CHAPTER FOUR

RESULTS

4.1 Biochemical presentations of FH patients

A total of 74 FH patients from 39 different families were identified according to Simon Broome criteria. The FH patient and NC subjects recruited were age and gender matched. Sixty-one (82.4 %) patients were categorised as definite FH and thirteen (17.6 %) were identified as possible FH. Out of 74 patients, 28 (37.8 %) were male patients while 46 (62.2 %) were female patients of Malay and Chinese ethnic groups (67.6 % and 32.4 %) respectively. The lipid profile of FH patients showed significantly higher mean TC, TG, and LDL levels of 8.6 ± 1.7, 2.0 ± 1.8, and 6.4 ± 1.7 mmol/L compared to NC subjects of 5.2 ± 0.9, 1.1 ± 0.5 and 3.2 ± 0.8 mmol/L ($p<0.001$) respectively. Significantly higher mean HDL levels were found in NC subjects compared to FH patients (1.4 ± 0.3 vs 1.3 ± 0.4 mmol/L; $p<0.001$). FH patients were found to have significantly higher waist to hip ratio compared to NC subjects (0.84 ± 0.07 vs 0.81 ± 0.06; $p<0.05$). There were no significant differences in smoking status, body mass index (BMI), systolic and diastolic blood pressure of the FH patients compared to NC subjects. The biochemical presentations of FH patients were tabulated in Table 4.1.1.

Table 4.1.1: Biochemical presentations of FH patients and normocholesterolaemic (NC) subjects.

Baseline Characteristics	FH (n=74)	NC (n = 77)	p value
Age (years)[a]	45.9 ± 12.0	46.3 ± 10.3	NS
Gender (%)[b] (Male /Female)	28 / 46 (37.8% / 62.2%)	26 / 51 (33.8% / 66.2%)	NS
Race (%)[c] Malay/Chinese/Indian	67.6% /32.4%/0%	89.9% / 10.1%/0%	p<0.05
Current smoker (%)[c]	14.5%	17.4%	NS
Systolic BP [a]	136.6 ± 22.3	133.2 ± 16.4	NS
Diastolic BP [a]	77.8 ± 11.1	76.7 ± 10.8	NS
BMI (kg/m^2) [a]	24.3 ± 4.6	23.0 ± 4.6	NS
Waist-hip ratio (WHR) [a]	0.84 ± 0.07	0.81 ± 0.06	p<0.05
Total Cholesterol (mmol/L) [a]	8.6 ± 1.7	5.2 ± 0.9	p<0.001
Triglycerides (mmol/L) [a]	2.0 ± 1.8	1.1 ± 0.5	p<0.001
LDL-cholesterol (mmol/L) [a]	6.4 ± 1.7	3.2 ± 0.8	p<0.001
HDL-cholesterol (mmol/L) [a]	1.3 ± 0.4	1.4 ± 0.3	p<0.001

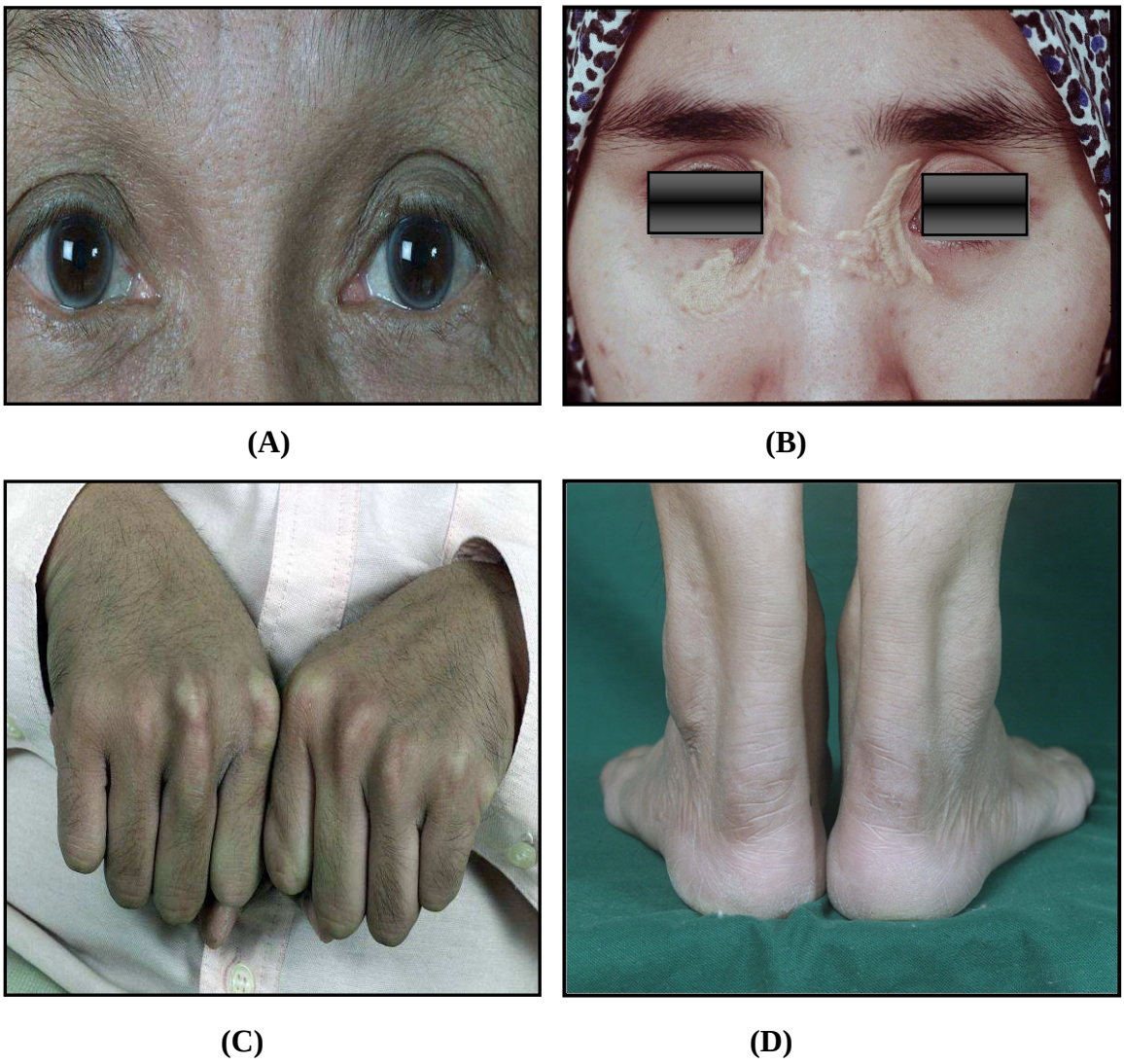
[a]Data expressed as mean ± SD; [b]Data expressed as proportion & percentage;

[c]Data expressed as percentage, NS = not significant.

BMI = Body mass index, BP = Blood pressure

4.2 Clinical Presentation of FH Patients

Clinical presentations of FH patients recruited as shown in Figure 4.2.1 (A to D)



(A) **(B)**

(C) **(D)**

Figure 4.2.1: Clinical presentations of FH patients showing:

(A) - Corneal Arcus

(B) - Xanthelasma

(C) - Xanthomata of the dorsum of hand

(D) - Xanthomata of Archilles tendon

4.3 DNA Extraction

Seventy four FH and 77 NC samples were extracted using commercial extraction kits (Maxwell Promega Blood Extraction Kit) to obtain the genomic DNA (gDNA). The concentration and purity of extracted DNA samples were measured spectrophotometrically using Nanodrop ND-1000 (Thermo Fisher Scientific). The gDNA purity and concentration obtained from a FH patient sample was shown in Figure 4.3.1. The extracted gDNA of obtained from 74 FH samples showed high purity and concentrations. The purity of the gDNA samples were within the range of 1.62 until 2.20 while the concentrations ranges from 3.2 ng/µl to 208.9 ng/µl. The concentration and purity of gDNA were shown in Table 4.1.2.

Table 4.1.2: Concentration and purity of genomic DNA obtained.

No	Patient ID	Concentration (ng/µl)	Purity (ratio A260/A280)	No	Patient ID	Concentration (ng/µl)	Purity (ratio A260/A280)
1	ZM	27.9	1.82	38	TTM	72.9	1.80
2	SFM	35.8	1.84	39	STS	5.6	1.48
3	PLH	24.5	1.94	40	MN	27.3	1.85
4	LKH	19.1	1.94	41	KM	52.8	1.79
5	MNI	7.3	1.83	42	SH	11.2	1.62
6	AK	61.8	1.91	43	YNL	6.5	1.73
7	AA	26.9	1.81	44	IM	70.9	1.82
8	WC	5.7	2.00	45	HMY	80.1	1.84
9	FK	25.0	1.84	46	CCM	40.2	1.92
10	WF	29.4	1.79	47	SA	80.9	1.86
11	MKA	37.7	1.84	48	NMR	95.7	1.78
12	NAH	29.7	1.86	49	SPL	35.7	1.93
13	NKH	6.4	2.00	50	NAZ	4.1	1.77
14	RMM	22.4	2.00	51	ANAS	29.4	1.84
15	JAK	13.2	1.99	52	NFZ	40.9	1.77
16	HAR	3.2	2.05	53	TCH	16.2	1.78
17	CSL	18.3	1.80	54	KAL	4.5	1.33
18	GSC	12.3	2.02	55	NPH	6.5	1.73
19	NKF	86.1	1.89	56	FSK10	17.6	2.18
20	TL	16.3	1.97	57	RI	158.9	1.85
21	PCM	23.1	1.88	58	HWAR	7.3	1.97
22	ZH	26.1	1.93	59	ASAR	208.9	1.84
23	RB	3.2	1.87	60	MSR	8.5	1.74
24	RWM	13.1	1.79	61	ANSA	193.3	1.87
25	KK	33.6	1.86	62	MH	35.3	1.91
26	NHM	131.1	1.83	63	CWK	30.8	1.85
27	LAH	18.4	1.80	64	CPL	7.0	1.69
28	FYC	19.8	1.77	65	SS	27.1	1.84
29	FNE	21.6	1.96	66	MSS	43.4	1.84
30	SZO	3.75	1.67	67	KCL	4.0	1.90
31	KCL	40.3	1.90	68	CSH	21.6	1.83
32	ARH	12.2	1.77	69	AZ	86.7	1.82
33	SPM	56.7	1.95	70	MI	3.7	1.94
34	NH	30.0	1.99	71	MYJ	3.2	1.94
35	SM	3.3	1.23	72	ZT	12.2	2.20
36	MK	16.0	1.50	73	MM	7.5	1.95
37	ZMD	43.5	1.83	74	MIY	34.3	1.80

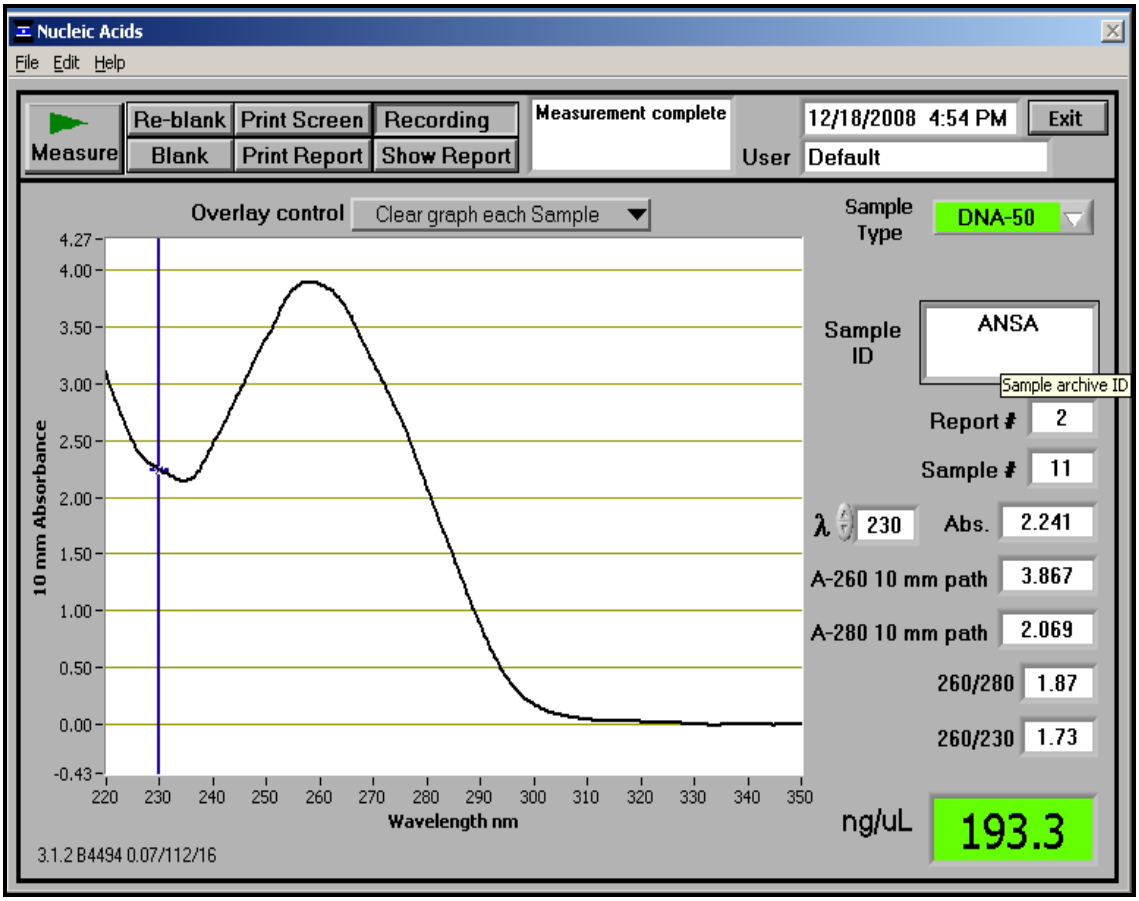


Figure 4.3.1: Genomic DNA quantitation using spectrophotometer Nanodrop ND-1000 showed the purity and the concentration of extracted DNA in ng/µL. This FH patient sample showed good purity of 1.87 and high concentration of extracted genomic DNA of 193.3 ng/µl.

4.4 PCR results

Amplification of promoter region and all exons of LDLR gene were performed on 74 FH patients and 77 NC subjects with the presence of negative controls. The PCR products obtained from the PCR reactions were detected using on-chip gel electrophoresis. LDLR promoter region amplification showed distinctive bands for FH and NC samples lanes and was detected at 275 bp, whereas no band was detected at the negative control lane (Figure 4.4.1).

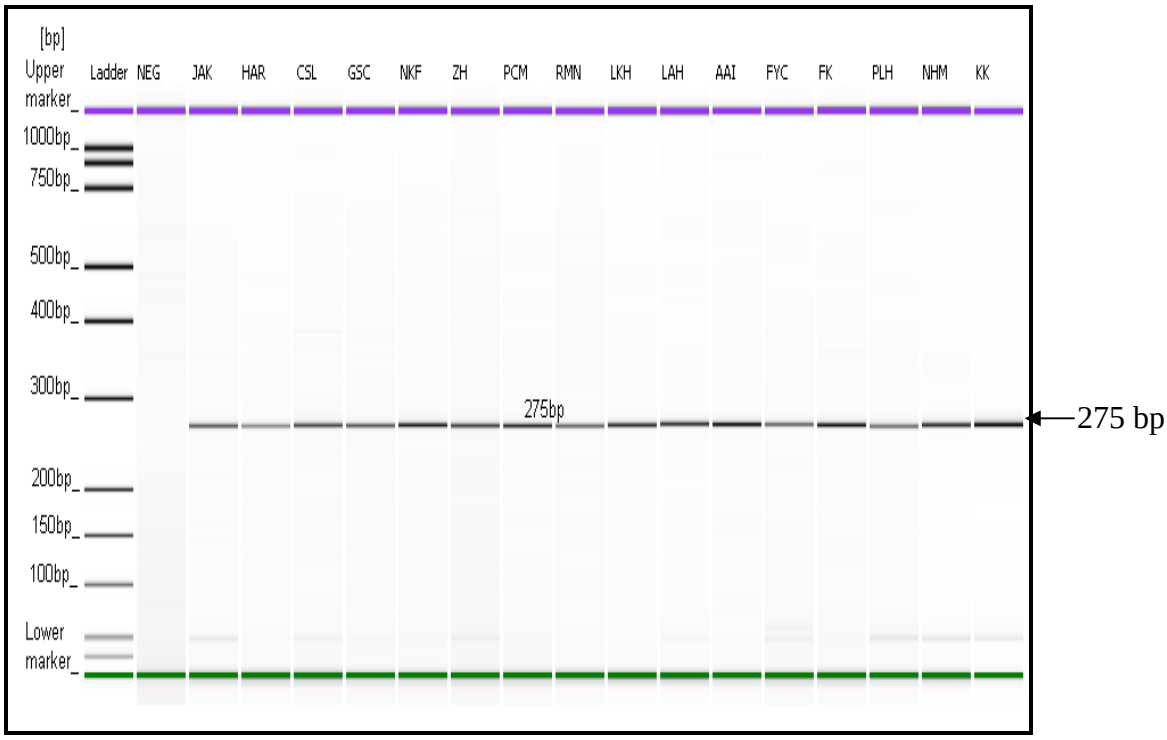


Figure 4.4.1: Amplification of promoter region of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patients samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 275 bp.

Amplification of exon 1, exon 2 and exon 3 of LDLR gene showed distinctive bands at 244 bp, 189 bp and 201 bp for FH and NC samples lanes respectively with no band was detected on the negative control lane (Figures 4.4.2 – 4.4.4).

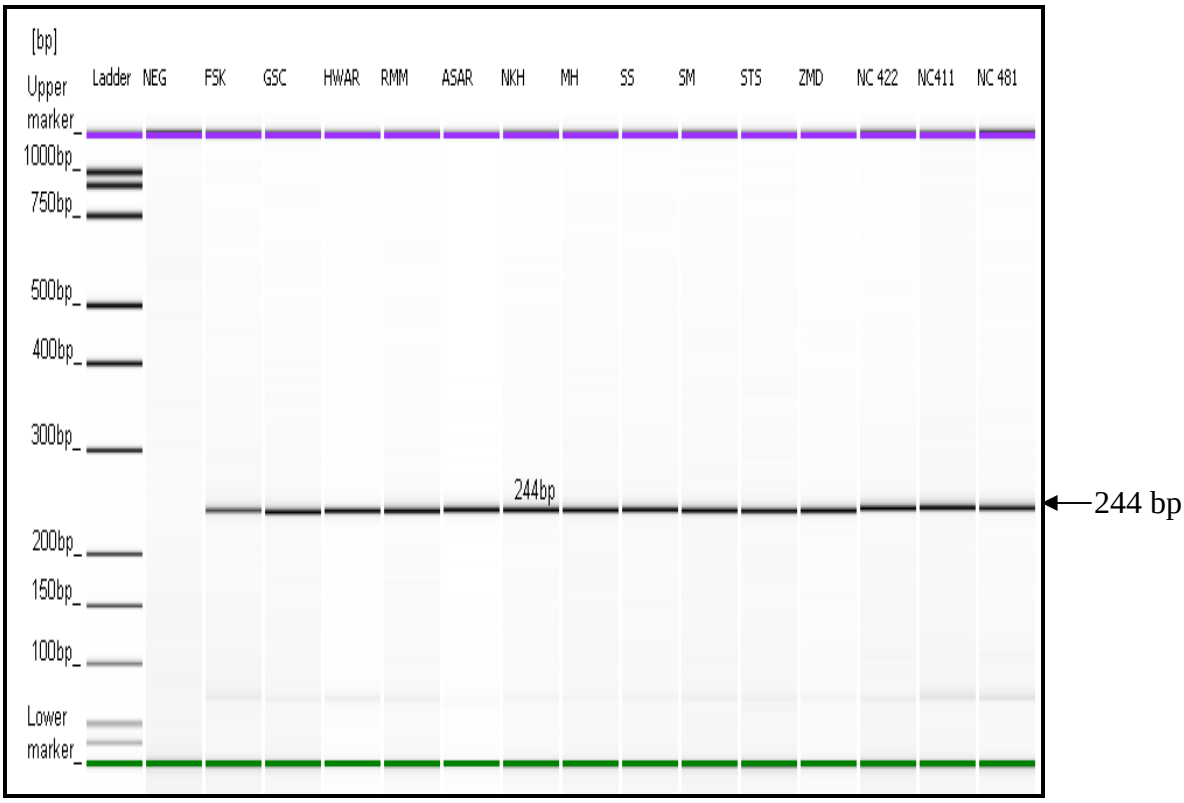


Figure 4.4.2: Amplification of Exon 1 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample, patient samples and normal control samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 244 bp.

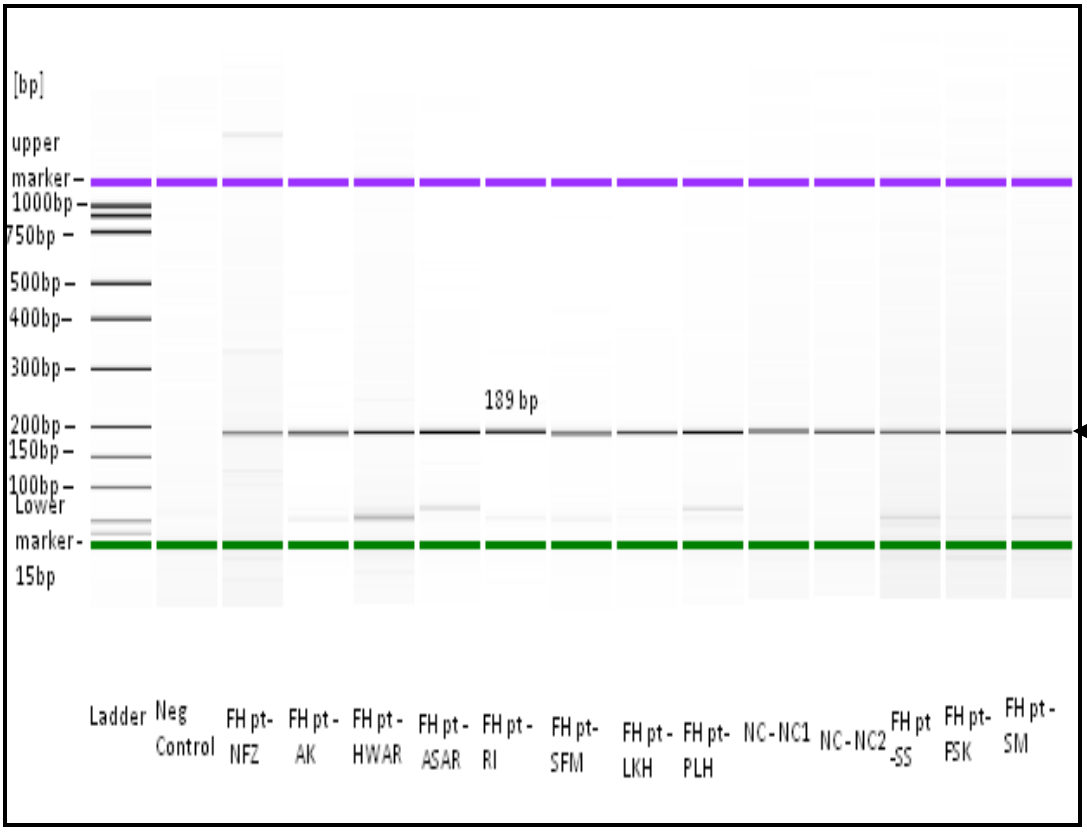


Figure 4.4.3: Amplification of Exon 2 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample, FH patient samples and normal control samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 189 bp.

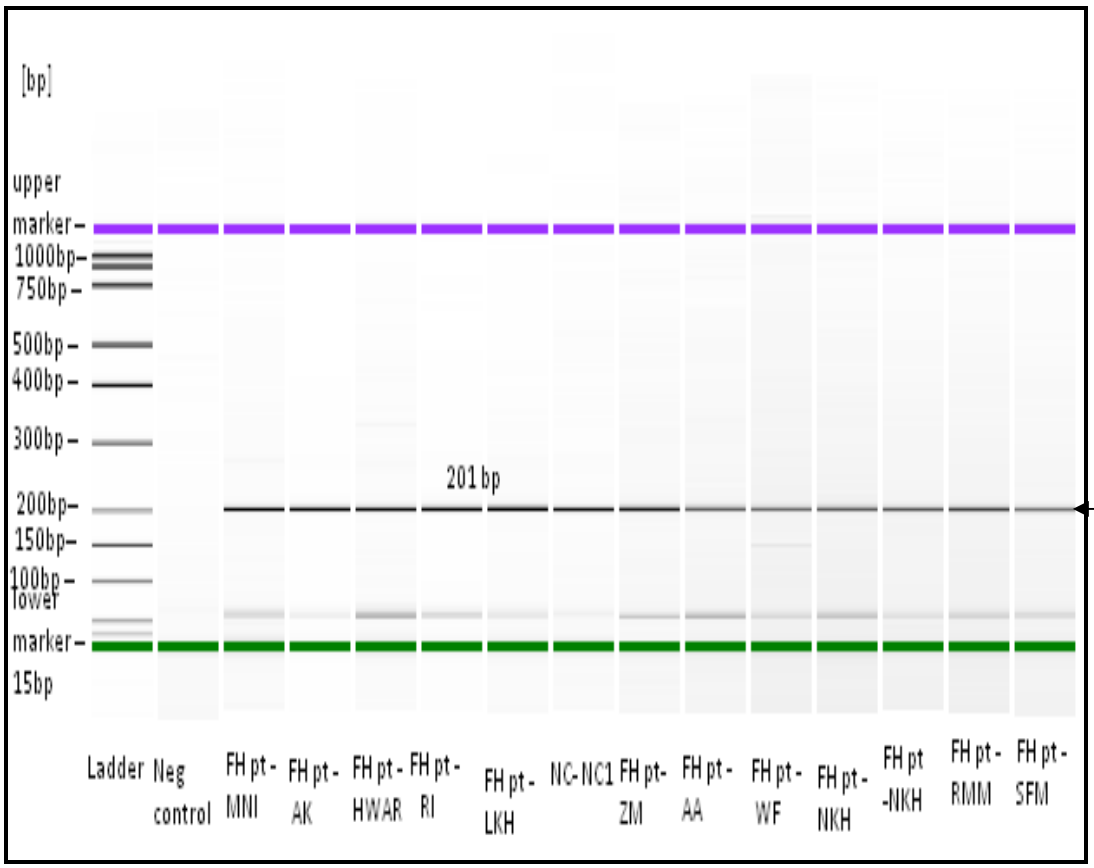


Figure 4.4.4: Amplification of Exon 3 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 201 bp.

The amplification of exon 4 using 3 sets of primers obtained distinctive bands at 187 bp, 186 bp and 240 bp respectively, for both samples of study subjects with no band was obtained on the negative control lane (Figures 4.4.5 – 4.4.7).

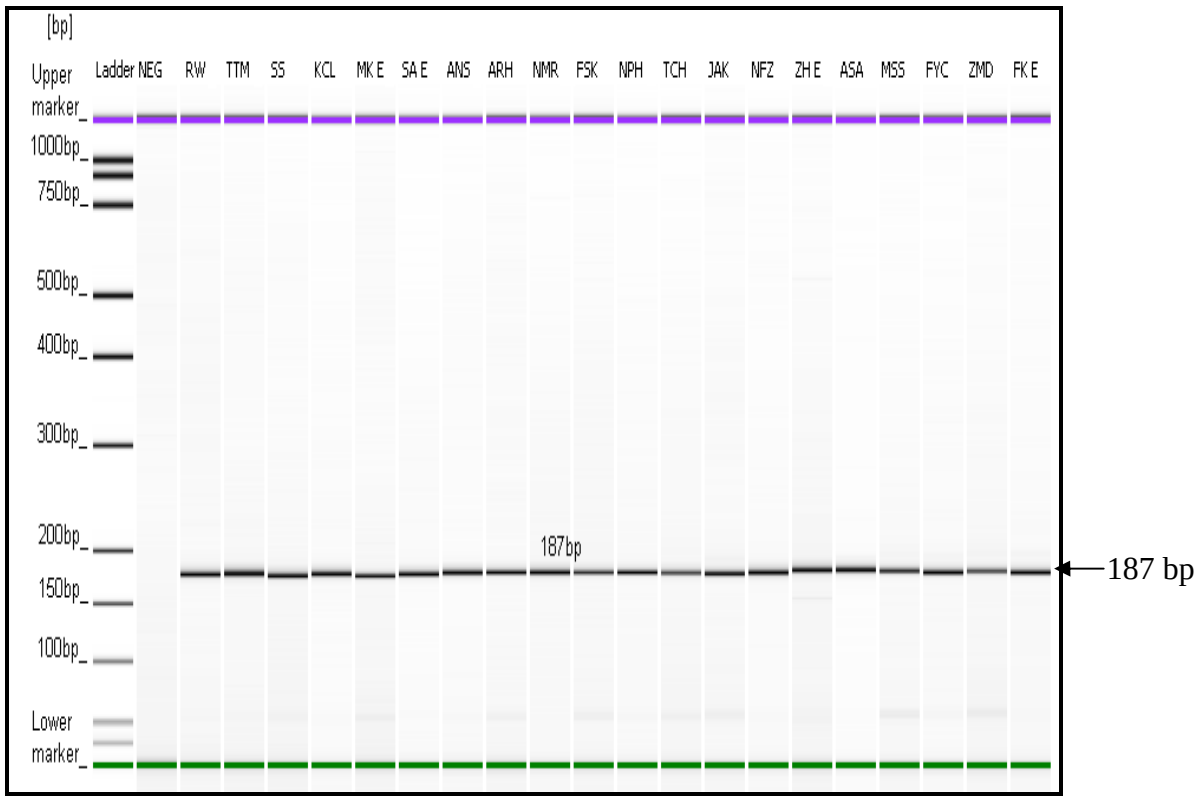


Figure 4.4.5: Amplification of Exon 4-5' of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 187 bp.

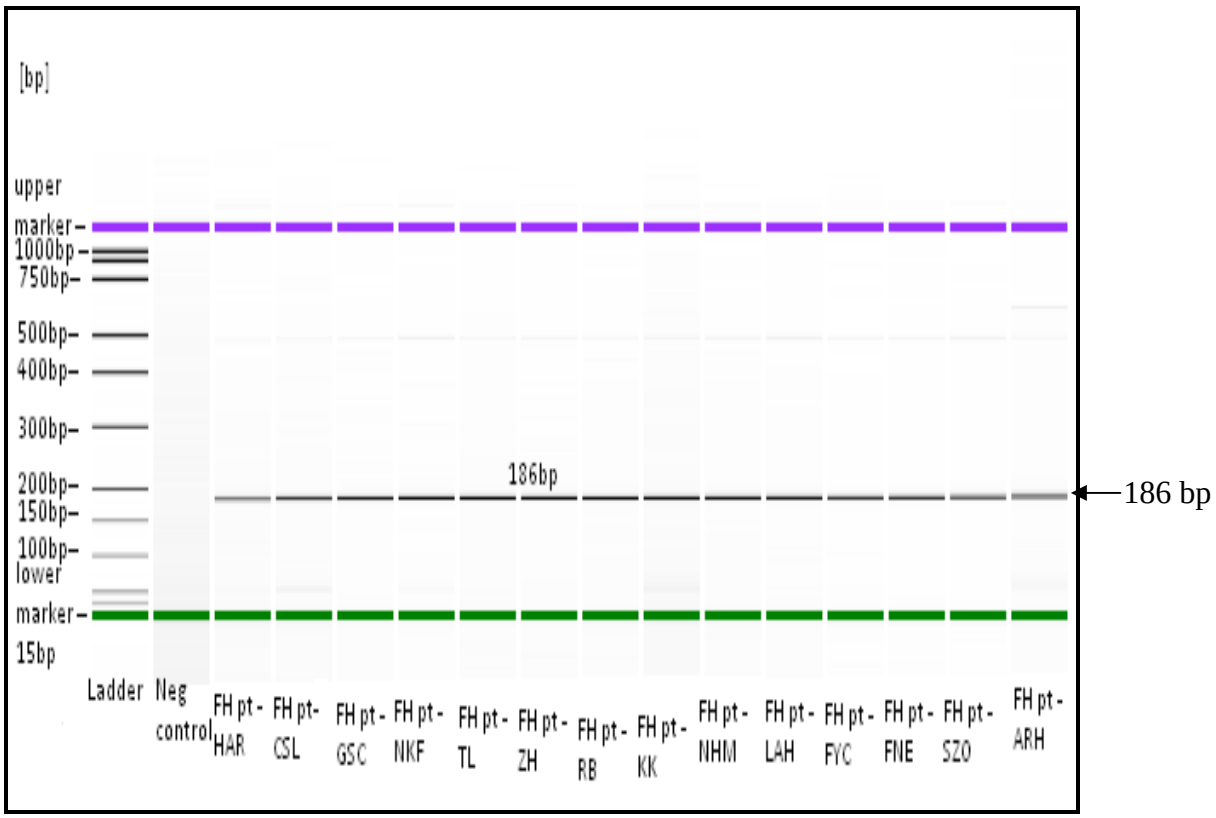


Figure 4.4.6: Amplification of Exon 4-mid of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 186 bp.

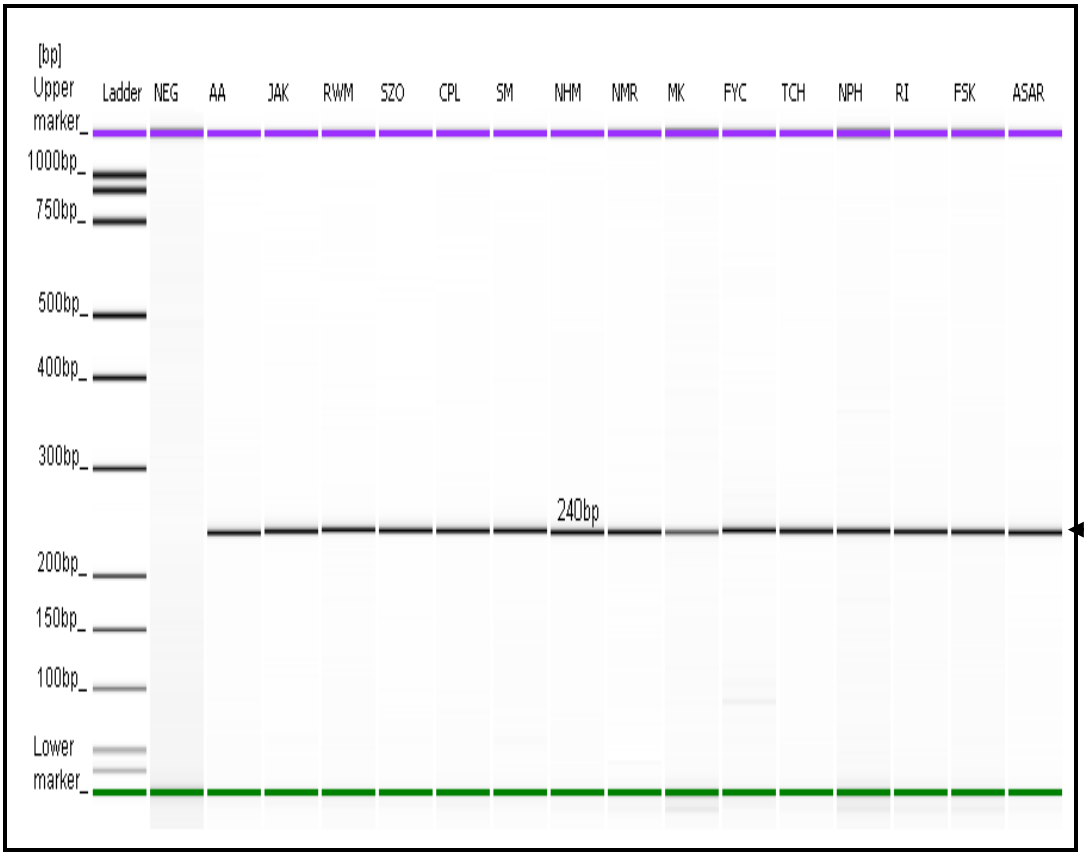


Figure 4.4.7: Amplification of Exon 4-3' of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 240 bp.

The PCR products for exon 5 to exon 9 were detected at 182 bp, 180 bp, 242 bp, 206 bp and 275 bp respectively (Figures 4.4.8 – 4.4.12). Distinctive bands were seen on the FH patients and NC samples lanes, whereas no band was detected at the negative control lane.

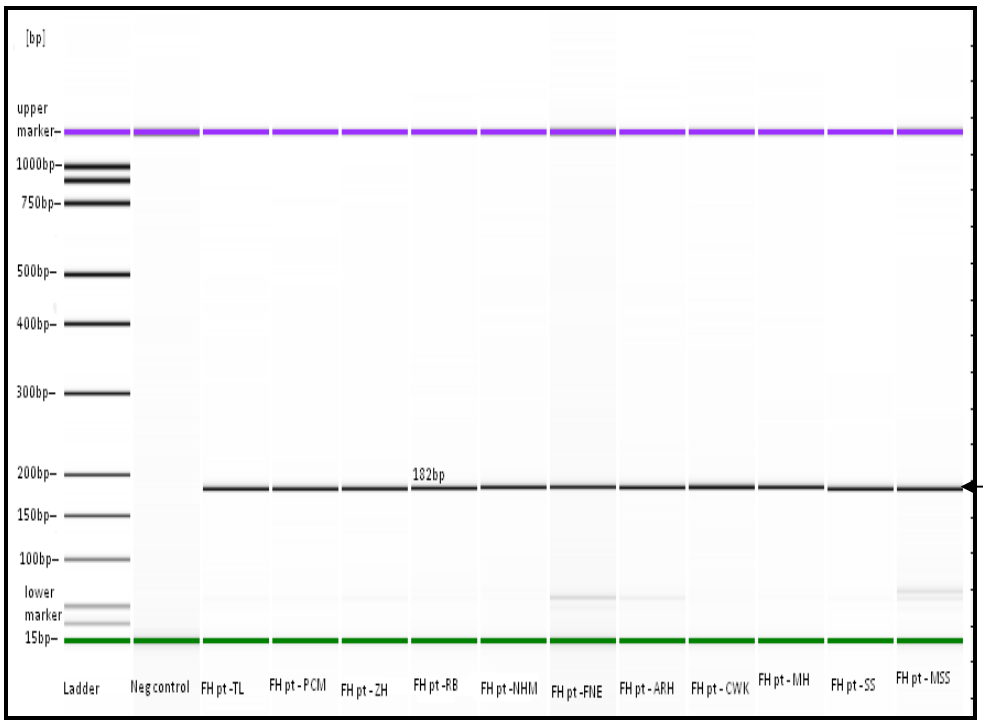


Figure 4.4.8: Amplification of Exon 5 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample, patient samples and normal control samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 182 bp.

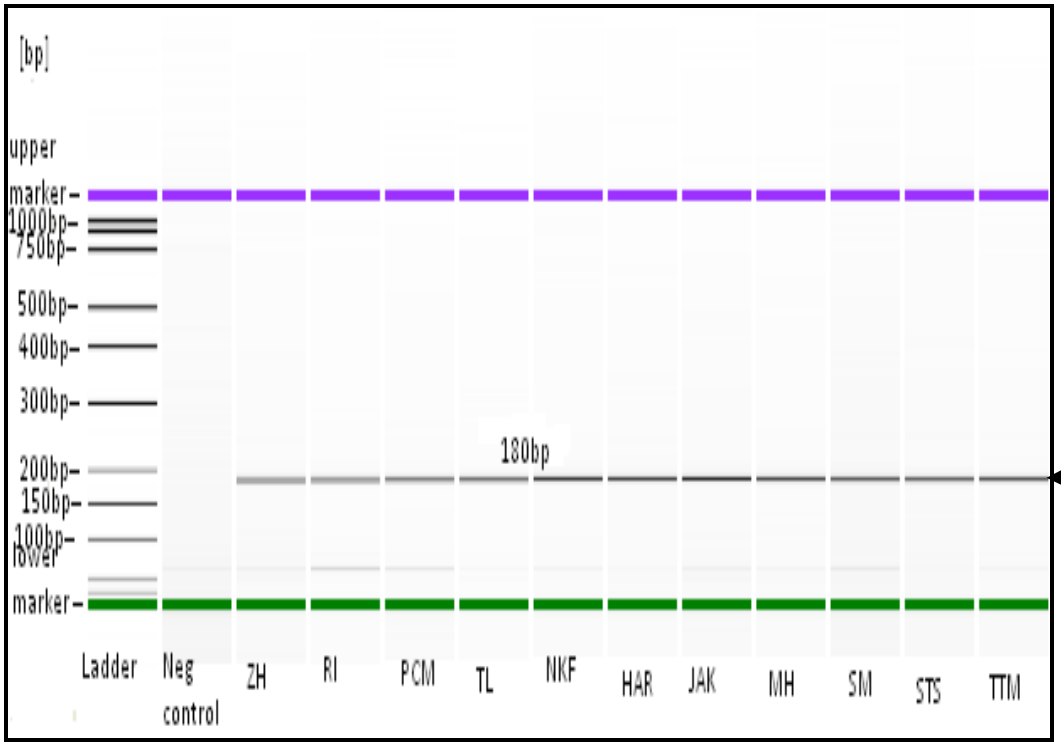


Figure 4.4.9: Amplification of Exon 6 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 180 bp.

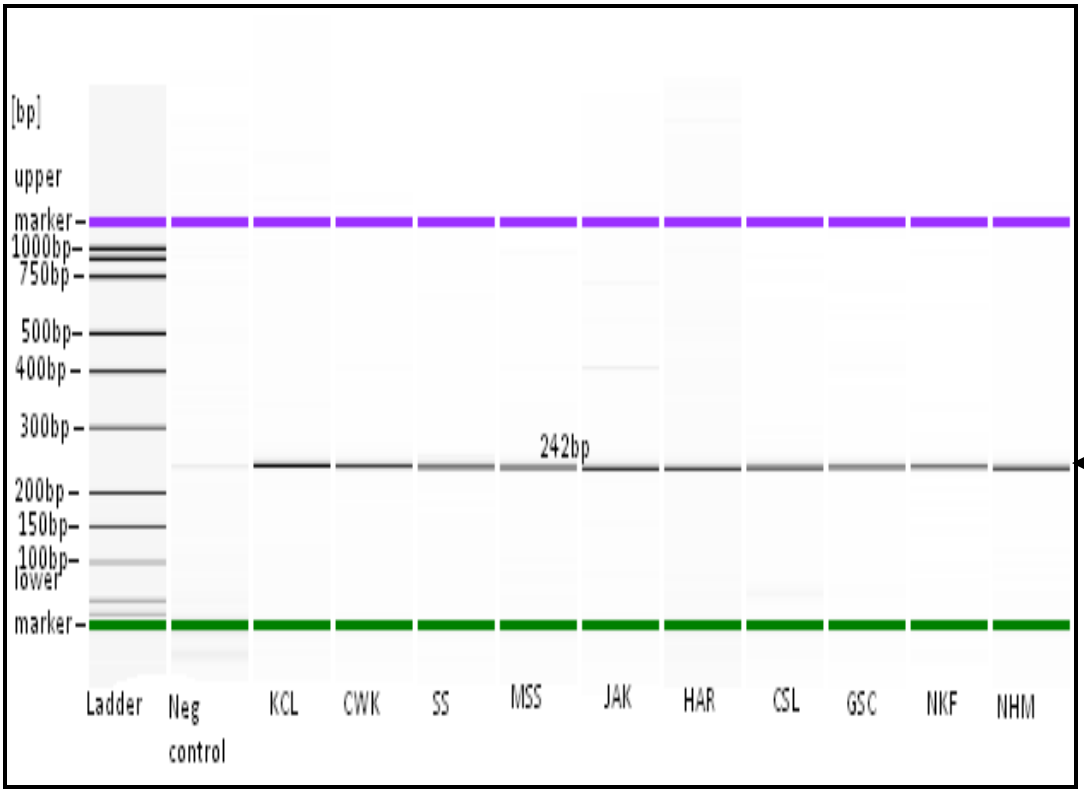


Figure 4.4.10: Amplification of Exon 7 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 242 bp.

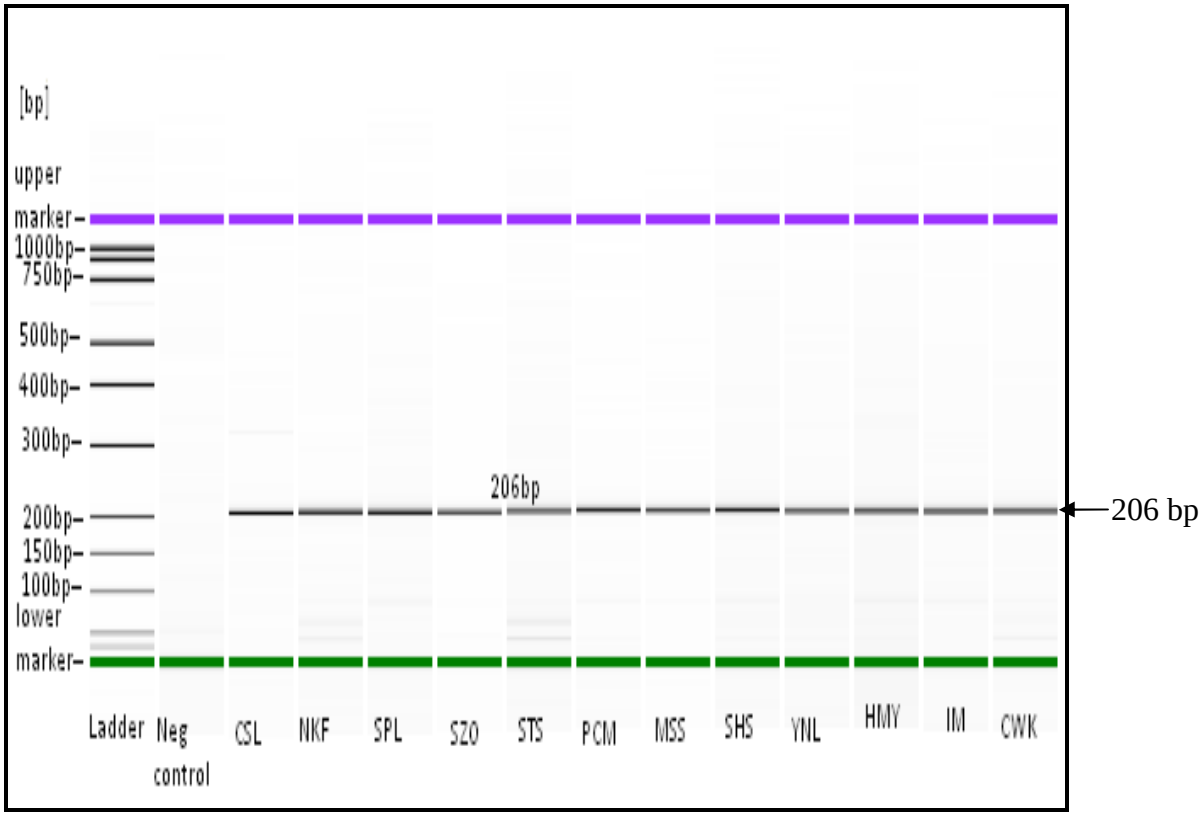


Figure 4.4.11: Amplification of Exon 8 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 206 bp.

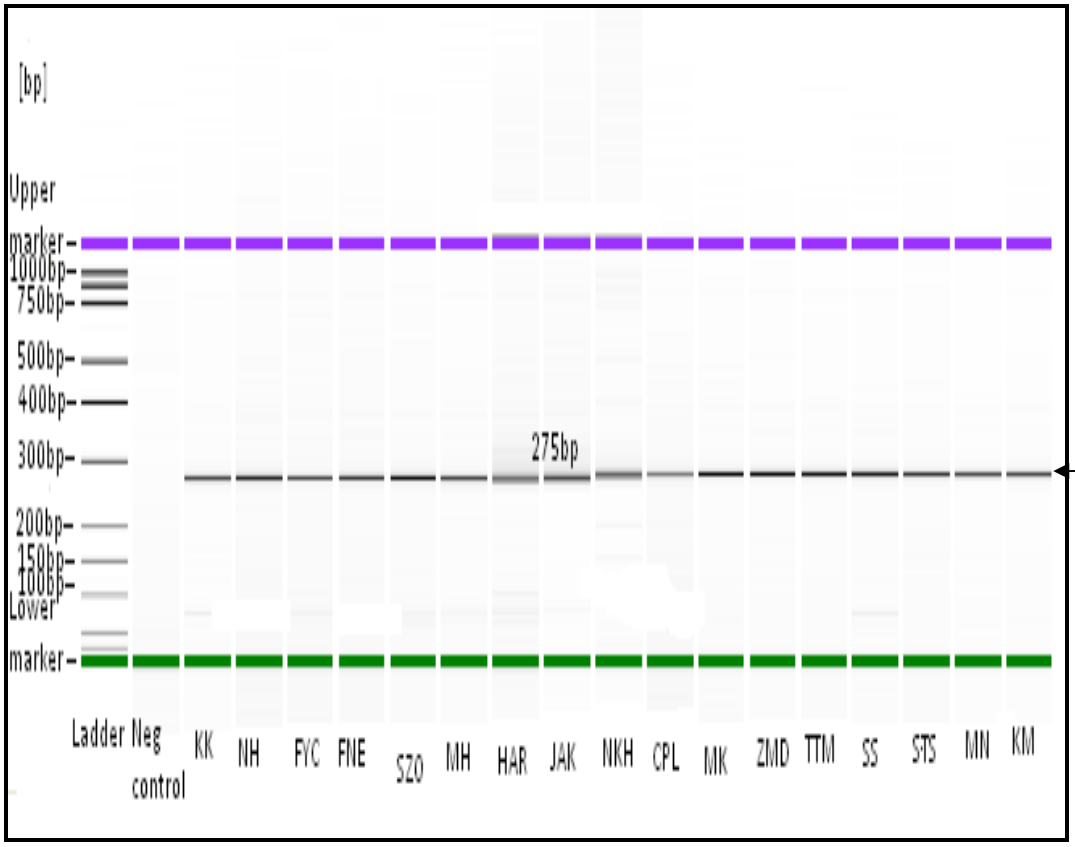


Figure 4.4.12: Amplification of Exon 9 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 275 bp.

Two sets of primer were used for the amplification of exon 10 and the PCR products were detected at 213 bp and 166 bp respectively (Figures 4.4.13 – 4.4.14). Distinctive bands were seen on the FH patients, whereas no band was detected at the negative control lane.

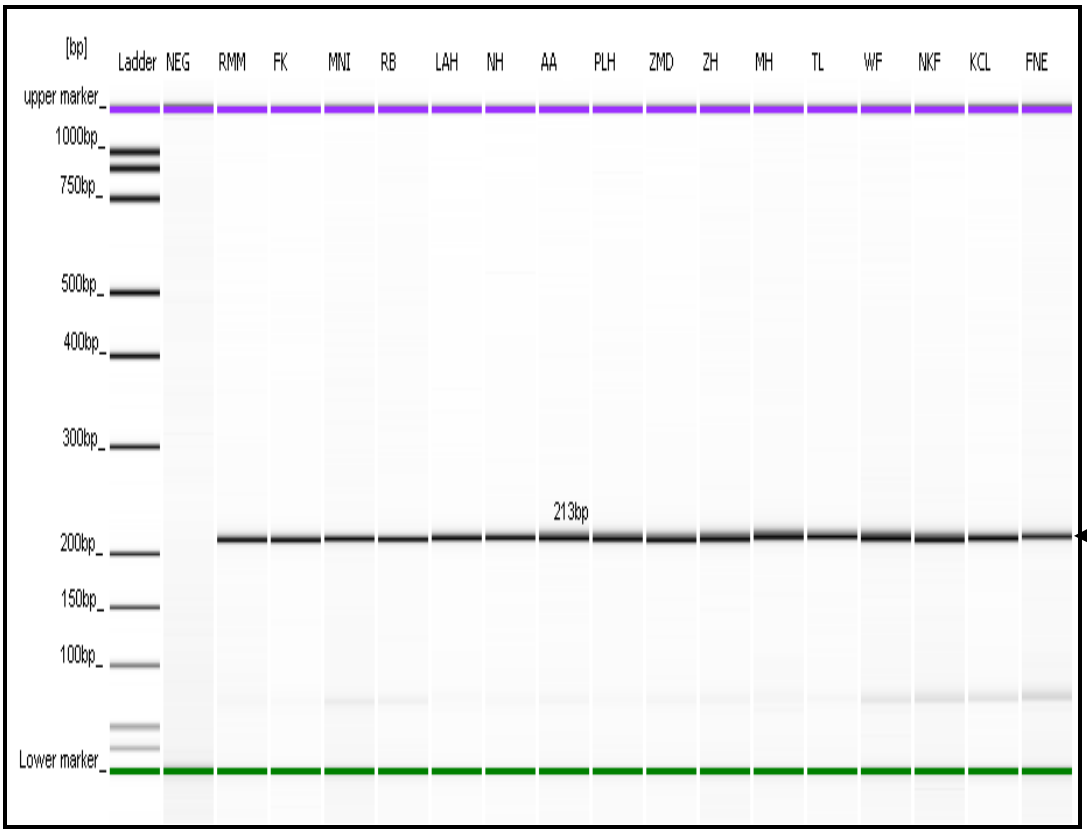


Figure 4.4.13: Amplification of Exon 10-5' of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 213 bp.

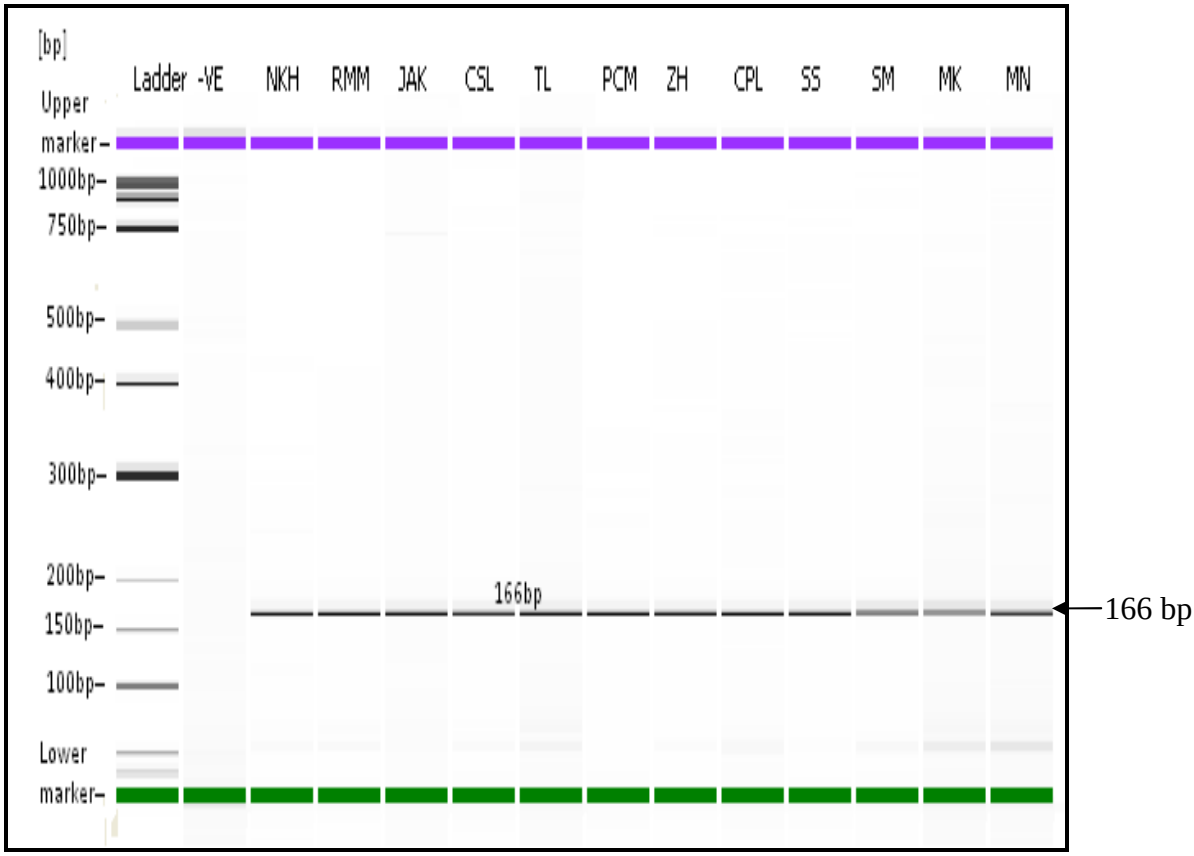


Figure 4.4.14: Amplification of Exon 10-3' of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 166 bp.

The amplifications of exon 11 to exon 18 obtained distinctive bands for FH and NC samples lanes at 177 bp, 213 bp, 271 bp, 284 bp, 255 bp, 134 bp, 245 bp and 140 bp respectively (Figures 4.4.15 – 4.4.22). The distinctive bands obtained indicated that the PCR protocols employed have amplified the specific target regions of exon 11 to exon 18 of LDLR gene.

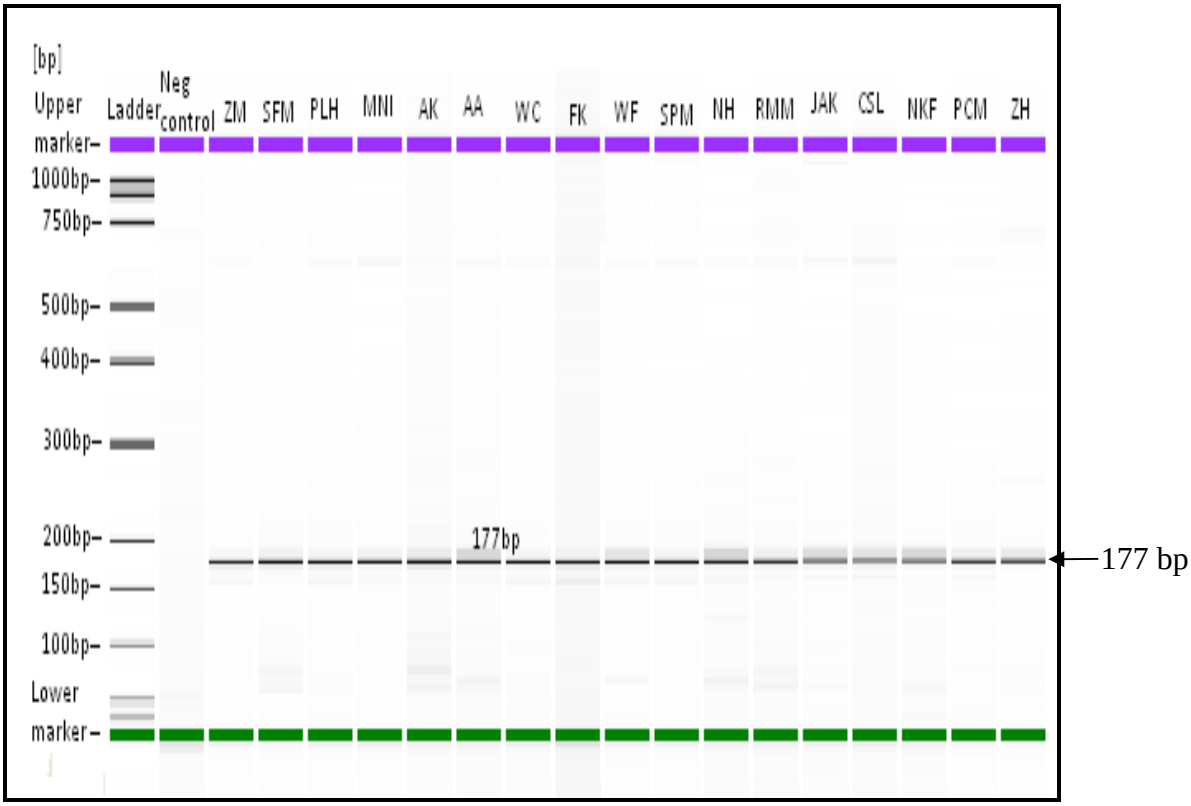


Figure 4.4.15: Amplification of Exon 11 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patiens samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 177 bp.

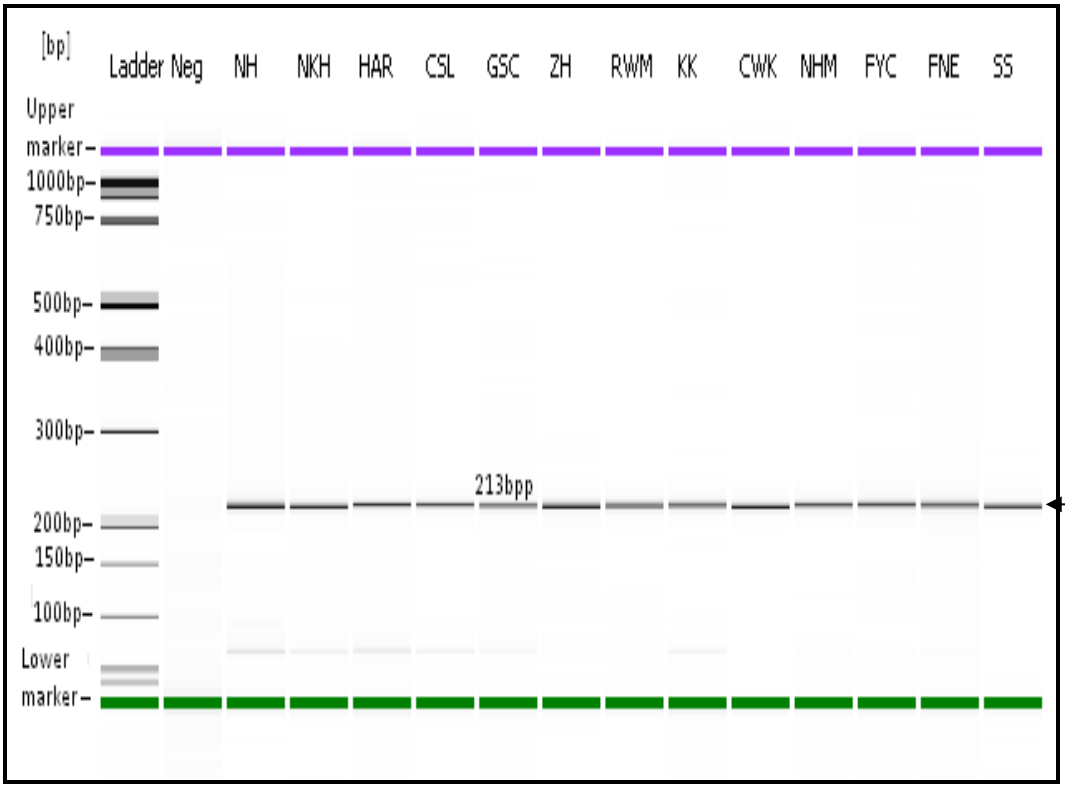


Figure 4.4.16: Amplification of Exon 12 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patiens samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 213 bp.

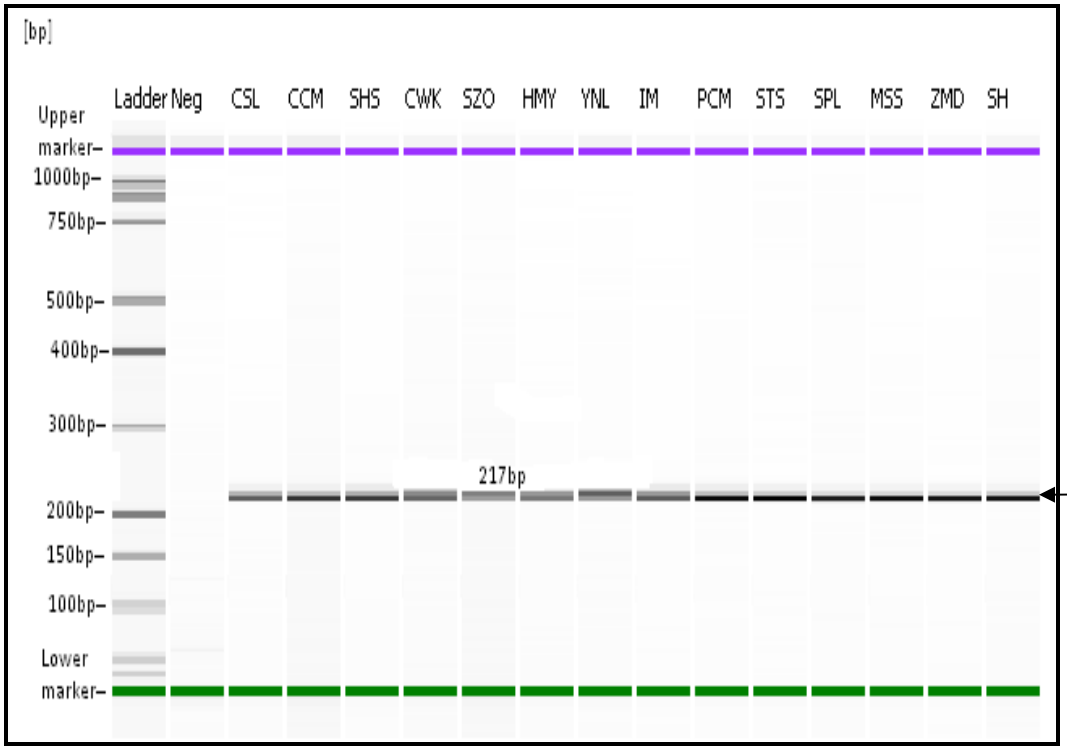


Figure 4.4.17: Amplification of Exon 13 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 217 bp.

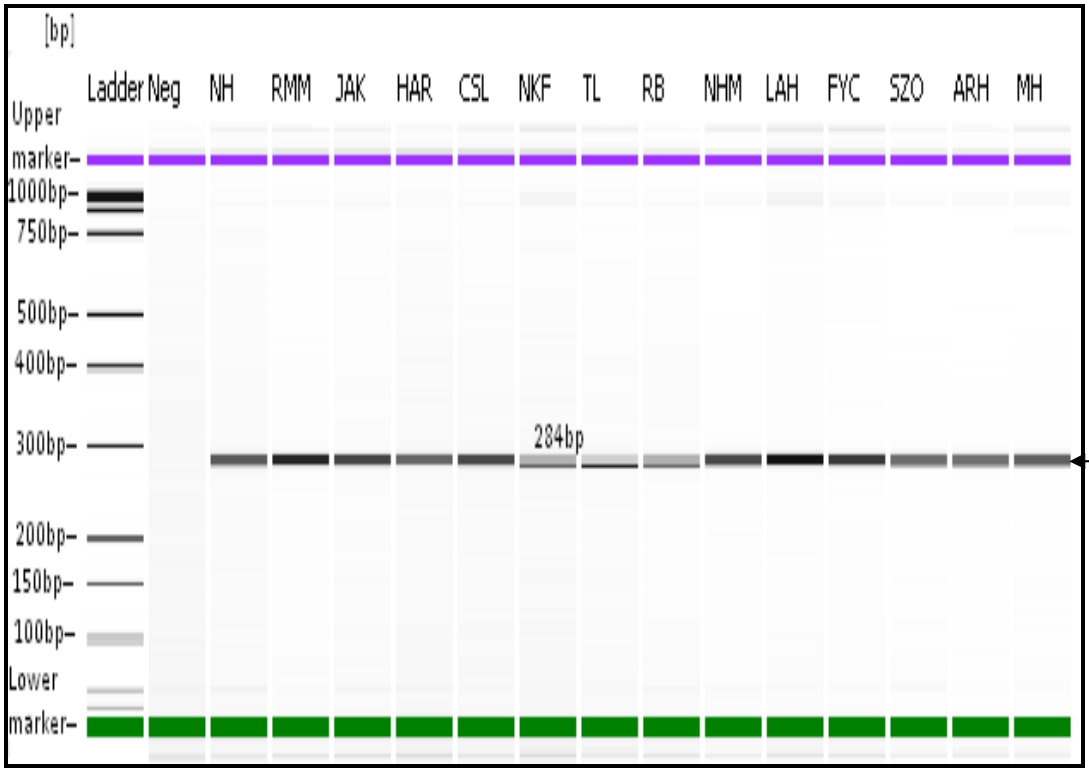


Figure 4.4.18: Amplification of Exon 14 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 284 bp.

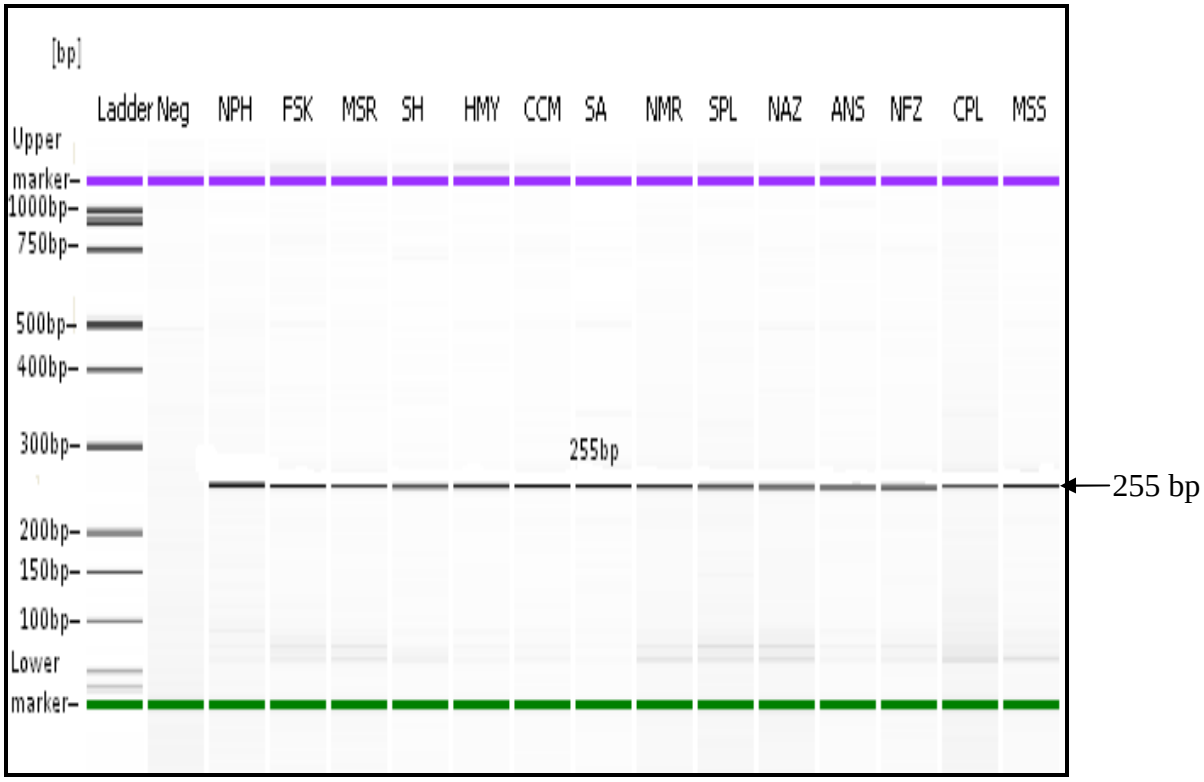


Figure 4.4.19: Amplification of Exon 15 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 255 bp.

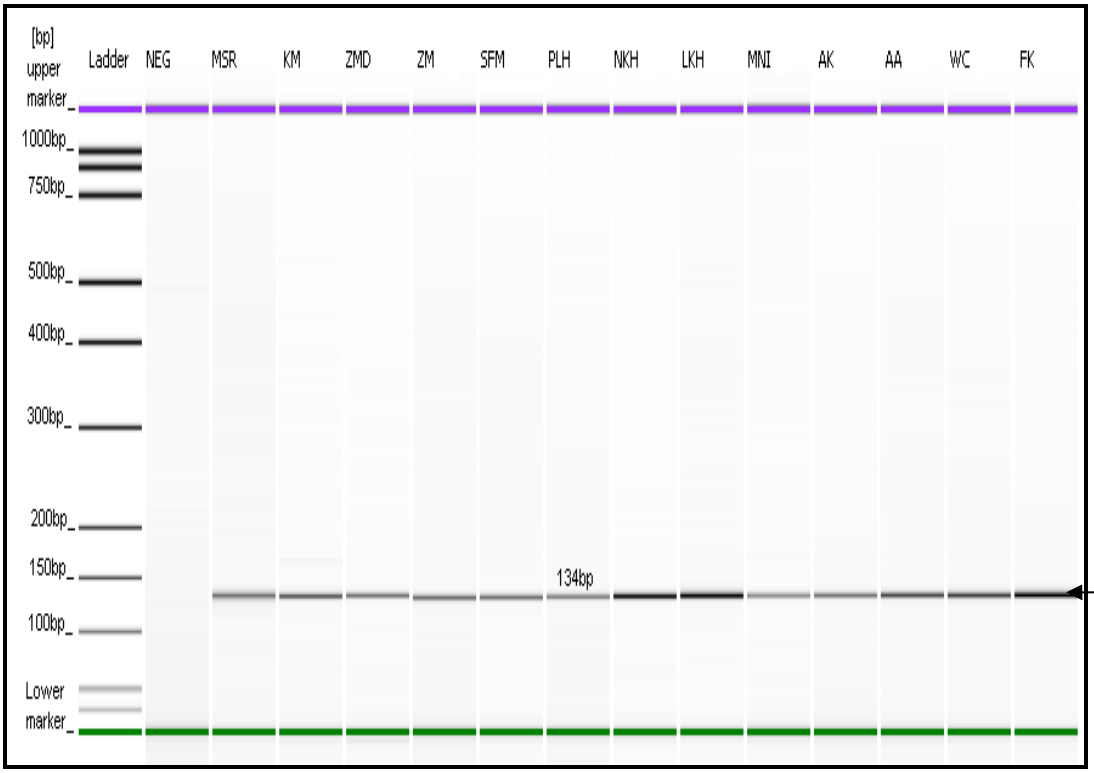


Figure 4.4.20: Amplification of Exon 16 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 134 bp.

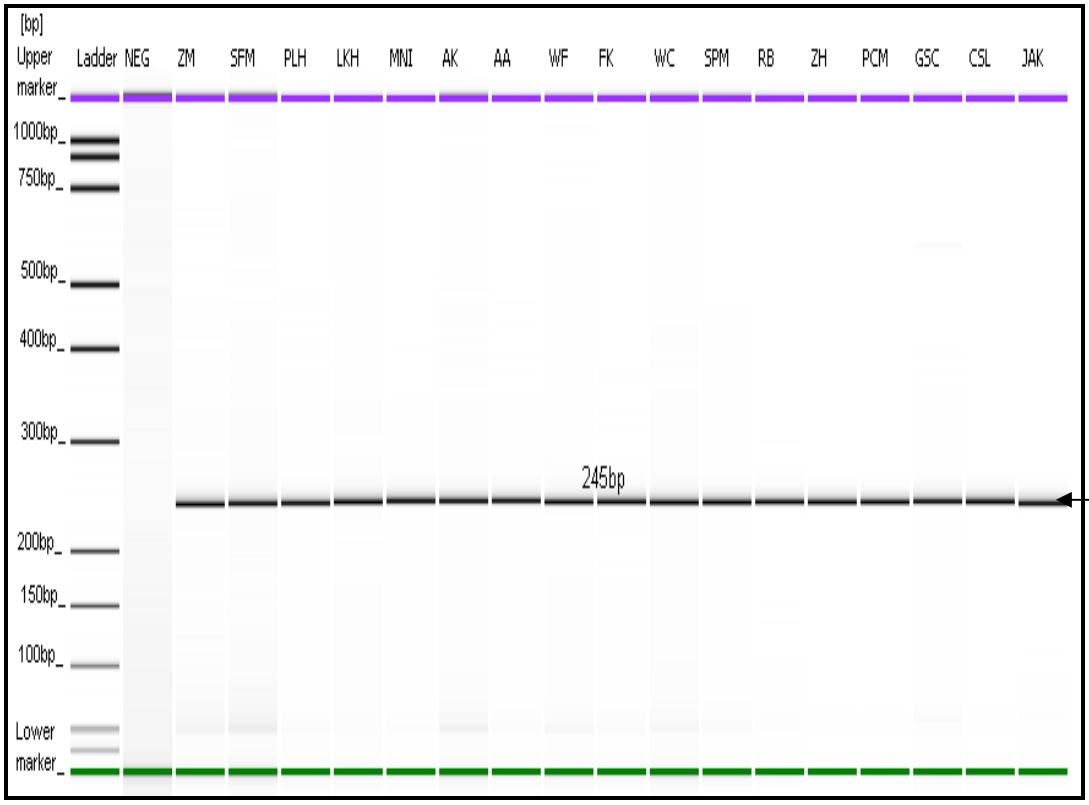


Figure 4.4.21: Amplification of Exon 17 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 245 bp.

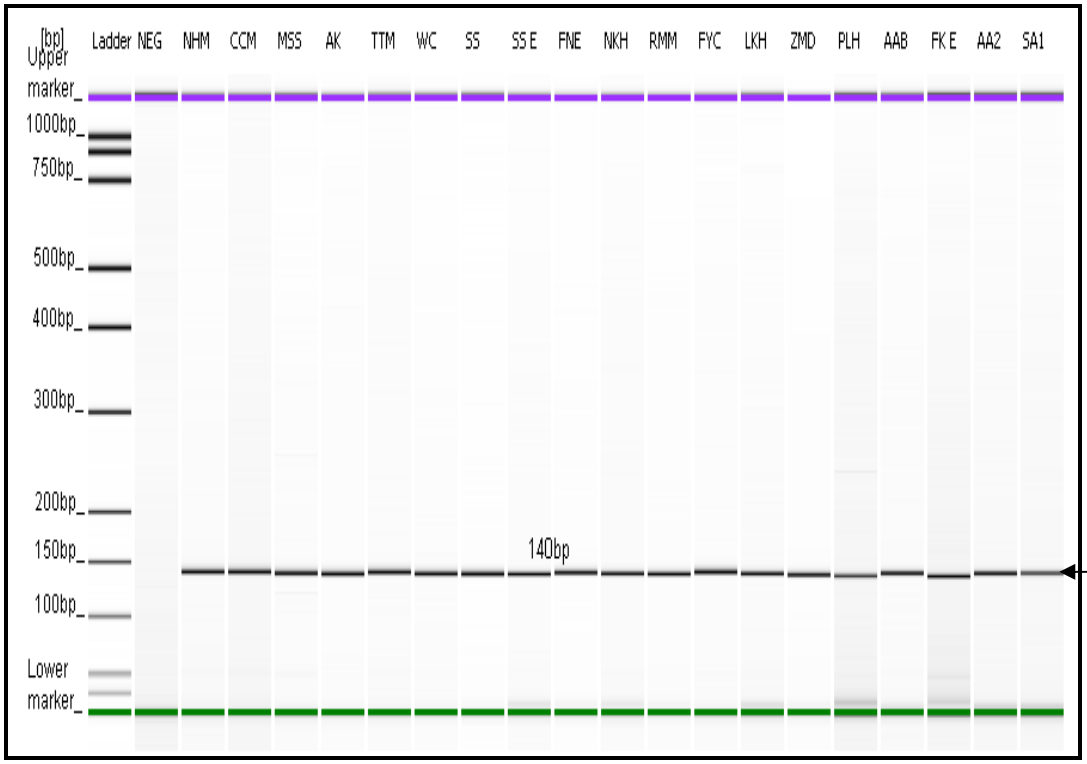


Figure 4.4.22: Amplification of Exon 18 of LDLR gene by PCR. The PCR products were analysed on an automated on-chip capillary gel electrophoresis (Agilent Bioanalyser). Lanes from left to right; 1-Kb Ladder, Negative control sample and patient samples. The green band represent lower marker and purple band represent upper marker. The PCR products were detected at 140 bp.

4.5 DHPLC results

Mutational screening of 74 FH patients samples were successfully identified using DHPLC method on Wave Nucleic Acid Fragment Analysis System. The system was calibrated using four different standards to ensure optimum cartridge separation, resolution performance and reproducibility between sample injections. The chromatograms of the four different standards were illustrated in Figure 4.5.1.

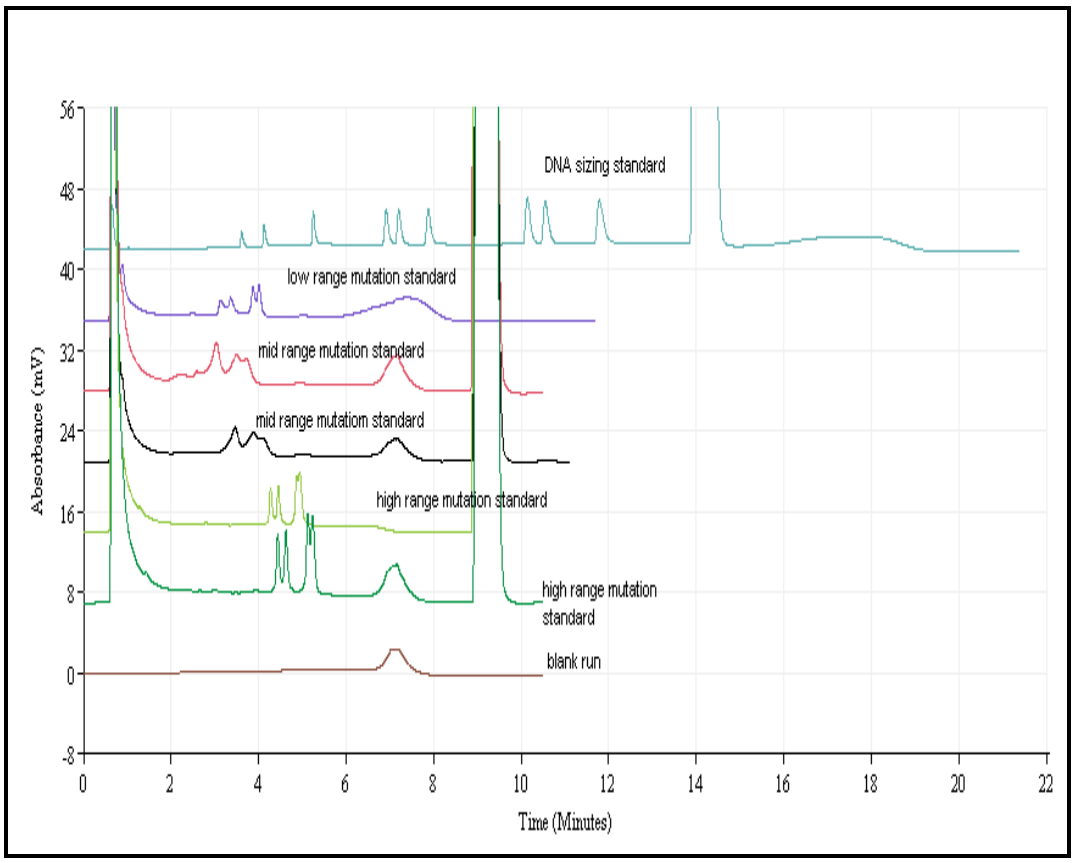


Figure 4.5.1: DHPLC Chromatogram profile showing the low range standard, mid range standard, high range standard, DNA sizing standards and blank run on DHPLC. These standard materials analysis were performed to check on the performance of the DHPLC.

Table 4.5.1 tabulated the 46 FH patients with their ethnicity that showed disease-causing variants detected by DHPLC. Mutational screening by DHPLC showed that the disease-causing variants were detected in exon 3 (was confirmed by DNA sequencing that the location was at the intron 3), exon 5, exon 9, exon 10 and exon 12. The identifications were done by comparing FH patient elution profiles with NC subject elution profile. These 46 samples were subjected sequencing to confirmation for the location and nature of variants.

Table 4.5.1: FH patient samples showed disease-causing variant screened by DHPLC.

No	Patients ID	Exons	Ethnic group	No	Patients ID	Exons	Ethnic group
1	LKH	3	C	24	SZO	10	M
2	SFM	5	M	25	SPL		M
3	FK		M	26	MK	12	M
4	WC		M	27	SS		M
5	KM		M	28	AA		M
6	CSL	9	C	29	RWM		M
7	ZM	10	M	30	NHM		M
8	ZMD		M	31	FNE		M
9	NAZ		M	32	SH		M
10	NFZ		M	33	SA		M
11	ASAR		M	34	KAL		C
12	MN		M	35	HAR		M
13	FSK		M	36	GSC		C
14	NPH		C	37	NKF		C
15	PLH		C	38	HWAR		M
16	SM		M	39	NKH		C
17	AK		M	40	JAK		M
18	MH		M	41	STS		M
19	LAH		C	42	YNL		C
20	KCL		C	43	TCH		C
21	MNI		M	44	CWK		C
22	MSS		M	45	CCM		C
23	PCM		C	46	HMY		C

M= Malay, C= Chinese

The presence of possible mutations or disease-causing variant of FH patients samples were distinguished from the normal control or wild type peak as the less stable hoteroduplex were eluted faster than the homoduplex. The heteroduplex peaks were detected earlier by observing the DHPLC chromatogram of absorbance in millivolts (mV) against retention time in minutes. The DHPLC chromatogram of exon 3 in Figure 4.5.2 showed heteroduplex peaks that were eluted earlier at 4.5 minutes and 5.0 minutes, whereas the homoduplex peaks were eluted at 5.4 minutes and 6.6 minutes at melting temperature of 63.7 ^{o}C. The wash peaks of both FH patient sample and NC subjects were eluted at 7.3 minutes.

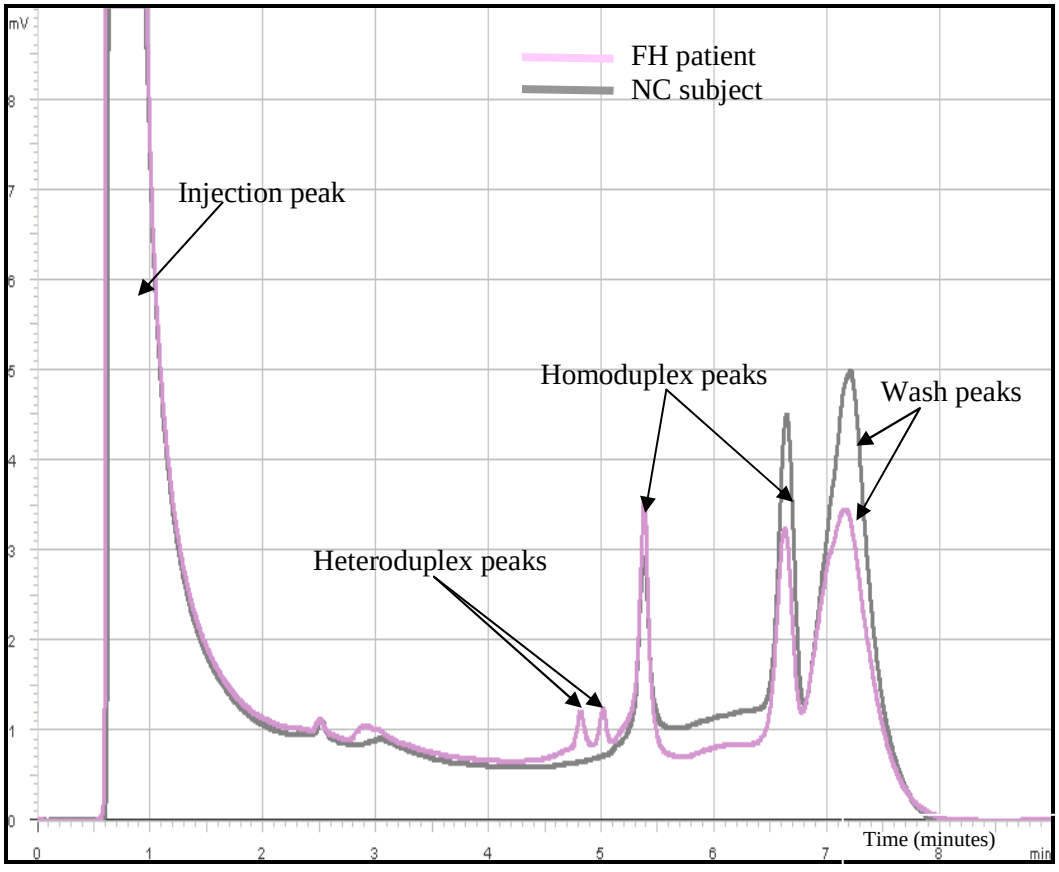


Figure 4.5.2: Graph absorbance (mV) against time (minutes) showing chromatogram profile at temperature 63.7 ^{o}C showing the disease-causing variant of exon 3 of LDLR in a patient with FH as compared to normal control subject.

The DHPLC chromatogram profile of exon 3 of a FH patient that showed presence of heteroduplex peaks was compared with a NC subject profile and another FH patient profile that showed only presence of homoduplex peak (Figure 4.5.3). The profile was suggestive of disease-causing variants and subjected to DNA sequencing to confirm.

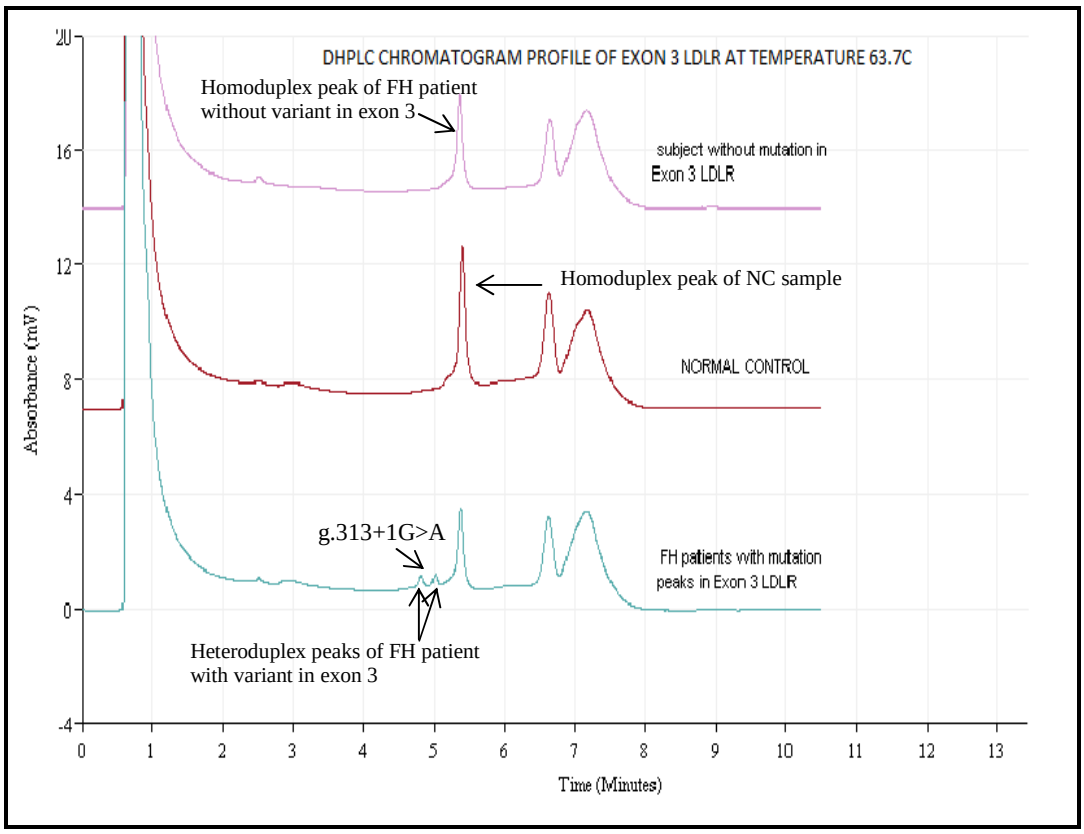


Figure 4.5.3: DHPLC Chromatogram profile showing the disease-causing variant of exon 3 of LDLR in a patient with FH as compared to normal control subject at temperature 63.7 ^{o}C.

The DHPLC chromatogram profiles of exon 5 in 4 FH patients that showed presence of fronting shoulder before the rise of another peak was considered as heteroduplex peaks (Sivakumaran *et al*., 2003) eluted at 5.2 minutes and 5.8 minutes whereas the NC subjects showed presence of homoduplex peak eluted at 6.2 minutes (Figure 4.5.4). The DHPLC chromatogram profiles of 4 FH patients that showed heteroduplex peaks were suggestive of disease-causing variants and subjected to DNA sequencing to confirm.

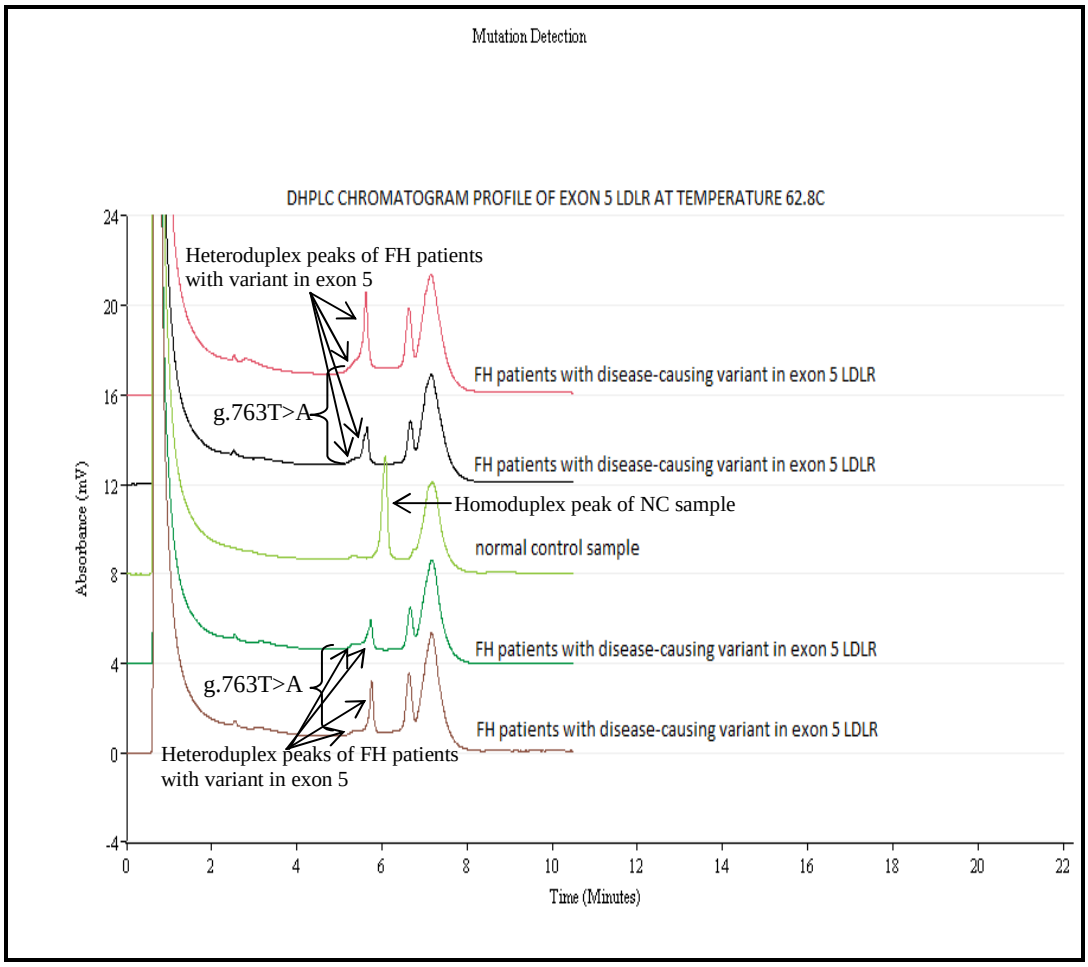


Figure 4.5.4: DHPLC Chromatogram profile showing the disease-causing variant of exon 5 of LDLR in a patient with FH as compared to normal control subject at temperature 62.8 ^{o}C.

Figure 4.5.5 showed DHPLC chromatogram profiles of FH patients with and without disease-causing variant in exon 9 compared with NC subject. The FH patient without disease-causing variant in exon 9 and NC subject showed presence of homoduplex peak eluted at 5.2 minutes when subjected to melting temperature of 62.2 ^{o}C. The FH patient with disease-causing variant at exon 9 showed presence of heteroduplex peaks eluted at at 4.5 minutes and 4.8 minutes. Wash peaks for all samples from FH patients and NC subject were eluted at 7.3 minutes. Blank run was performed to ensure the stability of the DHPLC system after every injection of samples.

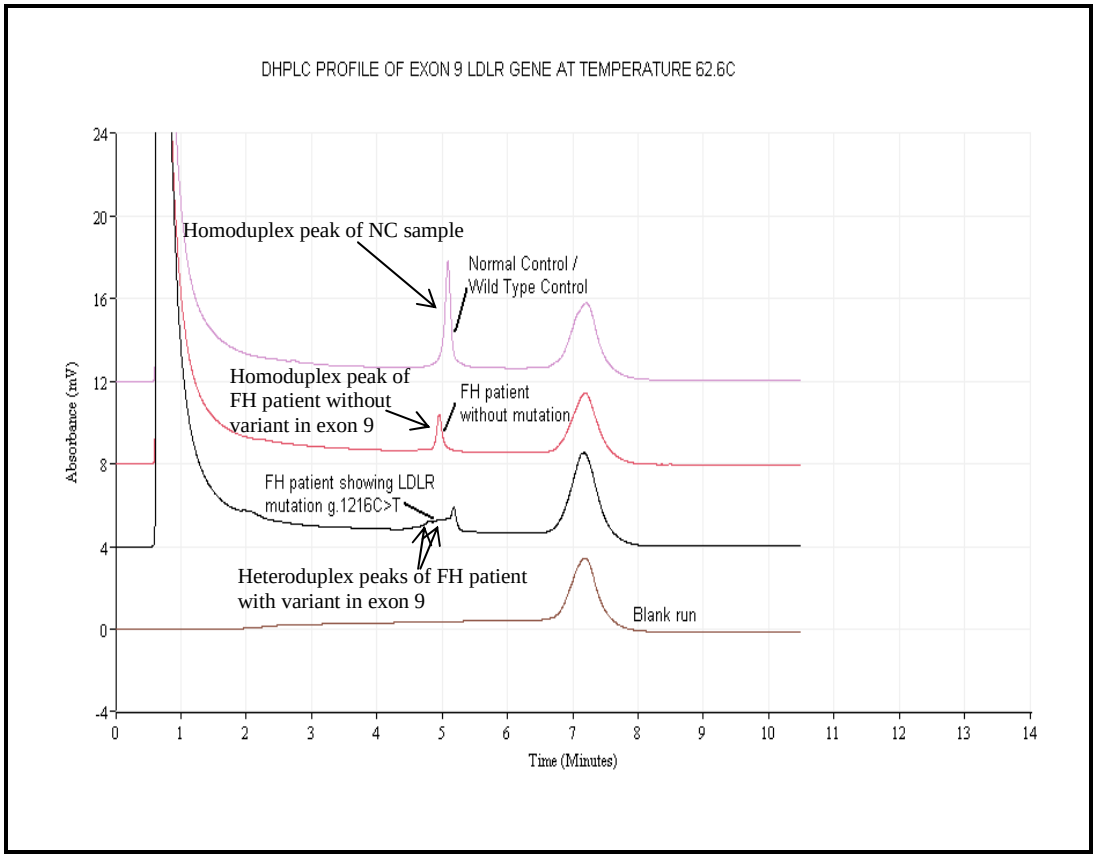


Figure 4.5.5: DHPLC Chromatogram profile showing the disease-causing variant of exon 9 of LDLR in a patient with FH as compared to normal control subject at temperature 62.2 ^{o}C.

DHPLC chromatogram profiles of exon 10 of FH patients and NC subject at melting temperature of 62.7 ^{0}C were illustrated in Figures 4.5.6 – 4.5.7. The chromatogram profile of NC subject showed homoduplex peak eluted at 5 minutes. DHPLC chromatogram profile of FH patients showed presence of heteroduplex peaks as the appearance of fronting shoulder and 2 unseparated peaks. The hetereduplex peaks were eluted at 4.4 minutes (fronting shoulder), 4.6 minutes and 4.8 minutes. The difference in elution profile of FH patients compared with the elution profile of NC subject warranted for subsequencing to confirm the variant.

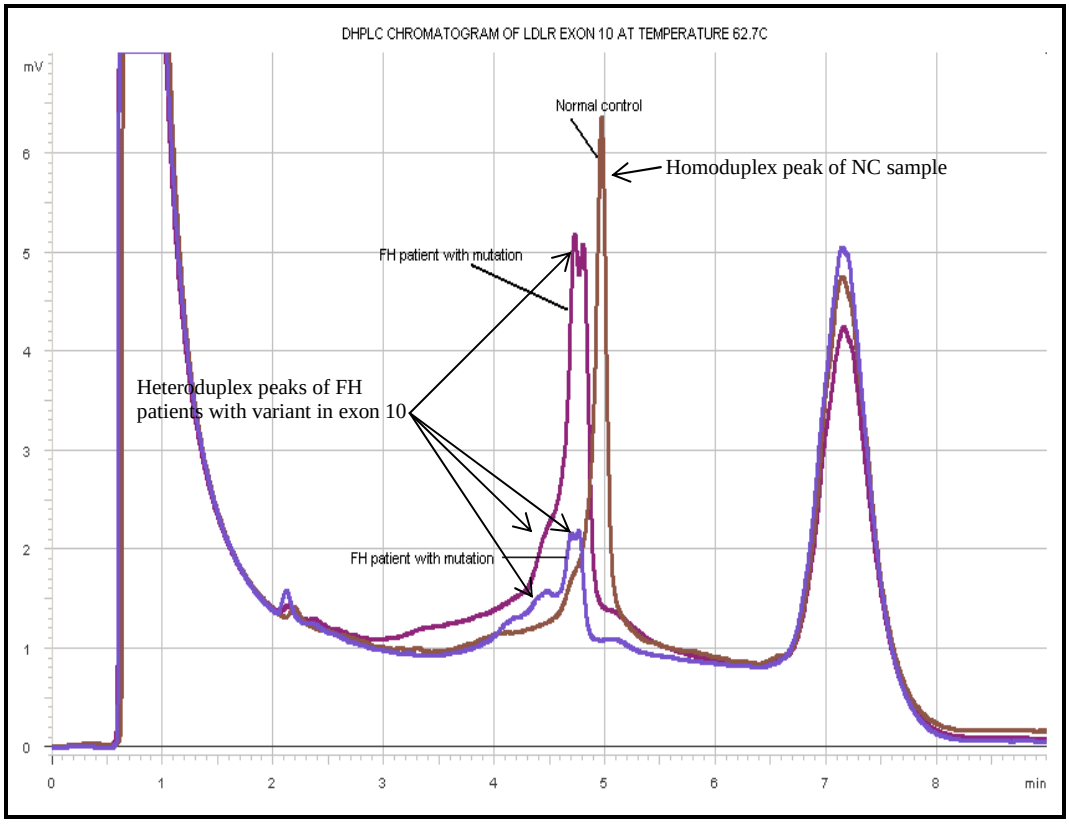


Figure 4.5.6: Graph absorbance (mV) against time (minutes) showing chromatogram profile at temperature 62.7 ^{o}C showing the disease-causing variant of exon 10 of LDLR in a patient with FH as compared to normal control subject.

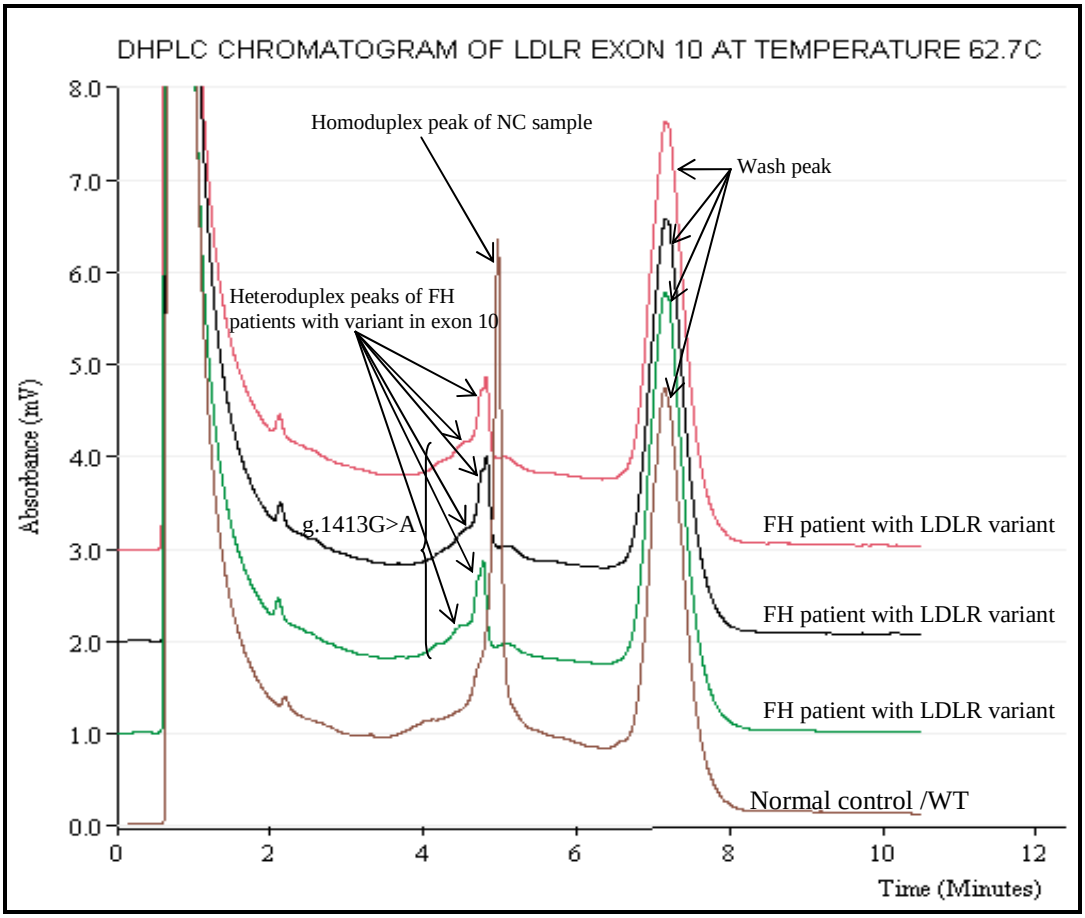


Figure 4.5.7: DHPLC Chromatogram profile showing the disease-causing variant of exon 10 of LDLR in a patient with FH as compared to normal control subject at temperature 62.7 ^{o}C.

Figure 4.5.8 showed the DHPLC chromatogram profile of exon 12 in FH patients compared with NC subject profile at melting temperature of 60.8 ^{o}C. The chromatogram of NC subject showed homoduplex peak eluted at 4.7 minutes. The FH patients with disease-causing variants in exon 12 showed heteroduplex peaks eluted at 4.1 minutes and 4.4 minutes. The difference in elution profile of FH patients compared with the elution profile of NC subject indicated for confirmation of location and nature of variants by sequencing.

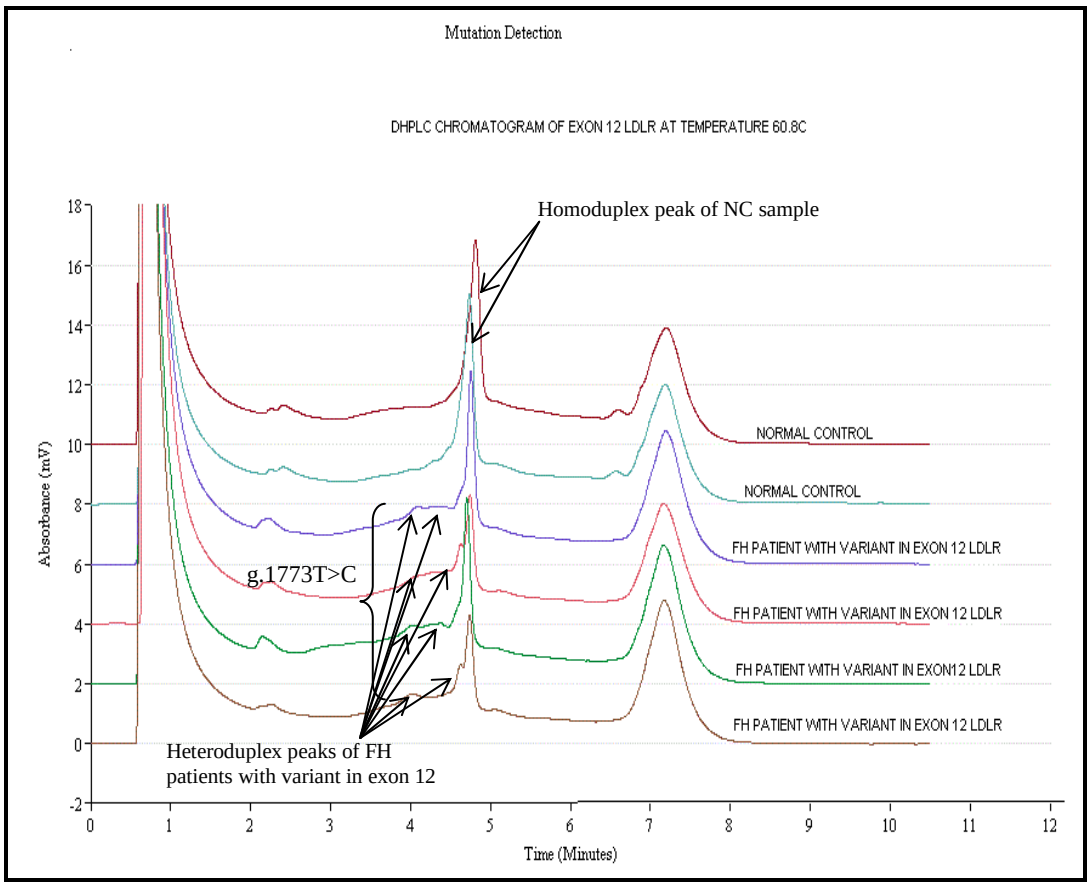


Figure 4.5.8: DHPLC Chromatogram profile showing the disease-causing variant of exon 12 of LDLR in a patient with FH as compared to normal control subject at temperature 60.8 ^{o}C.

4.6 Sequencing results

The 46 FH samples showing mismatched DNA fragments on DHPLC chromatograms were confirmed successfully using DNA sequencing on ABI Prism 377 sequencer to identify the nature of the mutations. The sequencing results from patients were aligned with the reference sequence obtained from nucleotide database of the National Centre for Biotechnology Information (NCBI) and were analysed using Invitrogen Vector NTI Advance Version 10 software. DNA sequencing has confirmed three different LDLR gene mutations in six FH patients (6/74; 8.1%) with FH while 34/74 (45.91%) have shown to have non-mutational SNPs. The remaining six FH (8.1%) patients that showed heteroduplex peaks on DHPLC were found to be normal when confirmed with sequencing. The results of sequencing analysis of affected LDLR regions were shown in Table 4.6.1.

Table 4.6.1: Results of DNA sequence analysis

No	Affected LDLR regions	DNA sequence	Description of variant and effect
1	Intron 3	Normal : GCAAGGCTGTC**G**TAAGT Variant : GCAAGGCTGTC**A**TAAGT	g.313+1G>A; 5'splice donor deleted
2	Exon 5	Normal : GCCGGCAG**T**GTGACCG Variant : GCCGGCAG**A**GTGACCG	g.763T>A; Cysteine (C) to Serine (S)
3	Exon 9	Normal : CTTCACCAAC**C**GGCACG Variant : CTTCACCAAC**T**GGCACG	g.1216C>T; Arginine (R) to Tryptophan (W)
4	Exon 10	Normal : CATCAGCAG**G**GACATCC Variant : CATCAGCAG**A**GACATCC	g.1413G>A; no amino acid change.
5	Exon 12	Normal : CGATGTCAA**T**GGGGGCA Variant : CGATGTCAA**C**GGGGGCA	g.1773T>C; no amino acid change.

The effects of all detected disease-causing variants on fasting serum lipid profile of the affected FH patients were tabulated in Table 4.6.2.

Table 4.6.2: Effects of LDLR variants on fasting serum lipid profile of affected FH patients

No	LDLR variants and affected regions	No of affected FH patients	TC levels (mmol/L)	Tg levels (mmol/L)	LDL levels (mmol/L)	HDL levels (mmol/L)
1	g.313+1G>A of Intron 3	1	11.9	1.9	9.8	1.2
2	g.763T>A of Exon 5	4	9.9 ± 3.1*	1.1 ± 0.2*	8.1 ± 3.1*	1.4 ± 0.3*
3	g.1216C>T of Exon 9	1	8.9	2.1	6.6	1.4
4	g.1413G>A of Exon 10	15	8.0 ± 0.8*	1.8 ± 0.7*	5.8 ± 0.9*	1.3 ± 0.3*
5	g.1773T>C of Exon 12	19	8.1 ± 1.3*	1.6 ± 0.7*	5.9 ± 1.2*	1.3 ± 0.3*

*Data expressed as mean ± SD

The confirmed mutations were point mutation which involved a single base nucleotide change in the genomic DNA segment and caused acid amino changes (Figures 4.6.2 – 4.6.6). Other variants found were non mutational single nucleotide polymorphism (SNPs), which the changes in the nucleotide in the genomic DNA segment did not caused any change in the acid amino (Figures 4.6.1, 4.6.7 & 4.6.8).

DNA sequencing confirmed that a male FH patient of Chinese ethnicity has variant in intron 3 (g.313+1g>A) (Figure 4.6.1). The mutation has caused the patient to have highly elevated TC and LDL levels of 11.9 mmol/L and 9.8 mmol/L respectively (Table 4.6.2).

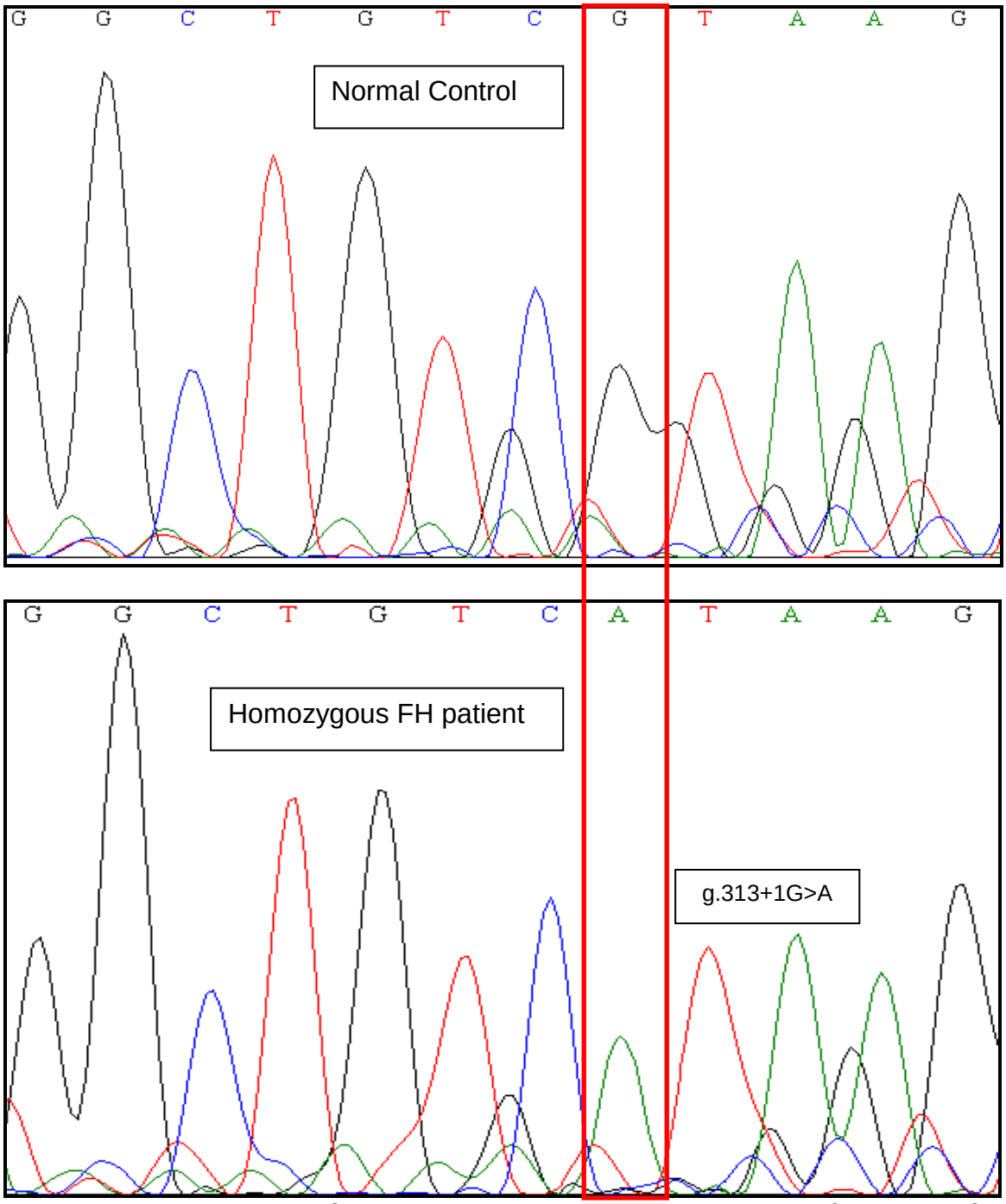


Figure 4.6.1: Sequence profile showing the disease-causing variant of intron 3 of LDLR in a patient with FH as compared to normal control subject. Substitution of G>A in intron 3 at nucleotide position 313+1G. The identified LDLR variant is 313+1G>A.

Four FH patients from a Malay family have been confirmed by DNA sequencing to have mutations in exon 5 (g.763T>A) with one member was homozygote and the other three members were heterozygotes (Figures 4.6.2 – 4.6.5). The substitution of T>A at nucleotide position 763 in exon 5 caused amino acid change from Cysteine (C) to Serine (S) at codon 234 [TGT>AGT]. The mutation was identified as C234S. The four FH patients with the mutations in exon 5 showed highly elevated TC and LDL levels of 9.9 ± 3.1 mmol/L and 8.1 ± 3.1 mmol/L (mean ± SD) respectively (Table 4.6.2).

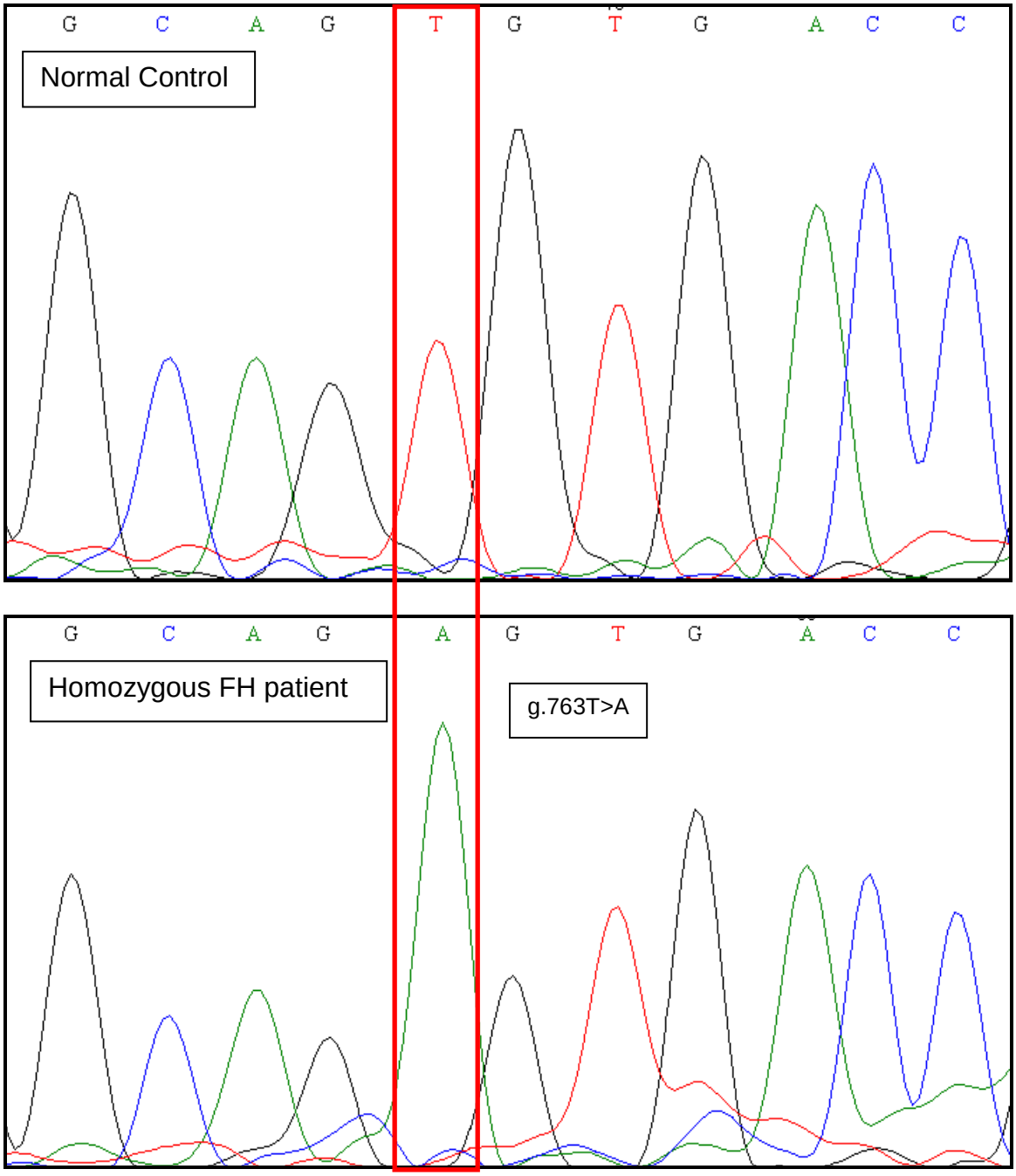


Figure 4.6.2: Sequence profile showing the disease-causing variant of exon 5 of LDLR in a patient with homozygous FH mutation as compared to normal control subject. Substitution T>A at nucleotide position 763 causes amino acid change from Cysteine (C) to Serine(S) at codon 234 [TGT>AGT]. The identified mutation is C234S.

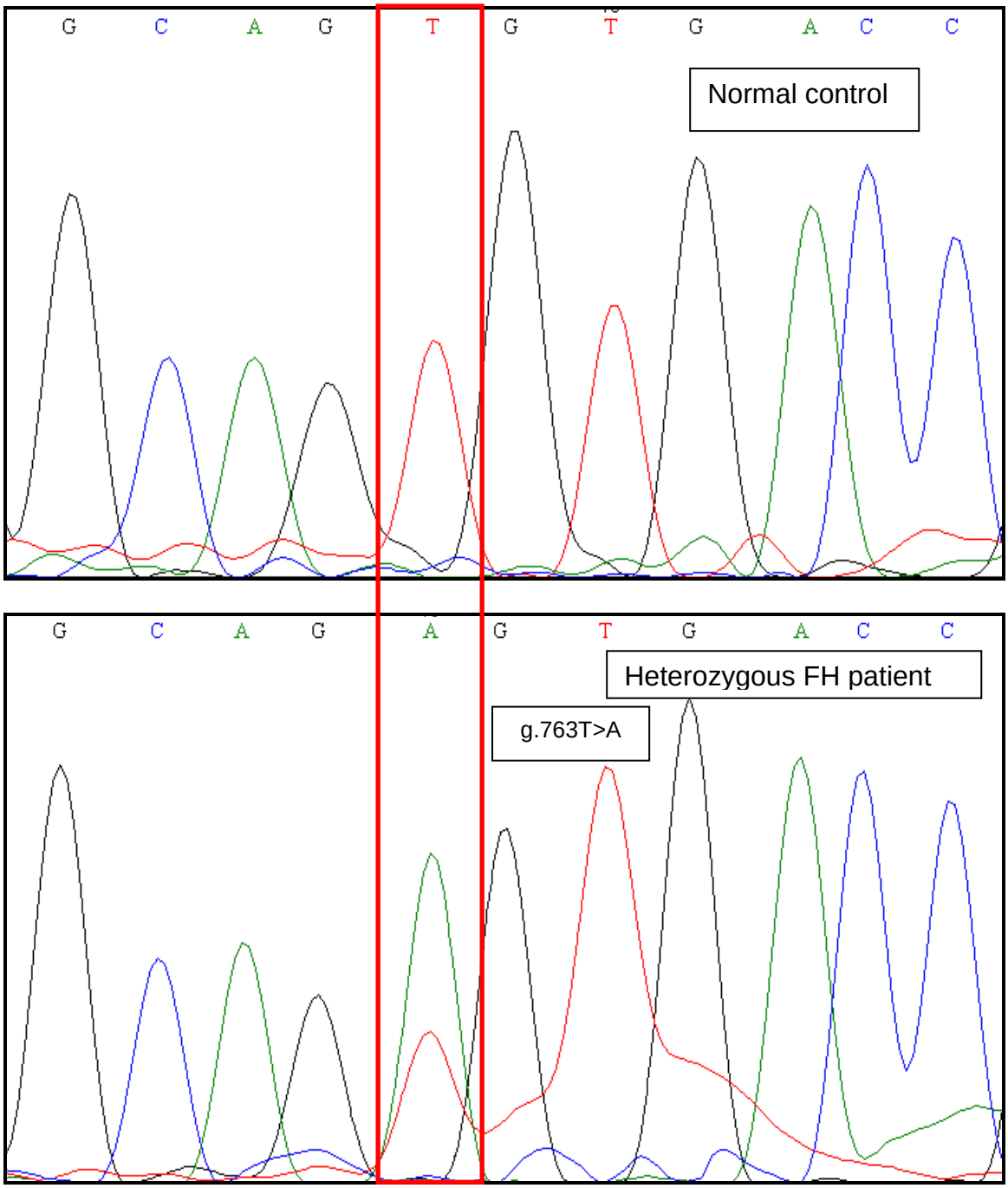


Figure 4.6.3: Sequence profile showing the disease-causing variant of exon 5 of LDLR in a patient with heterozygous FH mutation as compared to normal control subject. Substitution T>A at nucleotide position 763 causes amino acid change from Cysteine (C) to Serine (S) at codon 234 [TGT>AGT]. The identified mutation is C234S.

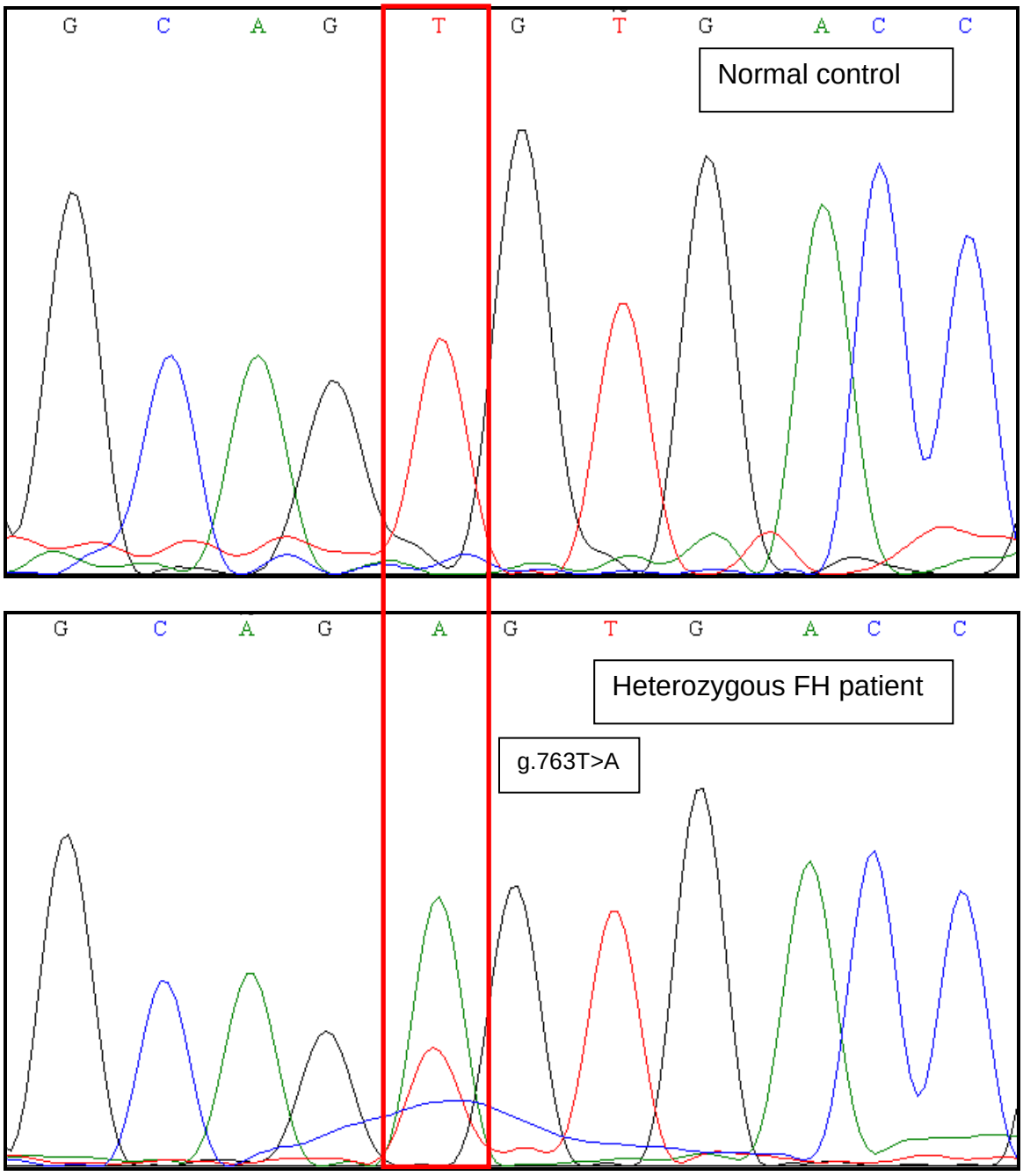


Figure 4.6.4: Sequence profile showing the disease-causing variant of exon 5 of LDLR in a patient with heterozygous FH mutation as compared to normal control subject. Substitution T>A at nucleotide position 763 causes amino acid change from Cysteine (C) to Serine(S) at codon 234 [TGT>AGT]. The identified mutation is C234S.

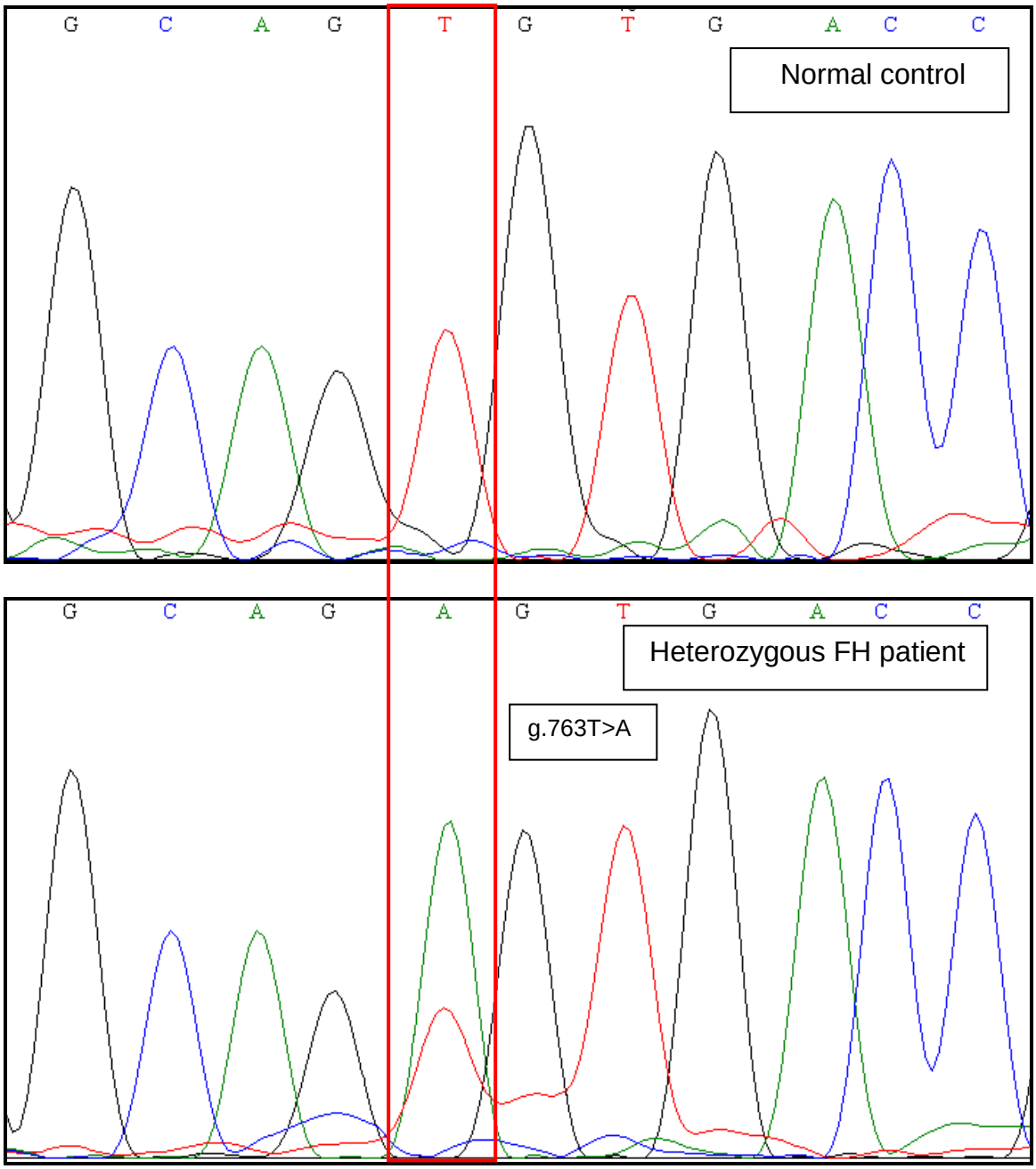


Figure 4.6.5: Sequence profile showing the disease-causing variant of exon 5 of LDLR in a patient with heterozygous FH mutation as compared to normal control subject. Substitution T>A at nucleotide position 763 causes amino acid change from Cysteine (C) to Serine(S) at codon 234 [TGT>AGT]. The identified mutation is C234S.

DNA sequencing has confirmed that a male FH patient of Chinese ethnicity has variant in exon 9 (g. 1216C>T) (Figure 4.6.6). The substitution of C>T at nucleotide position 1216 of exon 9 caused amino acid change from Arginine (R) to Tryptophan (W) at codon 385 [CGG>TGG]. The mutation was identified as R385W. The identified FH patient with this mutation in exon 9 was presented with highly elevated TC and LDL levels of 8.9 mmol/L and 6.6 mmol/L respectively (Table 4.6.2).

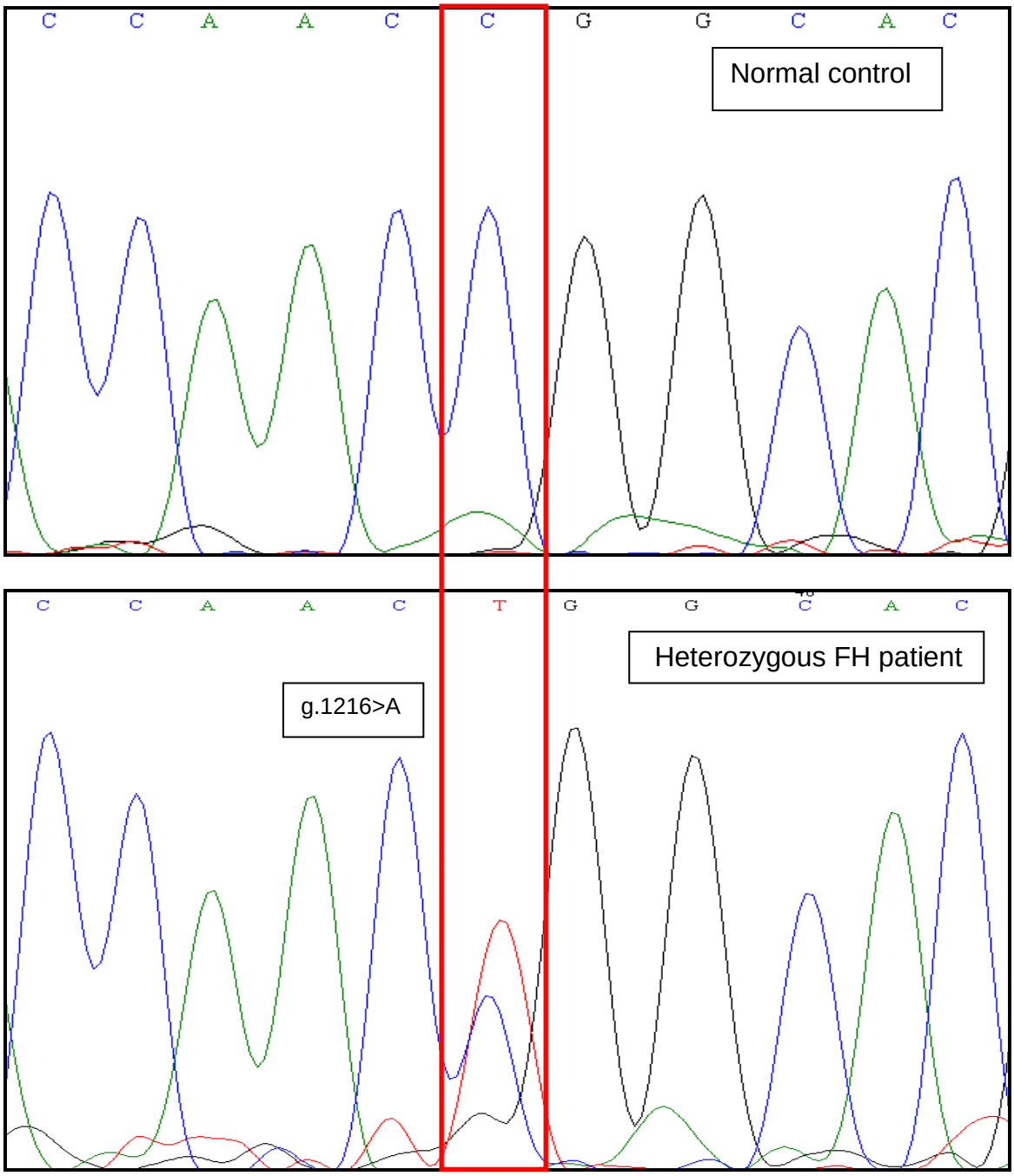


Figure 4.6.6: Sequence profile showing the disease-causing variant of exon 9 of LDLR in a patient with heterozygous FH mutation as compared to normal control subject. Substitution of C>T at nucleotide position 1216 causes amino acid change from Arginine (R) to Tryptophan (W) at codon 385 [CGG>TGG]. The identified mutation is R385W.

Fifteen FH patients have been confirmed by DNA sequencing to have variants in exon 10 (g.1413G>A) (Figure 4.6.7). The substitution of G>A at nucleotide position 1413 in exon 10 but the substitution caused no change to amino acid Arginine (R) at codon 450 [AGG>AGA]. The identified variant in exon 10 caused elevated TC and LDL levels of 8.0 ± 0.8 mmol/L and 5.8 ± 0.9 mmol/L (mean ± SD) respectively (Table 4.6.2).

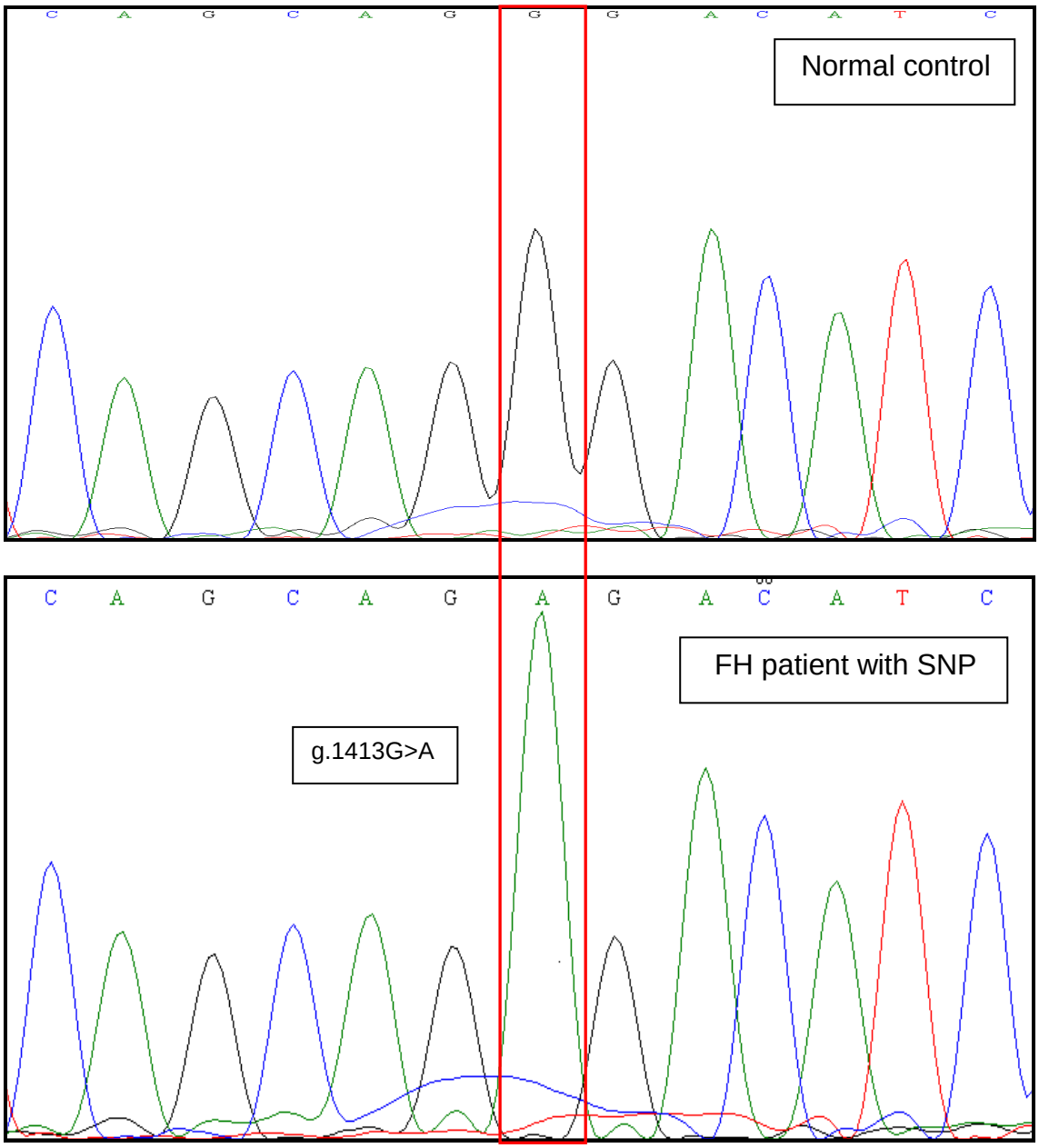


Figure 4.6.7: Sequence profile showing the disease-causing variant of exon 10 of LDLR in a patient with FH as compared to normal control subject. Single nucleotide polymorphism identified as substitution of G>A at nucleotide position 1413. This nucleotide substitution does not cause any change to amino acid Arginine (R) at codon 450 [AGG>AGA].

Nineteen FH patients have been confirmed by DNA sequencing to have variants in exon 12 (g.1773T>C) (Figure 4.6.8). The substitution of T>C at nucleotide position 1773 in exon 12 but the substitution cause no change to amino acid Proline (N) at codon 570 [AAT>AAC]. The identified variant in exon 12 caused elevated TC and LDL levels of 8.1 ± 1.3 and 5.9 ± 1.2 mmol/L (mean ± SD) respectively (Table 4.6.2).

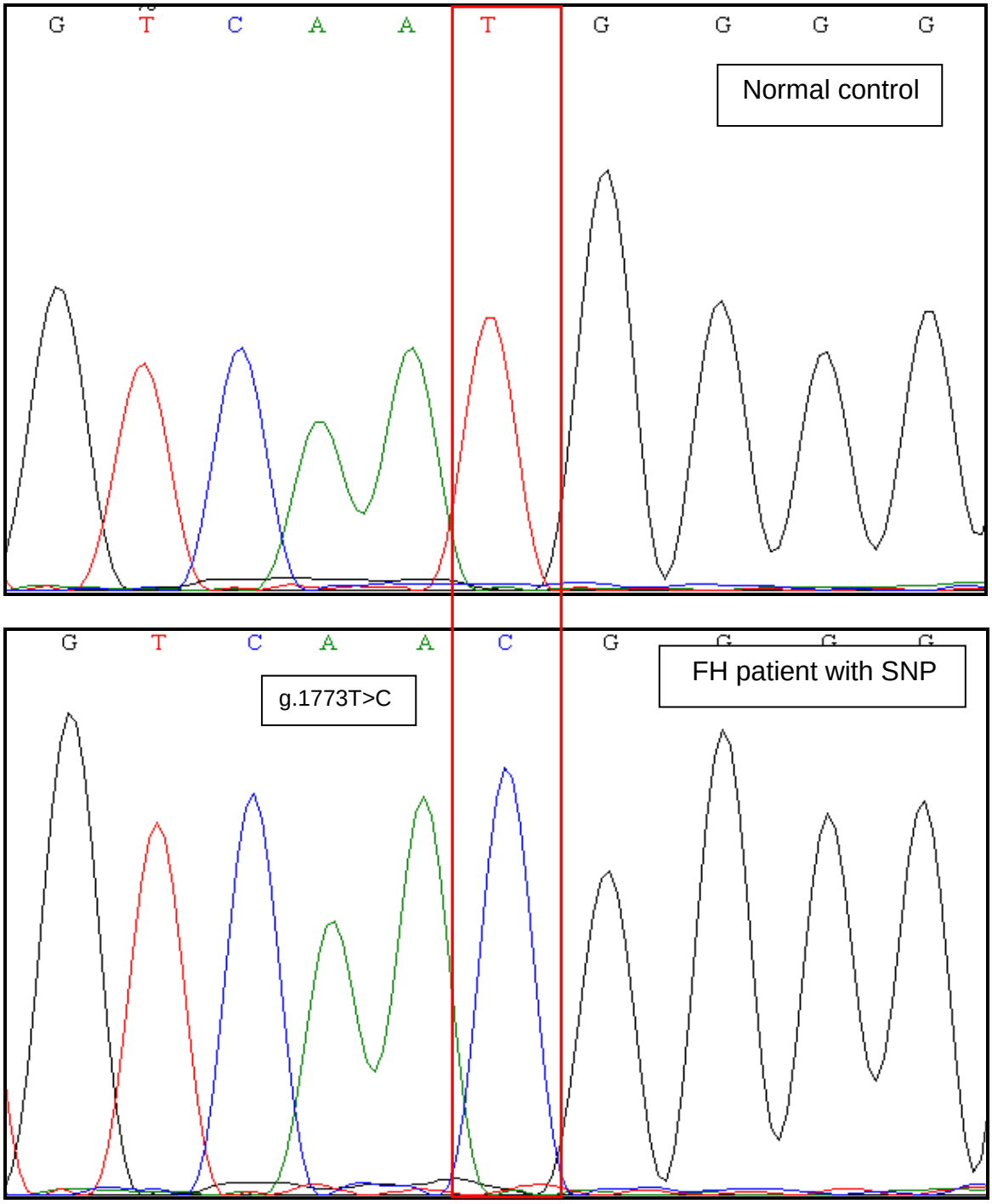


Figure 4.6.8: Sequence profile showing the disease-causing variant of exon 12 of LDLR in a patient with FH as compared to normal control subject. The single nucleotide polymorphism identified as substitution of T>C at nucleotide position 1773. This nucleotide substitution does not cause any change to amino acid Proline (N) at codon 570 [AAT>AAC].