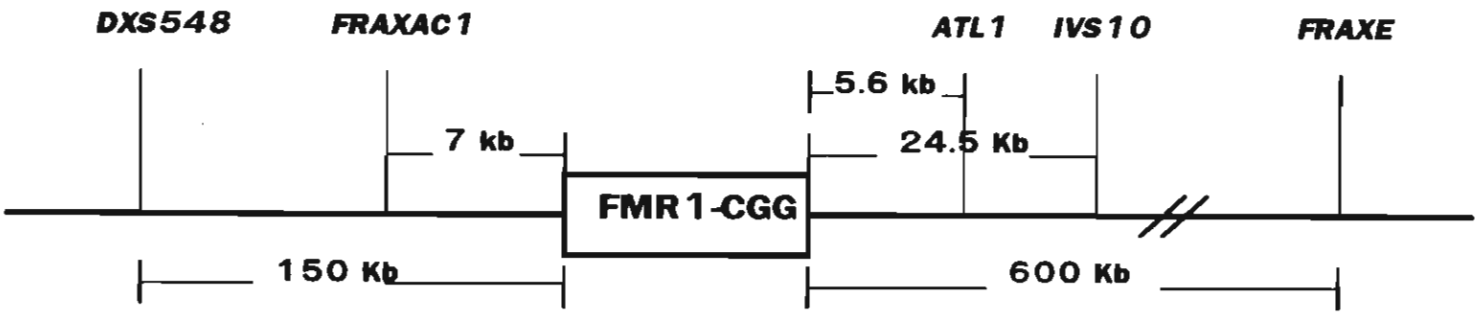
Haplotypes/CGGs	20-28	29	30	31-35	36	37-50	FXS
A-C	2	1	**22**	1	0	2	8
A-T	2	1	**6**	1	0	1	0
G-C	1	5	**0**	1	3	0	1
G-T	3	**27**	4	7	22	13	16
Total	8	**34**	32	10	**25**	16	25

Table 4.13 Comparative significant haplotypes among different populations using *DXS548* and *FRAXAC1* (shown in partial haplotypes)

	Haplotypes (*DXS548-FRAXAC1*) Percentage in Normal/Percentage in FXS							
Populations (Numbers of Normal/FXS)	**20-18**	**20-19**	**21-18**	**21-19**	**25-21**	**25-20**	**25-19**	**20-21**
Thai[1] (125/25)	60.0/64.0	29.6/24.0	4.0/0	1.6/8.0	-	-	-	-
Chinese[2] (206/24)	54.9/20.8	26.2/4.2	9.7/62.5*	2.4/8.3	-	-	-	-
Caucasian[3] (157/70)	19.7/20.0	66.2/34.3	5.1/24.3*	4.5/8.6	-	-	-	-
Caucasian[4] (564/152)	6.9/2.6	62.2/20.4	7.1/32.9*	6.1/4.6	3.9/23.0*	0.2/0	1.9/3.2	1.4/3.2
Caucasian[5] (188/44)	7.9/0	64.9/18.2	9.0/31.8*	5.9/0	2.7/15.9*	-	3.2/2.3	0.5/6.8
Finn[6] (54/36)	13.0/0	55.6/0	13.0/11.1	3.7/83.3*	5.6/5.6	-	-	-
Italian[7] (202/125)	9.9/4.0	63.9/20.8	7.4/16.8*	5.4/1.6	2.5/24.0*	-	-	-
Czech[8] (33/35)	3.0/0	66.7/8.6	0/17.1*	6.1/11.4	6.1/22.9*	-	0/8.6	-
Brazilian[9] (60/71)	3.3/2.8	58.3/4.2	8.3/ 23.9*	3.3/1.4	3.3/40.8*	-	0/12.7	-
Argentine[10] (168/42)	20.8/7.1	54.8/28.6	6.0/7.1	4.2/2.4	3.0/14.3*	-	1.8/4.8	0.6/26.2*

1 = This study, 2 = Zhong et al(1999), 3 = USA, Zhong et al (1999), 4 = USA, Gunter et al (1998), 5 = UK, Macpherson et al (1994), 6 = Zhong et al (1996), 7 = Chiurazzi et al (1996), 8 = Pekarik et al (1999), 9 = Mingroni-Netto et al (1999), 10 = Bonaventure et al (1999)

* significant founder FXS chromosomes



It is not in proportional scale.

Figure 4.1 Diagram of microsatellites using for haplotype analysis (Gunter et al,1998; Gene bank accession: L29074))

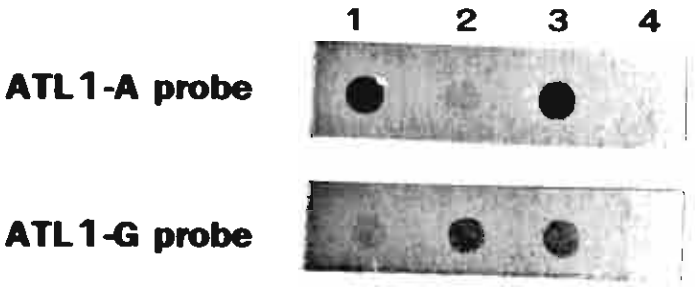


Figure4.2 Dot blots probed with allele-specific oligonucleotides (ATL1-A or ATL1-G probes). Cross-hybridizations are seen as faint background in 1 and 2, however, the real hybridization is much more intense.
1 = homozygous A, 2= homozygous G, 3= heterozygous A/G, 4= No DNA

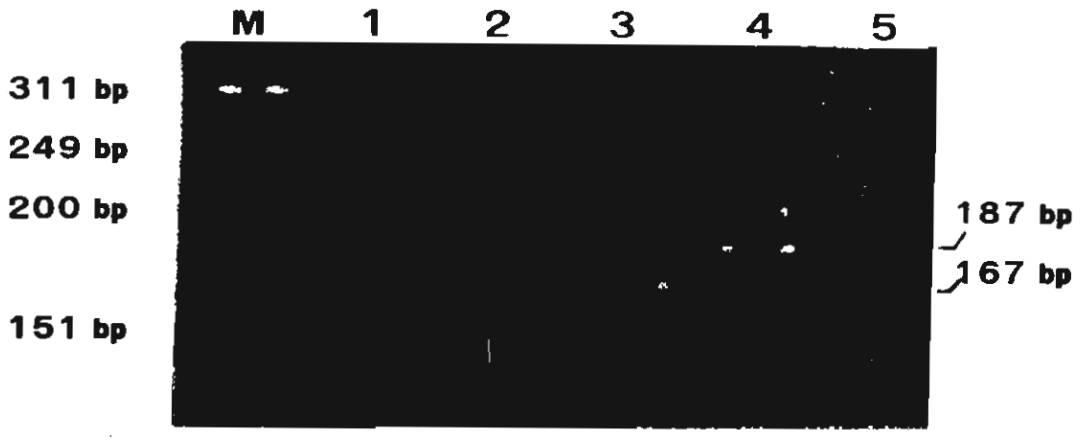


Figure 4.3 PCR products of the *IVS10* were cut by BStUI. The T allele is not cut by the enzyme (187 bp) whereas the C allele is cut into 167 and 20 bp. The 20 bp was run out of the gel, therefore it is not seen in the figure.
M = Φ X174/HinfI fragments, 1,3 and 5 = C allele, 2 and 4 = T allele

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1. Limprasert P, Ruangdaraganon N, Sura T, Vasiknanonte P, Jinorose U (1999). Molecular Screening for fragile X syndrome in Thailand. Southeast Asian J Trop Med and Pub Health 30 (supp2) (in press).

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