CHAPTER 3

RESULTS

Identification of microsatellite and SNP alleles

The alleles of each polymorphic marker were genotyped by gel analysis (Figures 4.1-4.8). The nomenclature of the microsatellite alleles was based on the number of AC dinucleotide repeats as determined by sequencing. The discrimination of each SNP allele corresponded to their PCR product fragment size following careful consideration of the different product sizes obtained from the primer designs of the Biallelic-ARMS PCR technique.

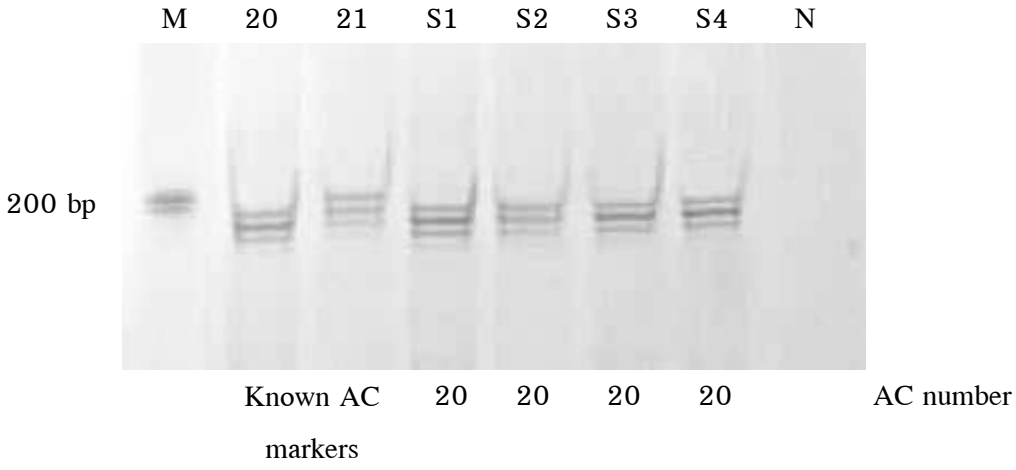

Figure 4.1. The representative polyacrylamide gel for DXS548. Marker (M) was the low molecular weight DNA ladder (200 ng). Lanes S1-S4 show individual males presenting a DXS548 microsatellite allele; the genotyping results are shown below each sample lane. There was no DNA template in Lane N (negative control). All lanes showed DXS548 allele 20 when compared with known alleles of 20 and 21 AC repeats (2^{nd} and 3^{rd} lanes from the left, respectively).

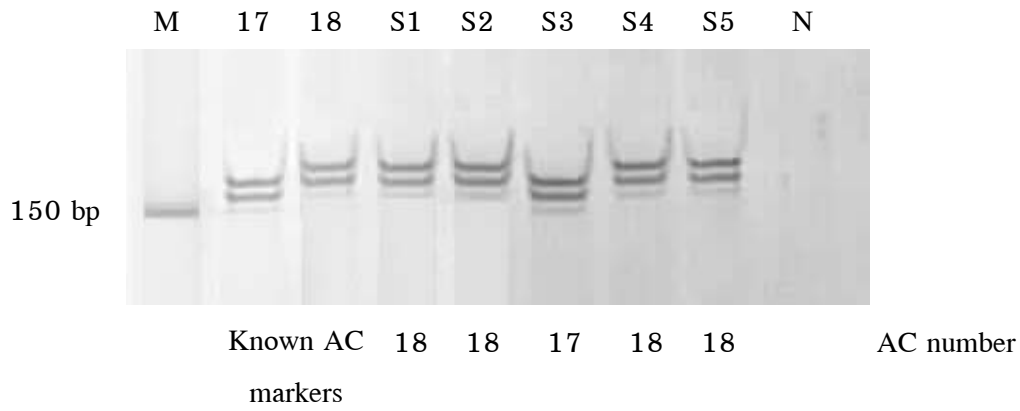

Figure 4.2. The representative polyacrylamide gel for FRAXAC1. Marker (M) was the low molecular weight DNA ladder (200 ng). Lanes S1-S5 were individual males presenting a FRAXAC1 microsatellite allele; the genotyping results are shown below each sample lane. There was no DNA template in Lane N (negative control). Lane S3 had FRAXAC1 allele 17 while lanes S1, S2, S4 and S5 showed FRAXAC1 allele 18 when compared with known alleles of 17 and 18 AC repeats (2^{nd} and 3^{rd} lanes from the left, respectively).

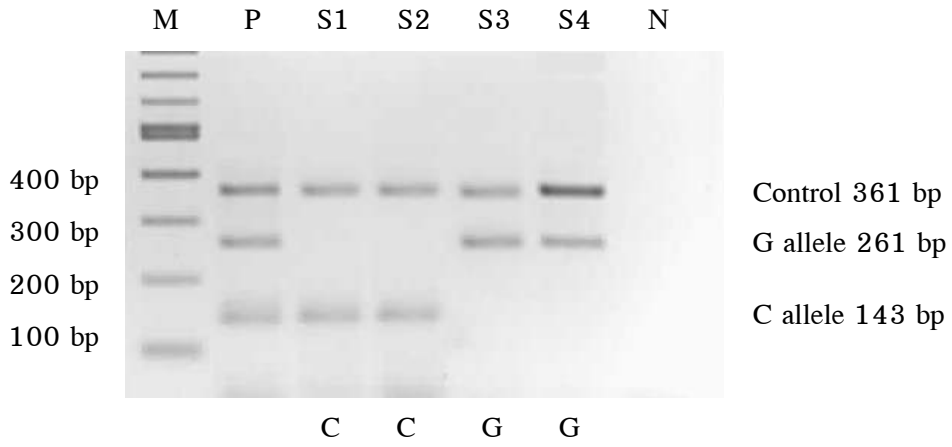

Figure 4.3. The representative agarose gel for WEX5. Marker (M) was the 100 bp DNA ladder (400 ng). Lane P (positive control) contained the DNA control of the heterozygous female indicating two different SNP alleles and there was no DNA template in Lane N (negative control). Lanes S1-S4 show samples of individual males presenting a particular SNP allele along with the genotyping results shown below each sample lane. The control product of 361 bp used as the internal control to prove the successful PCR amplification appears in every lane. Lanes S1 and S2 exhibit the fragment size of 143 bp resulting from the WEX5 genotype C as well as lanes S3 and S4 indicate the PCR product of 261 bp scoring of WEX5 genotype G.

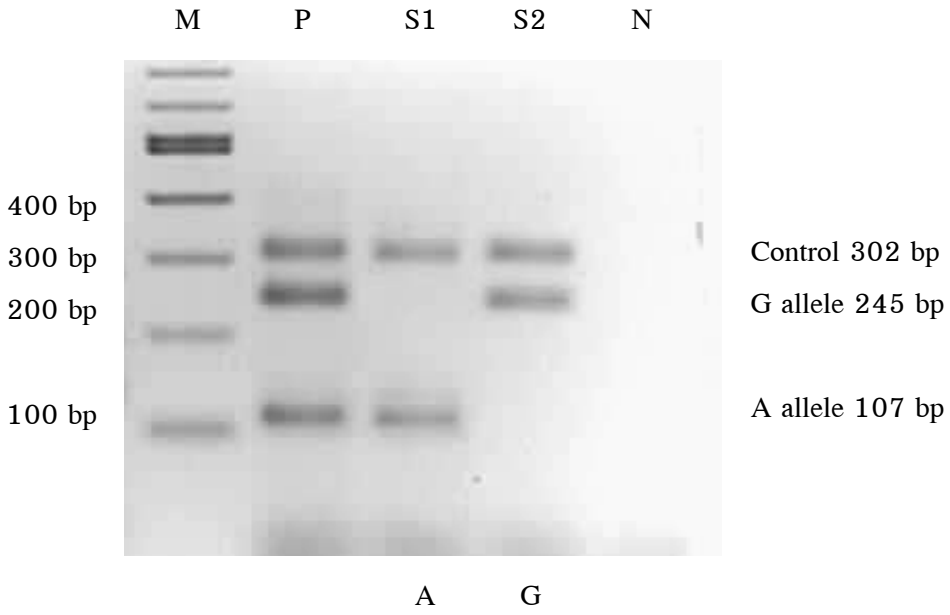

Figure 4.4. The representative agarose gel for ATL1. Marker (M) was the 100 bp DNA Ladder (400 ng). Lane P (positive control) contains the DNA control of the heterozygous female indicating two different SNP alleles and there is no DNA template in Lane N (negative control). Lanes S1 and S2 are samples of individual males presenting a particular SNP allele along with the genotyping results shown below each sample lane. The control product of 302 bp was used as an internal control to prove the successful PCR amplification which appears in every lane. Lane S1 exhibits the fragment size of 107 bp resulting in the ATL1 genotype A and lane S2 shows the PCR product of 245 bp scoring of ATL1 genotype G.

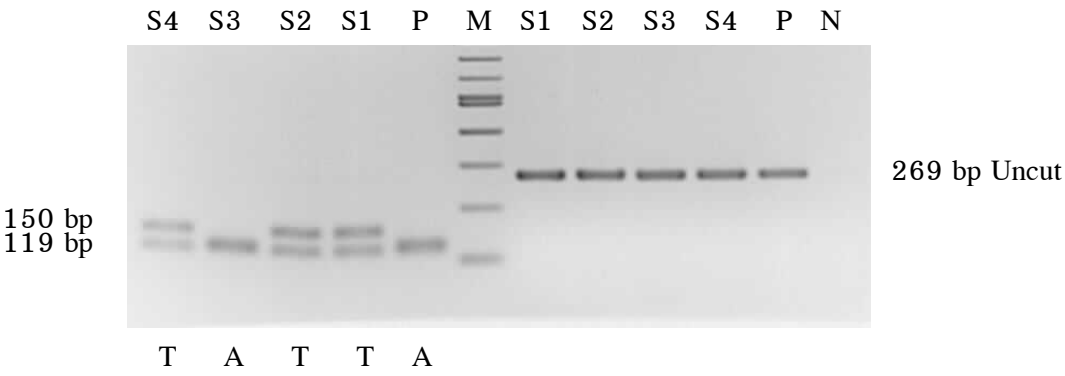

Figure 4.5. The representative agarose gel for rs25731. Marker (M) was the 100 bp DNA ladder (400 ng). Lane P (positive control) contains the male DNA control and there is no DNA template in Lane N (negative control). Lanes S1–S4 show samples of individual males presenting a particular SNP allele along with the genotyping results shown below each sample lane. Unlike the multiplex PCR of other SNP loci, the singleplex PCR with only control forward and reverse primer of SNP rs25731 amplified a PCR product of 269 bp (lanes S1–S4 and P on the right part of the gel). After these products were digested with restriction endonuclease, *Dra* I, the A allele of rs25731 displayed three bands of 125, 119 and 25 bp and the pattern looked like one band on 2.5% gel (lanes P and S3 on the left side of gel) because the 2.5 percent of the gel could not resolve the 6-bp difference fragments and 25-bp fragment was run out of gel. The distinguishing pattern of the T allele presented two obvious distinct bands of 150 and 119 bp (lanes S1, S2 and S4 on the left side of the gel).

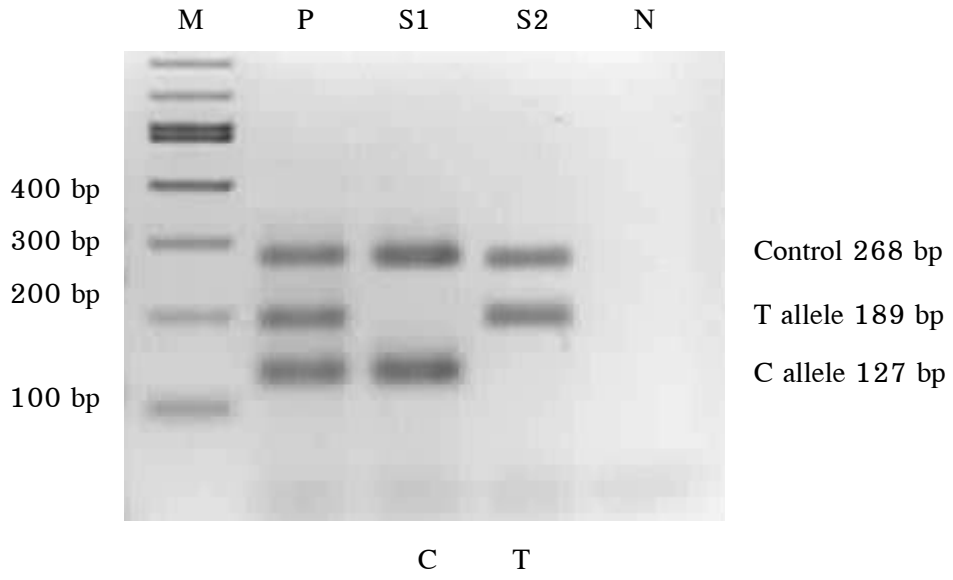

Figure 4.6. The representative agarose gel for IVS10. Marker (M) was the 100 bp DNA Ladder (400 ng). Lane P (positive control) contains the DNA control of the heterozygous female indicating two different SNP alleles and there is no DNA template in Lane N (negative control). Lanes S1 and S2 are samples of individual males presenting a particular SNP allele along with the genotyping results shown below each sample lane. The control product of 268 bp used as the internal control to prove the successful PCR amplification appears in every lane. Lane S1 exhibits the fragment size of 127 bp resulting in IVS10 genotype C and lane S2 shows the PCR product of 189 bp scoring of IVS10 genotype T.

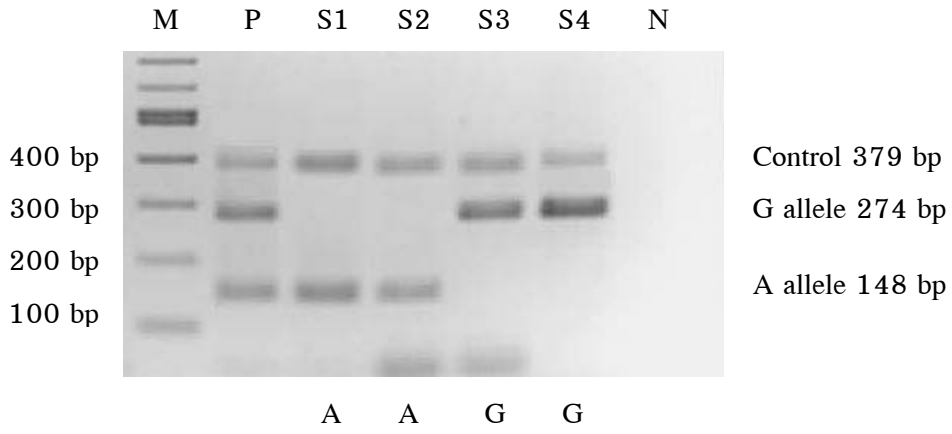

Figure 4.7. The representative agarose gel for rs25702. Marker (M) was the 100 bp DNA ladder (400 ng). Lane P (positive control) contains the DNA control of the heterozygous female indicating two different SNP alleles and there is no DNA template in Lane N (negative control). Lanes S1-S4 are samples of individual males presenting a particular SNP allele along with the genotyping results shown below each sample lane. The control product of 379 bp used as the internal control to prove the successful PCR amplification appears in every lane. Lanes S1 and S2 exhibit the fragment size of 148 bp resulting in rs25702 genotype A as well as lanes S3 and S4 show the PCR product of 274 bp scoring of rs25702 genotype G.

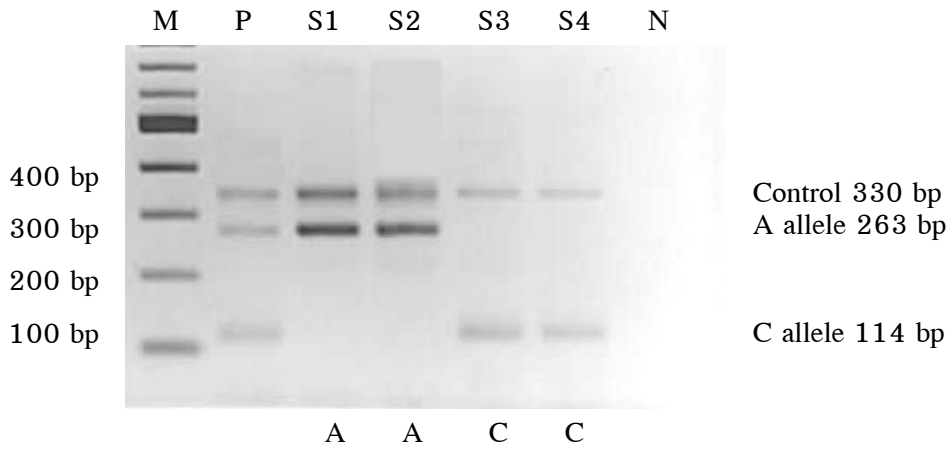

Figure 4.8. The representative agarose gel for rs25723. Marker (M) was the 100 bp DNA ladder (400 ng). Lane P (positive control) contains the DNA control of the heterozygous female indicating two different SNP alleles and there is no DNA template in Lane N (negative control). Lanes S1–S4 are samples of individual males presenting a particular SNP allele along with the genotyping results shown below each sample lane. The control product of 330 bp used as the internal control to prove the successful PCR amplification appears in every lane. Lanes S1 and S2 exhibit the fragment size of 263 bp resulting in rs25723 genotype A as well as lanes S3 and S4 which show the PCR product of 114 bp scoring of rs25723 genotype C.

Allele distributions and frequencies of the microsatellite and SNP markers

We evaluated two types of markers, microsatellites and single nucleotide polymorphisms (SNPs). The microsatellite marker is more polymorphic than SNP which has only two alleles. As can be seen in Table 6, five DXS548 alleles of 18, 20, 21, 22 and 25 AC repeats presented in normal subjects, while only two alleles of 20 and 21 repeats observed in FXS subjects. The AC 20 repeat allele was the most common allele accounting for 90.2% (120/133) of all alleles in the normal groups and 96% (48/50) in the FXS groups. The AC alleles of FRAXAC1 in both cohorts had a similar allele distribution pattern of 17, 18 and 19 alleles (there was no allele 19 in the FXS samples). The 17 AC allele followed by the 18 AC allele were the most prevalent genotypes of both normal and FXS groups with similar allele frequencies, 61.7% as compared with 62% for 17 AC repeats and 36.8% as compared with 38% for 18 AC repeats, respectively. We found no statistically significant differences in allele distributions and frequencies of all polymorphic markers between the normal and FXS groups ($P > 0.05$, Table 6). In both sample groups, DXS548 was less polymorphic than other markers. The heterozygosity (Het) of DXS548 in normal chromosomes was 18.1%, while the remaining markers presented similar heterozygosities ranging from ~46-48%. Additionally on FXS chromosomes, the smallest heterozygosity was 7.7% in the DXS548 locus, which greatly differed from other loci (~46-47%). We observed that the heterozygosity of FRAXAC1 and all SNPs in normal samples were similar to the FXS groups.

Table 6. Allele distributions and frequencies of the microsatellite and SNP markers in normal controls and FXS patients.

Locus	Allele	Normal		FXS	
		NO.	%	NO.	%
DXS548	18	1	0.8	0	0.0
	20	120	90.2	48	96.0
	21	9	6.8	2	4.0
	22	1	0.8	0	0.0
	25	2	1.5	0	0.0
	total	133	100.0	50	100.0
	Het		18.1		7.7

Normal vs FXS: Chi-square = 2.10, df = 4, P = 0.72 (not significant)

Locus	Allele	Normal		FXS	
		NO.	%	NO.	%
FRAXAC1	17	82	61.7	31	62.0
	18	49	36.8	19	38.0
	19	2	1.5	0	0.0
	total	133	100.0	50	100.0
	Het		48.4		47.1

Normal vs FXS: Chi-square = 0.77, df = 2, P = 0.68 (not significant)

Locus	Allele	Normal		FXS	
		NO.	%	NO.	%
WEX5	C	84	63.2	31	62.0
	G	49	36.8	19	38.0
	total	133	100.0	50	100.0
	Het		46.5		47.1

Normal vs FXS: Chi-square = 0.02, df = 1, P = 0.89 (not significant)

Table 6. (continued)

Locus	Allele	Normal		FXS	
		NO.	%	NO.	%
ATL1	A	48	36.1	18	36.0
	G	85	63.9	32	64.0
	total	133	100.0	50	100.0
	Het		46.1		46.1

Normal vs FXS: Chi-square = 0.00, df = 1, P = 0.99 (not significant)

Locus	Allele	Normal		FXS	
		NO.	%	NO.	%
rs25731	A	48	36.1	19	38.0
	T	85	63.9	31	62.0
	total	133	100.0	50	100.0
	Het		46.1		47.1

Normal vs FXS: Chi-square = 0.06, df = 1, P = 0.81 (not significant)

Locus	Allele	Normal		FXS	
		NO.	%	NO.	%
IVS10	C	52	39.1	19	38.0
	T	81	60.9	31	62.0
	total	133	100.0	50	100.0
	Het		47.6		47.1

Normal vs FXS: Chi-square = 0.02, df = 1, P = 0.89 (not significant)

Locus	Allele	Normal		FXS	
		NO.	%	NO.	%
rs25702	A	81	60.9	31	62.0
	G	52	39.1	19	38.0
	total	133	100.0	50	100.0
	Het		47.6		47.1

Normal vs FXS: Chi-square = 0.02, df = 1, P = 0.89 (not significant)

Table 6. (continued)

Locus	Allele	Normal		FXS	
		NO.	%	NO.	%
rs25723	A	81	60.9	31	62.0
	C	52	39.1	19	38.0
	total	133	100.0	50	100.0
	Het		47.6		47.1

Normal vs FXS: Chi-square = 0.02, df = 1, P = 0.89 (not significant)

Haplotype analysis

We established several series of combined haplotype and analyzed all of them (Table 7). This analysis revealed that all sets of combined haplotypes showed no statistically significant differences of haplotype associations between the normal and FXS groups ($P > 0.05$), similar to the study of Thai subjects reported by Limprasert (2001), even though this study included more polymorphic markers and sample pools than the previous study. Nevertheless, we clearly observed a common haplotype background (bold characters) accounting for the majority of both groups in every haplotype set. Thus, we divided the combined haplotype of seven markers (FRAXAC1-WEX5-ATL1-rs25731-IVS10-rs25702-rs25723) into 4 groups including 17-G-G-A-T-A-A (designated as Hap A), 18-C-A-T-C-G-C (designated as Hap B), 17-C-G-T-T-A-A (designated as Hap C) and other haplotypes (Table 8). Reanalysis of such major and other haplotypes still detected no specific haplotype association ($P = 0.88$). In addition, Hap A was similar to Hap C with minor differences of only 2 SNP loci (WEX5 and rs25731), whereas Hap B was different from Hap A in all polymorphic markers and from Hap C in 5 markers (FRAXAC1, ATL1, IVS10, rs25702 and rs25723). Hap B was slightly different from the haplotypes of the OTHER group at 1 or 2 markers (FRAXAC1, WEX5 and ATL1). If it was assumed that these common haplotypes occurred on a related background, we could group them into 2 major haplotype backgrounds. The majority of samples in normal and FXS individuals occurred on haplotype backgrounds Hap A and Hap C (60.9% and 62%, respectively) while the minority of samples occurred on haplotype backgrounds Hap B and OTHER (39.1% and 38%, respectively).

Table 7. Frequencies of each haplotype set in normal and FXS groups. (D = DXS548, AC = FRAXAC1, W = WEX5, AT = ATL1, 1 = rs25731, IV = IVS10, 2 = rs25702, 3 = rs25723)

Haplotype set	Combined haplotypes	Normal		FXS	
		NO.	%	NO.	%
D-AC	18-17	1	0.8	0	0.0
	20-17	**74**	**55.6**	**31**	**62.0**
	20-18	**44**	**33.1**	**17**	**34.0**
	20-19	2	1.5	0	0.0
	21-17	5	3.8	0	0.0
	21-18	4	3.0	2	4.0
	22-18	1	0.8	0	0.0
	25-17	2	1.5	0	0.0
	total	133	100.0	50	100.0

Normal vs. FXS: Chi-square = 4.51, df = 7, P = 0.72 (not significant)

Haplotype set	Combined haplotypes	Normal		FXS	
		NO.	%	NO.	%
D-AC-W-AT-1-IV-2-3	18-17-G-G-A-T-A-A	1	0.8	0	0.0
	20-17-C-G-T-C-G-C	1	0.8	0	0.0
	20-17-C-G-T-T-A-A	**30**	**22.6**	**12**	**24.0**
	20-17-G-G-A-T-A-A	**43**	**32.3**	**19**	**38.0**
	20-18-C-A-T-C-G-C	**41**	**30.8**	**16**	**32.0**
	20-18-C-G-T-C-G-C	2	1.5	1	2.0
	20-18-G-A-T-C-G-C	1	0.8	0	0.0
	20-19-C-A-T-C-G-C	1	0.8	0	0.0
	20-19-C-G-T-C-G-C	1	0.8	0	0.0
	21-17-C-G-T-T-A-A	3	2.3	0	0.0
	21-17-G-G-A-T-A-A	2	1.5	0	0.0
	21-18-C-A-T-C-G-C	4	3.0	2	4.0
	22-18-C-A-T-C-G-C	1	0.8	0	0.0
	25-17-G-G-A-T-A-A	2	1.5	0	0.0
	total	133	100.0	50	100.0

Normal vs. FXS: Chi-square = 5.44, df = 13, P = 0.96 (not significant)

Table 7. (continued)

Haplotype set	Combined haplotypes	Normal		FXS	
		NO.	%	NO.	%
AC-W-AT-1-IV-2-3	**17-G-G-A-T-A-A**	**48**	**36.1**	**19**	**38.0**
	17-C-G-T-C-G-C	1	0.8	0	0.0
	17-C-G-T-T-A-A	**33**	**24.8**	**12**	**24.0**
	18-C-A-T-C-G-C	**46**	**34.6**	**18**	**36.0**
	18-C-G-T-C-G-C	2	1.5	1	2.0
	18-G-A-T-C-G-C	1	0.8	0	0.0
	19-C-A-T-C-G-C	1	0.8	0	0.0
	19-C-G-T-C-G-C	1	0.8	0	0.0
	total	133	100.0	50	100.0

Normal vs. FXS: Chi-square = 1.63, df = 7, P = 0.98 (not significant)

Table 8. Common haplotypes among normal and FXS subjects using seven polymorphic markers (FRAXAC1-WEX5-ATL1-rs25731-IVS10-rs25702-rs25723).

Haplotype AC-W-AT-1-IV-2-3	Designated name	Normal		FXS	
		NO.	%	NO.	%
17-G-G-A-T-A-A	Hap A	48	36.1	19	38.0
17-C-G-T-T-A-A	Hap C	33	24.8	12	24.0
18-C-A-T-C-G-C	Hap B	46	34.6	18	36.0
OTHER	-	6	4.5	1	2.0
total		133	100.0	50	100.0

Normal vs. FXS: Chi-square = 0.67, df = 3, P = 0.88 (not significant)

Haplotype association

When we divided the normal cohort into 6 subgroups according to the number of CGG repeats (19-28, 29, 30, 31-35, 36 and 37-56), we found a striking and significant association between the common CGG repeats (29, 30 and 36) and haplotype (FRAXAC1-WEX5-ATL1-rs25731-IVS10-rs25702-rs25723) (P = 0.00, Table 9). The 29 CGG alleles were associated with the Hap A (27/32 or 84.4%), the 30 CGG alleles were associated with the Hap B (29/31 or 93.5%) and the 36 CGG alleles were associated with the Hap C (23/28 or 82.1%). We also found, however, these common haplotypes in the FXS chromosomes (38% of Hap A, 36% of Hap B and 24% of Hap C). We observed that there was no Hap B in the 29 and 36 CGG repeats subgroups. Also, Hap C was not present in the 30 CGG repeats subgroup. Only one chromosome with Hap A was observed in the 30 CGG repeats subgroup. Interestingly, the majorities of large CGG repeats (36-56) were related to Hap A and Hap C (32/35 or 91.4%), while the other subgroups of the smaller CGG alleles (19-28 and 31-35) had only 43.8% (7/16) and 54.5% (12/22) of Hap A and Hap C, respectively. Nevertheless, no association of Hap A and Hap C with the FXS groups (31/50 or 62%) was found.

Table 9. Haplotype associations in subgroups of normal CGG repeats. (AC = FRAXAC1, W = WEX5, AT = ATL1, 1 = rs25731, IV = IVS10, 2 = rs25702, 3 = rs25723)

Haplotype (Hap)	19-28		29		30		31-35		36		37-56		FXS	
AC-W-AT-1-IV-2-3	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
17-G-G-A-T-A-A (A)	5	31.3	27	84.4	1	3.2	5	38.5	5	17.9	5	38.5	19	38.0
17-C-G-T-T-A-A (C)	2	12.5	2	6.3	0	0.0	1	7.7	23	82.1	5	38.5	12	24.0
18-C-A-T-C-G-C (B)	8	50.0	0	0.0	29	93.5	6	46.2	0	0.0	3	23.1	18	36.0
OTHER	1	6.3	3	9.4	1	3.2	1	7.7	0	0.0	0	0.0	1	2.0
total	16	100.0	32	100.0	31	100.0	13	100.0	28	100.0	13	100.0	50	100.0

29 vs 30: Chi-square = 56.14, df = 3, P = 0.00 (significant)

29 vs 36: Chi-square = 36.03, df = 2, P = 0.00 (significant)

29 vs FXS: Chi-square = 24.78, df = 3, P = 0.00 (significant)

30 vs 36: Chi-square = 55.66, df = 3, P = 0.00 (significant)

30 vs FXS: Chi-square = 27.85, df = 3, P = 0.00 (significant)

36 vs FXS: Chi-square = 26.53, df = 3, P = 0.00 (significant)

AGG Interruption

The results from the sequencing analysis in each subgroup of normal chromosomes revealed some variety in both the number of AGGs and the AGG substructures within the FMR1 CGG alleles (Figure 5 and Table 10). Most normal alleles had two interspersed AGG triplets (49/95 or 51.6%). Alleles with a single or more (3-4) AGG interspersions had similar frequencies of 20% (19/95) and 24.2% (23/95), respectively. Alleles devoid of AGG were rare in this study, accounting for only 4.2% (4/95). The position of the first AGG interruption varied, but in 91.2% (83/91) of normal alleles having one or more AGGs it punctuated at the tenth (9A) or eleventh (10A) position within the repeat. The most common CGG lengths between the first and second AGG for alleles with two or more AGG interruptions were nine or ten CGG repeat units (63/72 or 87.5%). Thus, the general pattern for the most normal alleles was two AGGs intervening every 9 or 10 CGG repeats with repeat length variability at the 3' end. Strikingly, the majority of the common CGG alleles 29, 30 and 36, had the specific AGG organization of 9A9A9 (15/17 or 88.2%), 10A9A9 (16/18 or 88.9%) and 9A9A6A9 (15/18 or 83.3%), respectively, whereas the uncommon CGG alleles (19-28, 31-35 and 37-56) presented various AGG interspersion patterns corresponding to their CGG lengths. We also observed an allele possessing a 5' tract with 20 CGG repeats (20A9; 1/95 or 1.1%) and alleles with a 3' pure CGG tract (9/10An, where $n \geq 20$; 9/95 or 9.5%).

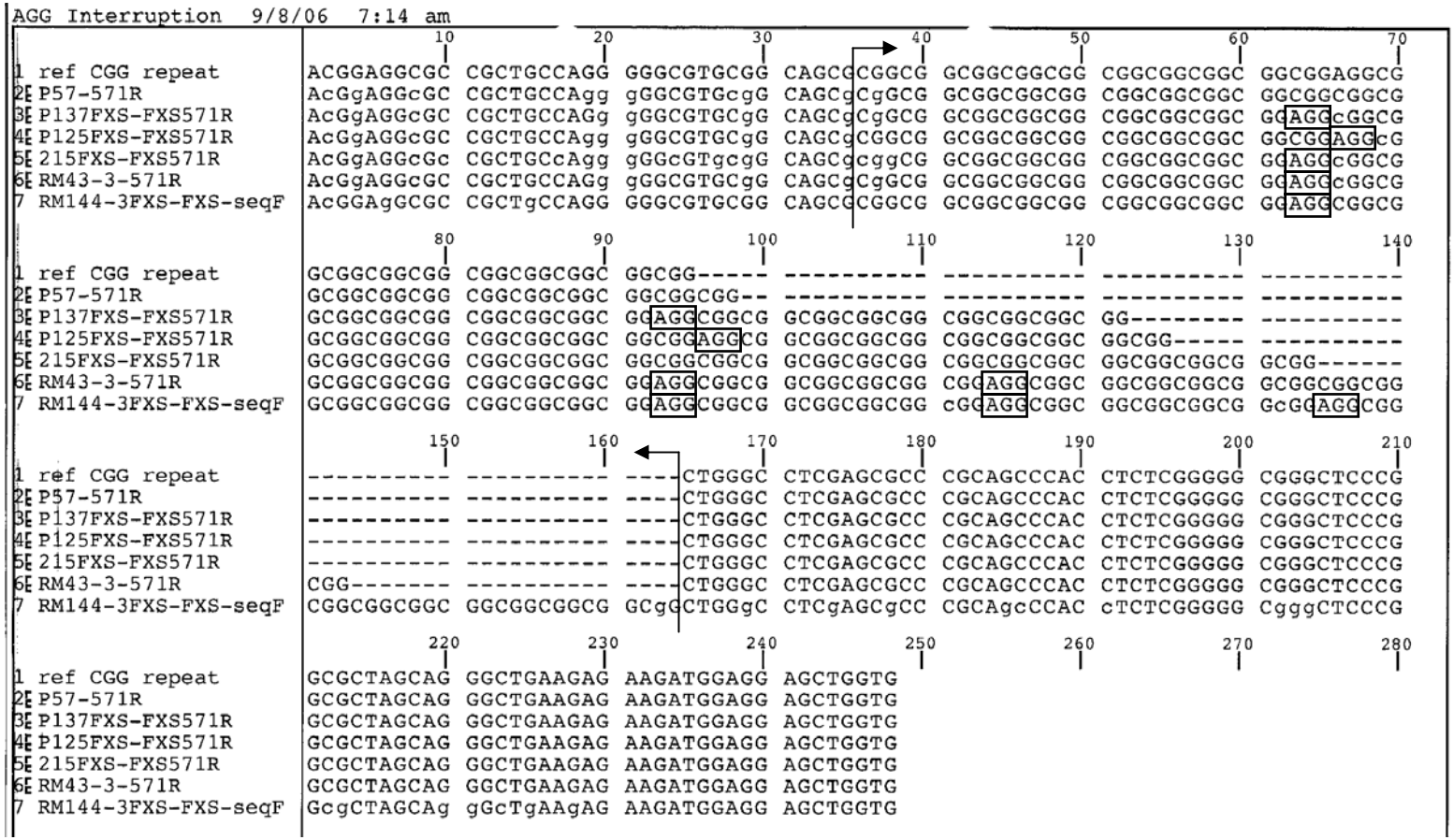

Figure 5. The alignment of CGG repeat regions resulting from the Sequence Navigator Software. The sequences of samples (P57, P137, P125, 215, RM43-3 and RM144-3) which shown different CGG repeat numbers (21, 29, 30, 33, 36 and 43 CGG repeats, respectively) are aligned with the reference sequences (ref CGG repeat) which were downloaded from GenBank (Accession number: L29074). The boundary of the CGG repeat regions are indicated with fishhook vertical arrows. The AGG triplet is squared.

Table 10. AGG Interruption patterns in each subgroup of control samples.

AGG Pattern	19-28 (%)	29 (%)	30 (%)	31-35 (%)	36 (%)	37-56 (%)
9A9	1 (6.3)					
9A13	2 (12.5)					
9A20			1 (5.6)			
9A21				1 (7.7)		
9A23				1 (7.7)		
9A25				1 (7.7)		
9A29						1 (7.7)
10A9	2 (12.5)					
10A23				2 (15.4)		
10A27						1 (7.7)
10A39						1 (7.7)
11A12	1 (6.3)					
12A9	3 (18.8)					
20A9			1 (5.6)			
9A9A9		15 (88.2)				
9A9A12				2 (15.4)		
9A9A15				1 (7.7)		
9A9A16					1 (5.6)	
10A6A9	2 (12.5)					
10A7A9	1 (6.3)					
10A9A3	1 (6.3)					
10A9A5	1 (6.3)					
10A9A9			16 (88.9)			
10A9A11				1 (7.7)		
10A12A9				1 (7.7)		
11A9A9				3 (23.1)		
12A6A9		2 (11.8)				
19A6A9					2 (11.1)	
9A9A6A9					15 (83.3)	
9A9A7A9						1 (7.7)
9A9A9A9						1 (7.7)
9A11A9A9						1 (7.7)
9A9A6A6A9						4 (30.8)
9A9A6A8A9						1 (7.7)

Table 10. (continued)

AGG Pattern	19-28 (%)	29 (%)	30 (%)	31-35 (%)	36 (%)	37-56 (%)
21	2 (12.5)					
43						1 (7.7)
56						1 (7.7)
Total (95)	16 (100.0)	17 (100.0)	18 (100.0)	13 (100.0)	18 (100.0)	13 (100.0)

Note: The position of an AGG is designated by A and the number refers to the triplet length of uninterrupted CGG repeats.

Relationship among CGG repeat numbers, haplotypes and AGG interruption patterns

When we analyzed all factors known to be responsible for repeat instability (CGG numbers, haplotypes and AGG interspersion patterns), we surprisingly found a strong association among the common CGG lengths (29, 30 and 36), the associated haplotypes (Hap A, Hap B and Hap C) and the specific AGG interspersion patterns (Figure 6 and Appendix A, Table 18). The 29 CGG repeats with Hap A showed the AGG pattern of 9A9A9 (12/17 or 70.6%), the 30 CGG repeats with Hap B showed the AGG pattern of 10A9A9 (16/18 or 88.9%) and the 36 CGG repeats with Hap C showed the AGG pattern of 9A9A6A9 (13/18 or 72.2%).

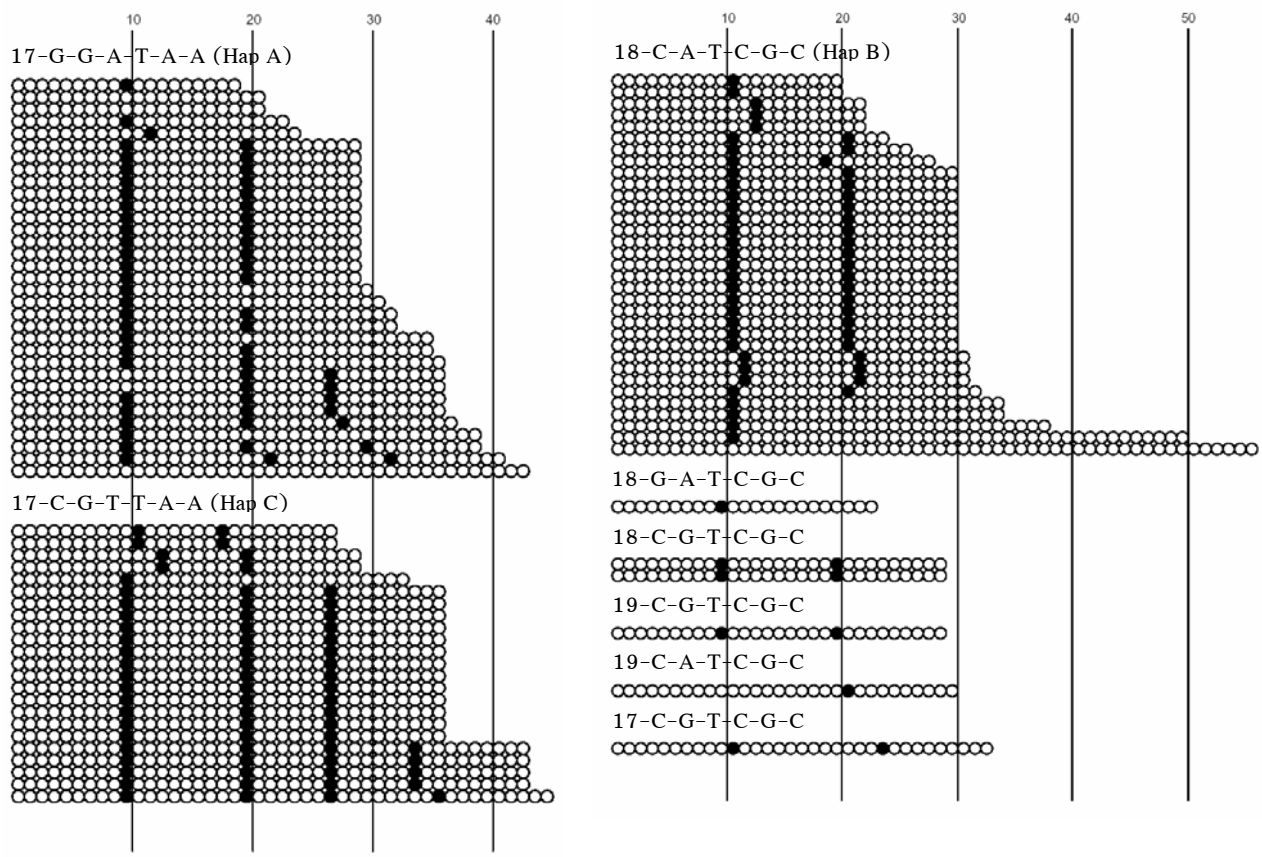

Figure 6. AGG interspersion patterns of 95 CGG alleles. The alleles are classified according to the associated FRAXAC1-WEX5-ATL1-rs25731-IVS10-rs25702-rs25723 haplotype. The AGG interspersion patterns are shown from the 5' to the 3' end. White circles represent CGG triplets and black circles represent AGG triplets. The numbers of triplets are indicated by the vertical lines.

Preliminary testing of DNA samples in affected families

We established the haplotype of seven markers (FRAXAC1-WEX5-ATL1-rs25731-IVS10-rs25702-rs25723) from each member of the affected families (Figure 7.1-7.2). Haplotype analysis of these members presented unsurprising results because common haplotype backgrounds (Hap A and Hap B) were found in each sample. The occurrence of recombination transmitted by maternal lineage was not observed in these affected families over the studied generations.

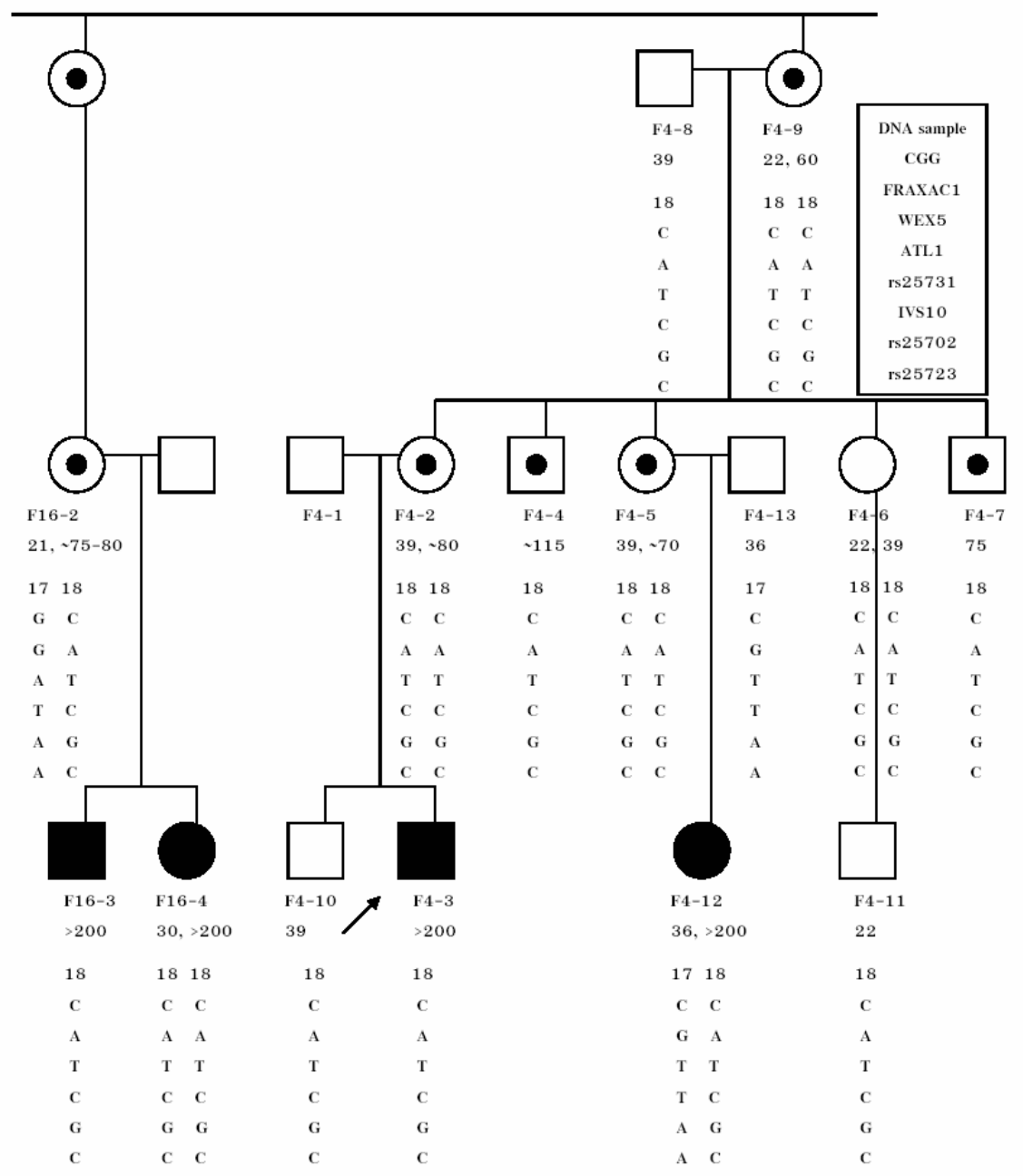

Figure 7.1. The pedigree of affected family 4 and 16. The number of CGG repeats and the results of haplotype analysis are indicated below each sample. This family had 3 generations and the inheritance of unstable chromosomes was through premutation carrier females.

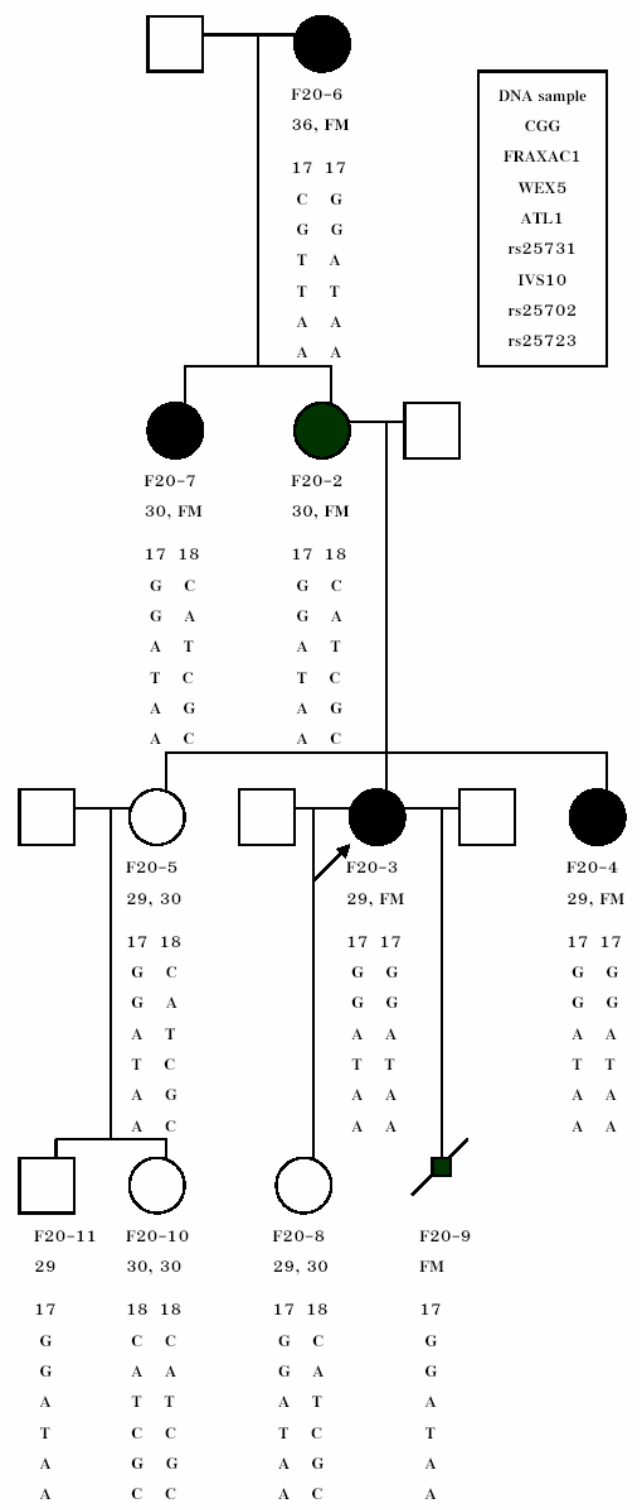

Figure 7.2. The pedigree of affected family 20. The number of CGG repeats and the results of haplotype analysis are indicated below each sample. There were 4 generations and the affected females passed the FXS mutation through the next successions.

Verification of microsatellite and SNP alleles

We confirmed the genotyping results of all polymorphic markers by sequencing in selected male samples. These sequencing results are shown in the right panel (square), and can be seen to be consistent with the genotyping alleles on the left panel obtained by PCR techniques as depicted in Figures 8.1-8.7 (DXS548 not analyzed).

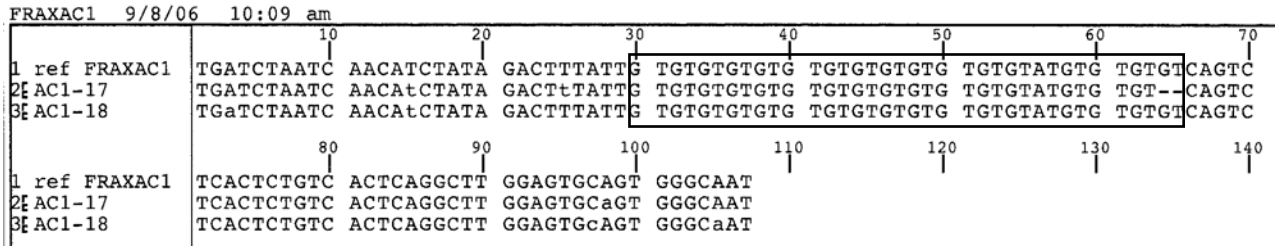

Figure 8.1. The alignment of AC repeat regions for FRAXAC1 using Sequence Navigator Software. The sequences of samples (AC1-17 and AC1-18) which shown different AC repeat numbers (17 and 18 AC repeats, respectively) are aligned with the reference sequences (ref FRAXAC1) which were downloaded from GenBank (Accession number: L29074). The boundary of the AC repeat regions is squared (seen as GT in the opposite strand).

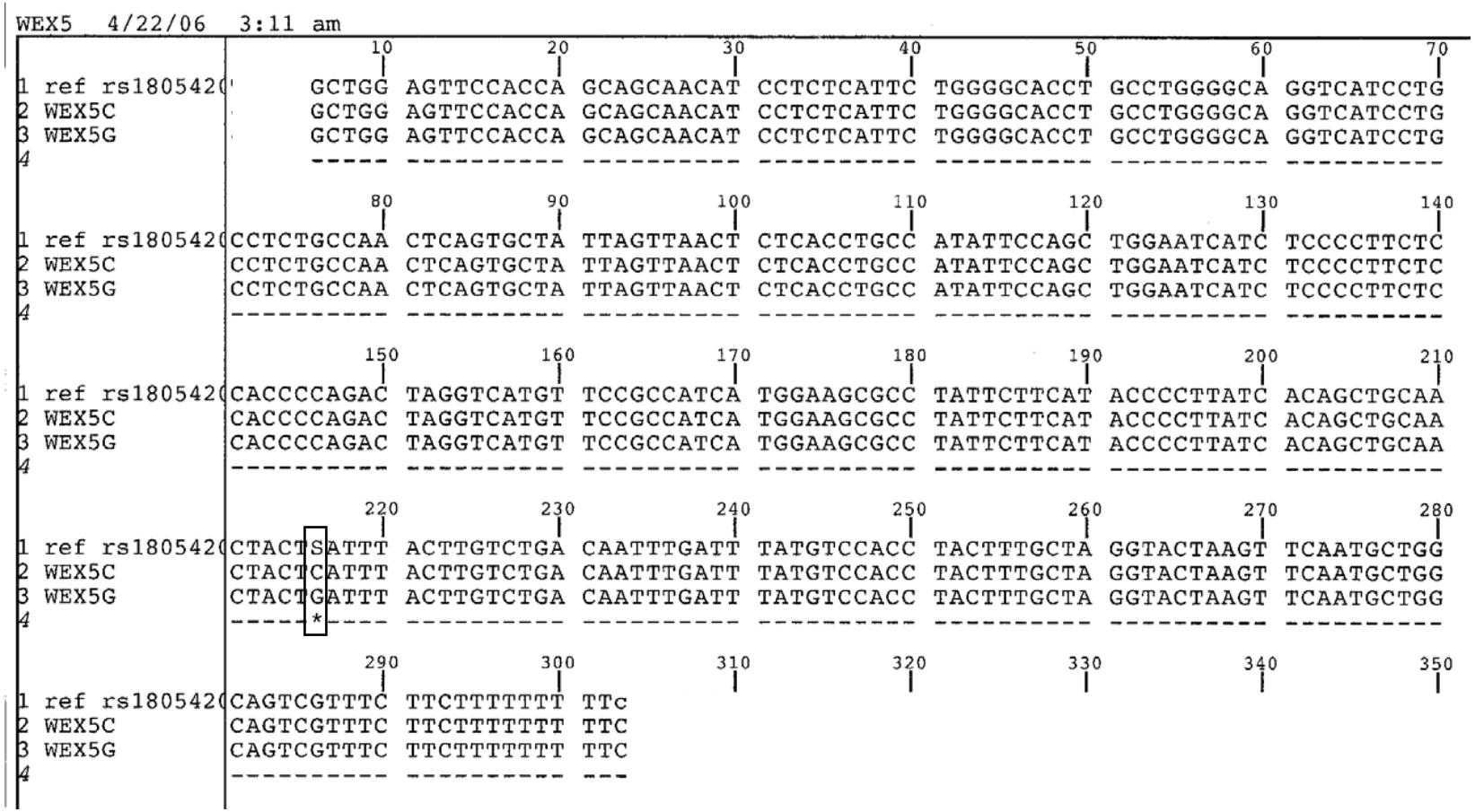

Figure 8.2. The alignment of WEX5 using Sequence Navigator Software. The sequences of samples (WEX5C and WEX5G) which shown different genotype (C and G) are aligned with the reference sequences (ref rs1805420) which were downloaded from the dbSNP of the NCBI. The asterisk (*) indicates two distinct alleles at the SNP site (the IUB code on the reference sequences). The dash (-) indicates the consensus of all nucleotides at that position.

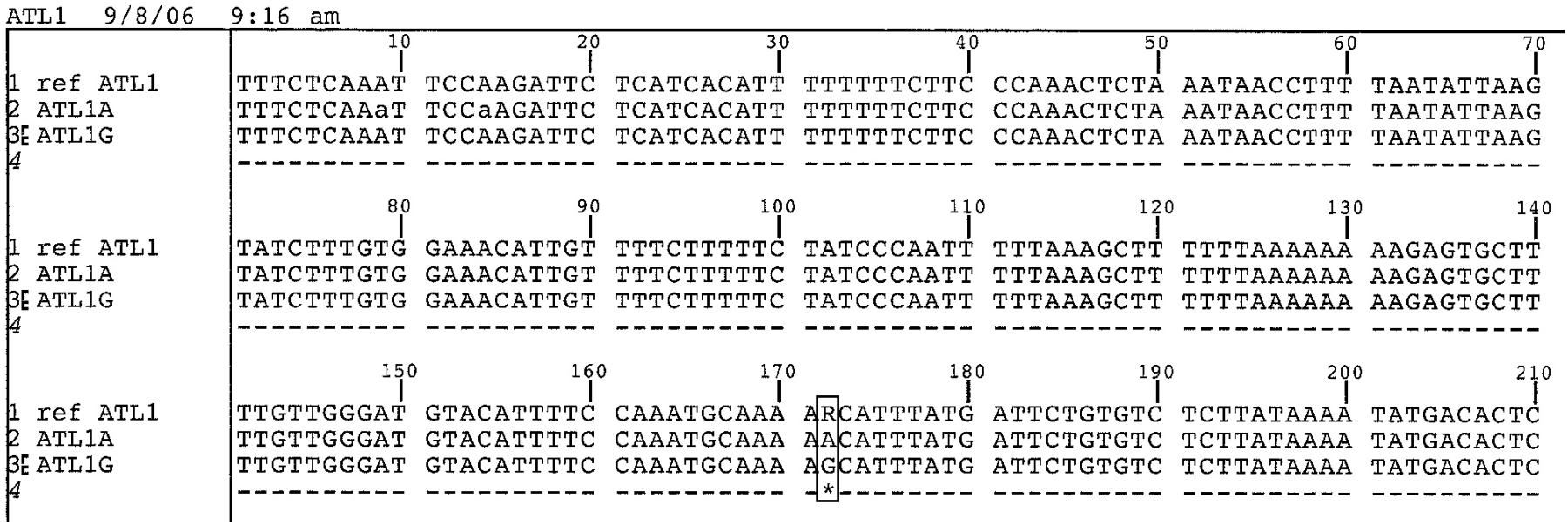

Figure 8.3. The alignment of ATL1 using Sequence Navigator Software. The sequences of samples (ATL1A and ATL1G) which shown different genotype (A and G) are aligned with the reference sequences (ref ATL1) which were downloaded from the dbSNP of the NCBI. The asterisk (*) indicates two distinct alleles at the SNP site (the IUB code on the reference sequences). The dash (-) indicates the consensus of all nucleotides at that position.

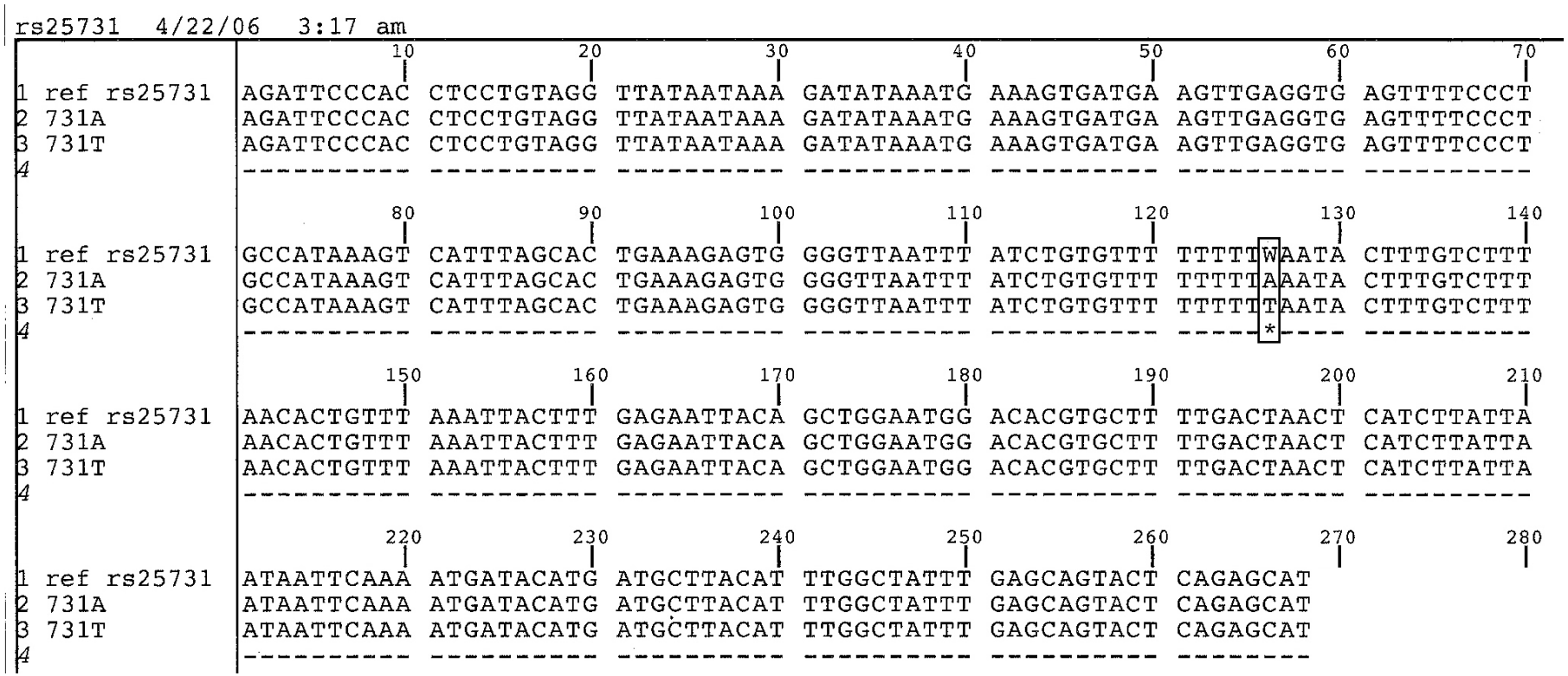

Figure 8.4. The alignment of rs25731 using Sequence Navigator Software. The sequences of samples (731A and 731T) which shown different genotype (A and T) are aligned with the reference sequences (ref rs25731) which were downloaded from the dbSNP of the NCBI. The asterisk (*) indicates two distinct alleles at the SNP site (the IUB code on the reference sequences). The dash (-) indicates the consensus of all nucleotides at that position.

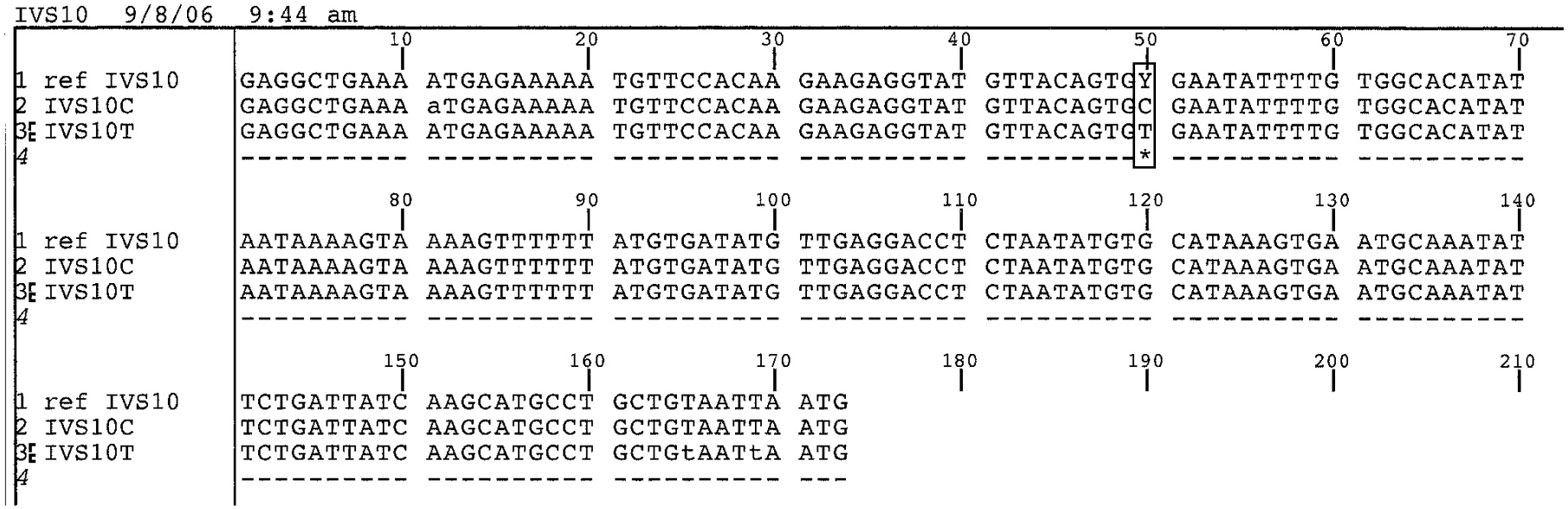

Figure 8.5. The alignment of IVS10 using Sequence Navigator Software. The sequences of samples (IVS10C and IVS10T) which shown different genotype (C and T) are aligned with the reference sequences (ref IVS10) which were downloaded from the dbSNP of the NCBI. The asterisk (*) indicates two distinct alleles at the SNP site (the IUB code on the reference sequences). The dash (-) indicates the consensus of all nucleotides at that position.

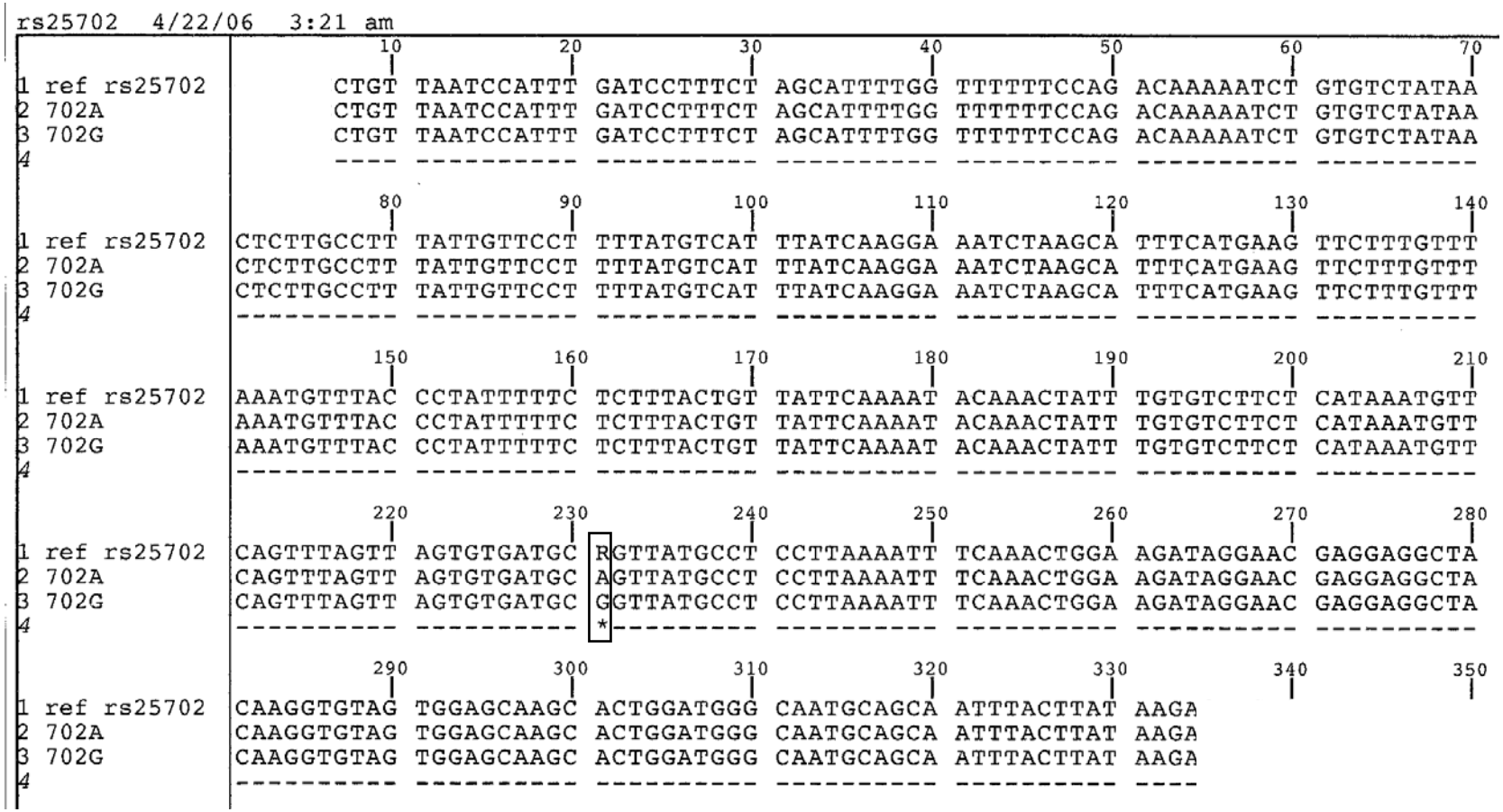

Figure 8.6. The alignment of rs25702 using Sequence Navigator Software. The sequences of samples (702A and 702G) which shown different genotype (A and G) are aligned with the reference sequences (ref rs25702) which were downloaded from the dbSNP of the NCBI. The asterisk (*) indicates two distinct alleles at the SNP site (the IUB code on the reference sequences). The dash (-) indicates the consensus of all nucleotides at that position.

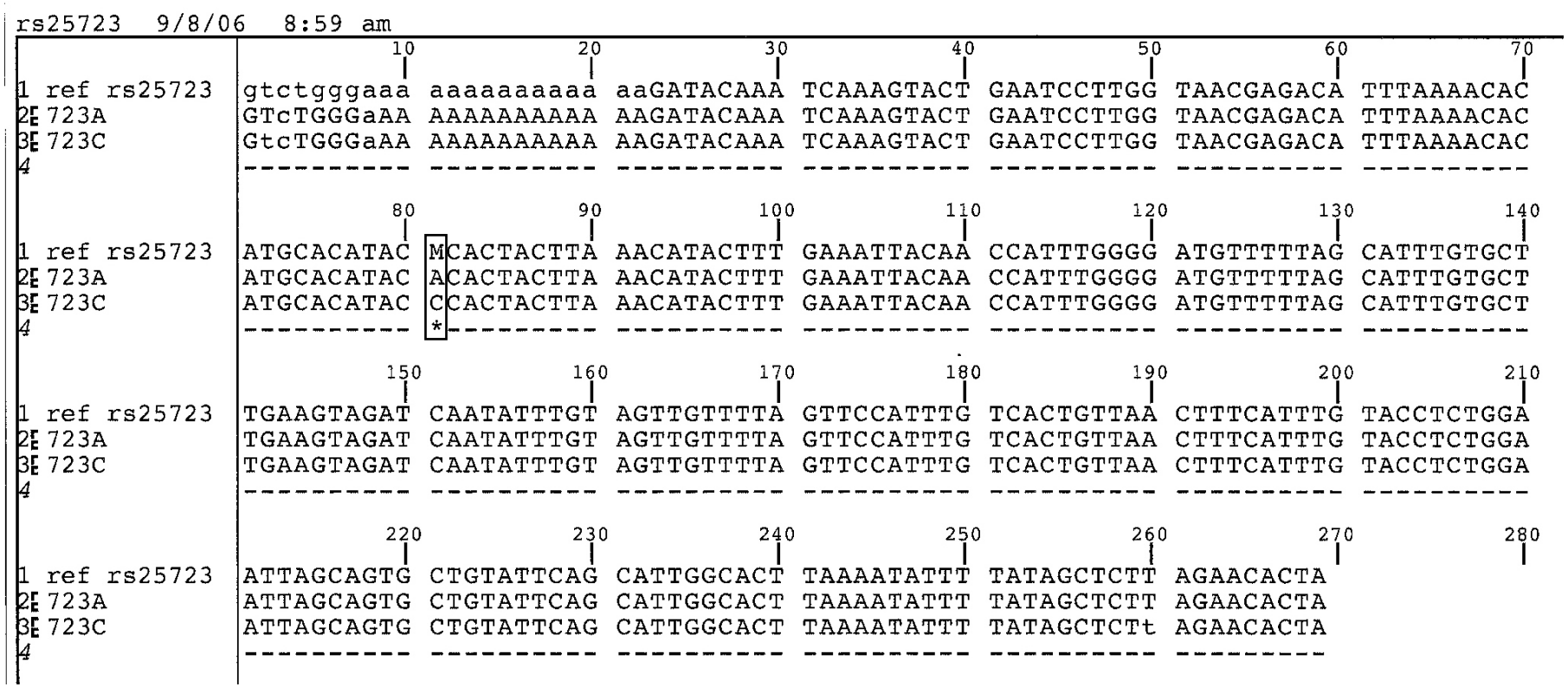

Figure 8.7. The alignment of rs25723 using Sequence Navigator Software. The sequences of samples (723A and 723C) which shown different genotype (A and C) are aligned with the reference sequences (ref rs25723) which were downloaded from the dbSNP of the NCBI. The asterisk (*) indicates two distinct alleles at the SNP site (the IUB code on the reference sequences). The dash (-) indicates the consensus of all nucleotides at that position.