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The Association between Methylenetetrahydrofolate Reductase (MTHFR) Mutations and Serum Biomarkers of Cardiac Health

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Abstract

Background: Homocysteine (tHcy) has emerged as a new risk factor for cardiovascular diseases (CVD) The Methylenetetrahydrofolate reductase (MTHFR) polymorphisms are seen to give rise to high levels of tHcy which can be a causative factor in the progression of CVD due to its thrombogenic effect. Serum cardiac biomarkers help in the diagnosis, prognosis, or surveillance of CVD. The present study evaluated the association of the two MTHFR mutations, rs1801133 and rs1801131 with 16 well-established serum cardiac markers. Additionally, the influence of age and gender on the association of the two MTHFR polymorphisms with serum cardiac marker levels was also investigated. **Methods:** The study was carried out on 1295 individuals who visited Vibrant America Clinical Lab for regular or suspected CVD check-ups. The serological markers and genomic variant analysis were carried out as per the standard laboratory protocol under CLIA. The association between serological markers and the rs1801133 and rs1801131 genetic variants with respect to age and gender was evaluated using a one-way ANNOVA test. **Results:** No significant association was observed in tHcy levels with respect to gender, however, plasma total tHcy levels were higher in males than females. tHcy levels increased with increasing age in the wild and heterozygous genotypes for the mutations, rs1801133 and rs1801131. Additionally, the serum cardiac markers, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Cholesterol (CHOL), Apolipoprotein A (APOA), Apolipoprotein B

(APOB), N-terminal (NT)-pro hormone BNP (BNPNT), LDL calculated (LDLCAL), Small Density Low Density Lipoprotein (SDLDL), APOBAR, Oxidised Low Density Lipoprotein (OXLDL), Lipoprotein (A) (LPA), Triglycerides (TRIG), and Lipoprotein-Associated Phospholipase (Lp-PLA2) Test (PLAC) showed significant associations with respect to gender and age for rs1801133 and rs1801131 ($P < 0.05$) However, these markers were seen to have different trends in correlation with gender and age. **Conclusions:** The present study reports the association of tHcy, HDL, LDL, CHOL, APOA, APOB, BNPNT, LDLCAL, SDLDL, APOBAR, OXLDL, LPA, TRIG, and PLAC with respect to age and gender for the mutations, rs1801133 and rs1801131. We observed that tHcy levels were high in males and the levels increased with increasing age in males for both polymorphisms. rs1801131 mutant males have high levels of triglyceride whereas rs1801133 mutant postmenopausal females showed high levels of cholesterol. Further analysis will be required to understand the pattern of association of the rest of the serum cardiac markers with age and gender for rs1801133 and rs1801131 mutations.

Keywords

Homocysteine, MTHFR, Cardiac Markers

1. Introduction

Homocysteine (tHcy) is a sulfur-containing amino acid that is emerging as a new risk factor for cardiovascular disease (CVD) Methylenetetrahydrofolate reductase (MTHFR), a key enzyme in tHcy metabolism is believed to play a role in CVD [1]. tHcy is metabolized by the intersection of the two pathways, transsulfuration and remethylation. During transsulfuration, tHcy and serine are irreversibly condensed to cystathionine by the enzyme cystathionine b-synthase (CBS3) in the presence of the cofactor, vitamin B6. In the remethylation pathway, the methyl donor for the conversion of tHcy to methionine is provided by the reduction of 5, 10-methylene-tetrahydrofolate to 5-methyl-tetrahydrofolate which is brought about by the MTHFR enzyme. 5-Methyltetrahydrofolate which is the predominant circulating form of folate acts as the methyl donor for the remethylation of tHcy to methionine. This conversion takes place with the help of the enzyme, methionine synthase, in the presence of vitamin B12. Low levels of tHcy are maintained by transsulfuration to cysteine or by remethylation to methionine [2].

CVD is a multifactorial disease and its pathogenesis is believed to be a fine interplay between environmental risk factors and multiple predisposing genes. Functional allelic variations or polymorphisms in humans may play a role in an individual's susceptibility to diseases. Elevated total plasma homocysteine (tHcy) concentrations have emerged as a major risk factor for thrombosis and atherosclerosis [2]. High values of plasma tHcy may occur as a consequence of deficiencies in the nutrients such as vitamin B-12, folic acid, and vitamin B-6, or from

genetic defects, particularly in the two enzymes MTHFR and CBS [3]. MTHFR is responsible for the circulating form of folate, 5-methyltetrahydrofolate, which provides methyl groups for the remethylation of homocysteine to methionine. A C-to-T substitution at nucleotide 677 (rs1801133) in the MTHFR-gene coding sequence results in the substitution of alanine to valine residue in the protein [4]. The polymorphism, particularly the homozygous trait correlates with reduced activity of the enzyme due to thermolability and increased levels of plasma tHcy. Another mutation in the MTHFR gene has also been recognized, where an A-to-C transition at nucleotide 1298 (rs1801131) leads to a glutamate-to-alanine substitution in the protein. Similar to the rs1801133 mutation, this mutation also results in decreased MTHFR activity, which is more pronounced in the homozygous mutant phenotype [5] [6].

McCully, 1969, reported that patients suffering from congenital homocystinuria developed premature vascular disease [7]. Thus, elevated levels of tHcy have developed as a strong risk factor for atherosclerotic CVDs. Cardiac biomarkers are a good representation of one's cardiac health, as they not only help in the diagnosis but aid in the prognosis and timely management of CVDs. tHcy along with other markers including LDL, HDL, CHOL, PLAC, and BNPNT are all effective indicators of CVDs. It is important to note that these risk assessment markers are subject to change owing to factors such as age, gender, other related health conditions, and lifestyle [8] [9] [10].

In the present study, we evaluated the association of the two MTHFR mutations, rs1801133 and rs1801131 with 16 well-established serum cardiac markers. We also examined the influence of age and gender on the association of the two MTHFR polymorphisms with serum cardiac marker levels.

2. Methods

2.1. Study Population

The study analyzed 1295 samples that were collected from subjects who visited Vibrant America for regular or suspected CVD check-ups. The study was conducted on a healthy population of non-smokers (self-reported) who were tested for genetic mutations along with the examination of their cardiovascular biomarker profile. The subjects were all Caucasian. The data on general variables such as age and sex obtained at the time of sampling were included in the study.

2.2. Serological Markers

A total of 16 serological markers were used in the study. The standard laboratory protocol was followed for the assessment of each marker. LDL (Direct), HDL, Triglyceride, PLAC, tHcy, and SDLDL were measured by the enzymatic-colorimetric method using Beckman Coulter AU680 clinical analyzer. MPO was measured by latex enhanced Immunoturbidimetric method and total cholesterol was measured by the cholesterol dehydrogenase method using Beckman Coulter AU680 clinical analyzer. OXLDL was measured by ELISA using Hamilton

Microlab STAR. Apo A-1 and Apo B were measured by the immunoturbidometric method, while HSCRP and Lp(a) were measured by the particle enhanced immunoturbidimetric assay using Roche Cobas 6000 c 501. NT-proBNP was measured by the electrochemiluminescence Immunoassay using Roche Cobas 6000 c 501. The concentration of LDL cholesterol (denoted as LDL-C) was calculated using triglycerides and HDL concentration using Friedewald's formula (LDL cholesterol = total cholesterol − HDL cholesterol − (triglycerides/5) [11].

2.3. Candidate SNPs and Genotyping

The two MTHFR polymorphisms, rs1801133 (C677T) and rs1801131 (A1298C) were analyzed in the study. For genotyping, genomic DNA was isolated from blood samples and genetic analysis was carried out by the PCR-RFLP method using Thermo Fisher's QuantStudio™ 5 Real-Time PCR System [12].

2.4. Statistical Analysis

All statistical analyses were done with the statistical software package, Graph Pad Prism version 9.00 (Graph pad Software, Inc., San Diego, USA) Genotype distributions and allele frequencies were calculated by the gene-counting method. Quantitative variables were expressed as mean ± standard deviation. The difference in general characteristics between males and females was analysed using the nonparametric, Mann-Whitney *U* test. The difference with respect to age was assessed using intergroup comparison of one-way ANNOVA. In order to evaluate the association of serum marker levels with genotypes, Pearson's correlation analysis was carried out with the genotypes, wild, heterozygous, and mutant with assigned values 0, 1, and 2, respectively. The association between age and serum marker levels was carried out by Pearson's correlation analysis. Assessment of the association between tHcy and serum marker levels was carried out by Pearson's correlation analysis. A P-value of 0.05 was considered statistically significant for the study.

3. Results

3.1. Basic Characteristics and Genotype Frequency

A total of 1295 samples met the eligibility criteria of the study and they were used for analysis. The basic characteristics of the study subjects are given in **Table 1**. The mean age of the study subjects was 48.2 ± 16.1 years (range: 5 - 91 years) for males and 48.5 ± 15.7 years (range: 11 - 90 years) for females. With respect to serum cardiac markers, CHOL, HDL and APOA were significantly higher (P = 0.002, <0.0001, <0.0001 respectively) in females than in males (**Table 1**) On the other hand, the markers, TRIG (P = 0.0005), APOBAR (P < 0.0001), BNPNT (P < 0.0001), SDLDL (P < 0.0001), and PLAC (P = 0.022) had significantly higher levels in males than in females. Similarly, tHcy (P < 0.0001) and visceral fat area (P = 0.001) levels were much lower in females than in males. The overall mean plasma tHcy was 9.7 ± 3.4 μmol/L (range: 3.3 μmol/L - 32.96 μmol/L) and was

Table 1. Basic characteristics of the study population.

Parameter	Total	Male (n = 503, 38.8%)	Female (n = 792, 61.1%)	P
Age (Yrs)	48.34 ± 15.98	48.53 ± 15.75	48.25 ± 16.11	
CHOL (mg/dL)	194 ± 44.6	189.1 ± 45.79	197.2 ± 43.56	0.002***
LDL (mg/dL)	124.4 ± 39.59	125.6 ± 40.09	123.7 ± 39.29	0.1622
LDLCAL (mg/dL)	116.1 ± 38.12	116.5 ± 39.4	115.9 ± 37.3	0.6097
HDL (mg/dL)	57.16 ± 16.3	50.11 ± 13.9	61.62 ± 16.14	<0.0001****
TRIG (mg/dL)	105.3 ± 83.42	116 ± 111.7	98.82 ± 58.31	0.0005***
APOA (mg/dL)	168.6 ± 37.13	153.7 ± 33.28	178 ± 36.38	<0.0001****
APOB (mg/dL)	98.37 ± 28.31	100 ± 29.11	97.33 ± 27.76	0.0636
APOBAR (U/L)	0.59 ± 0.2	0.63 ± 0.45	0.55 ± 0.18	<0.0001****
BNPNT (pg/mL)	85.27 ± 351.3	97.11 ± 562.1	78.18 ± 91.77	<0.0001****
HOMOC (μmol/L)	9.723 ± 3.429	10.67 ± 3.6	9.12 ± 3.17	<0.0001****
HSCRP (mg/dL)	2.7 ± 6.608	2.705 ± 7.09	2.69 ± 6.27	0.2701
SDLDL (mg/dL)	31.51 ± 14.48	34.54 ± 15.66	29.58 ± 13.33	<0.0001****
OXLDL (U/L)	46.82 ± 20.34	47.48 ± 21.02	46.41 ± 19.9	0.4574
LPA (mg/dL)	35.71 ± 34.92	34.63 ± 34.01	36.39 ± 35.5	0.3101
MPO (pmol/L)	1116 ± 971.3	1082 ± 921.4	1137 ± 1001	0.7838
PLAC (ng/mL)	163.3 ± 43.46	185.3 ± 44.02	149.4 ± 36.98	<0.0001****

The values are presented as mean ± standard deviation. ****indicates the significant P < 0.0001 and ***indicates the significant P < 0.001 between serum markers levels of males and females in the study population. Abbreviations are provided in **Appendix 1**.

significantly higher in males than in females ($P < 0.0001$) (Table 1).

3.2. Frequency of MTHFR

The mutant variant frequency for rs1801133 was 9.8% (TT) while that for rs1801131 was 10.7% (CC) in the overall population. However, the distribution was marginally higher in women (10.9% and 12.1%) compared to men (8.1% and 8.5%) The heterozygous allele carriers were about the same size as the wild-type allele carriers in the study population for both SNPs (Table 2).

3.3. Relation between MTHFR Polymorphisms and tHcy Levels

3.3.1. Distribution of tHcy in Association with Gender

Plasma tHcy concentrations did not differ significantly between wild and mutant genotypes for the mutation, rs1801133 in both males ($P = 0.08$) (wild type: 10.21 μmol/L vs mutant type: 11.49 μmol/L) and females ($P = 0.91$) (wild type: 9.19 μmol/L vs 9.03 μmol/L) (Table 3) A similar observation was made for the

Table 2. Genotype frequency for C677T and A1298C SNPs.

677C > T	Genotype			
	N (No. of individuals)	CC (wild)	CT (Het)	TT (Mutant)
	1295	573 (44.1)	596 (45.9)	128 (9.8)
Male	503 (38.8)	232 (46.1)	230 (45.7)	41 (8.1)
Female	792 (61.1)	340 (42.9)	365 (46.1)	87 (10.9)
1298A > C	N (No. of individuals)	AA (wild)	AC (Het)	CC (Mutant)
	1295	594 (45.9)	562 (43.3)	139 (10.7)
Male	503 (38.8)	236 (46.9)	224 (44.5)	43 (8.5)
Female	792 (61.1)	358 (45.2)	338 (42.6)	96 (12.1)

Percentage of Number of subjects in parentheses.

Table 3. Homocysteine levels (μmol/L) among genotypes.

677C > T	CC (W)	CT	TT	P
Male	10.21	10.98	11.49	0.08
Female	9.19	9.08	9.03	0.91
1298A > C	AA	AC	CC	
Male	10.71	10.68	10.35	0.81
Female	8.92	9.20	9.55	0.20

mutation, rs1801131, wherein plasma tHcy levels were not significantly different for wild and mutant genotypes, in males ($P = 0.81$) (wild type: 10.71 μmol/L vs 10.35 μmol/L) as well as females ($P = 0.20$) (wild type: 8.92 μmol/L vs 9.55 μmol/L) (Table 3) However, males were seen to have higher tHcy levels when compared to females for both the mutations, rs1801133 and rs1801131.

3.3.2. Distribution of tHcy in Association with Age

When associated with age, increase in tHcy levels correlated with increasing age. A significant increase in tHcy levels with respect to age was observed in the wild and heterozygous genotypes for both the mutations, rs1801133 and rs1801131 ($P < 0.05$) (Table 4) No significant difference was observed in the tHcy levels for the mutant genotypes of rs1801133 and rs1801131. However, high tHcy values were seen in the younger age groups for the mutant genotypes of both the mutations.

3.4. Correlation between Plasma tHcy and Serum Cardiac Markers

On carrying out Pearson's correlation between plasma tHcy levels and serum cardiac markers, BNPNT ($P < 0.0001$), SDLDL ($P = 0.018$), and MPO ($P = 0.004$) were seen to be statistically significant in males. On the other hand, tHcy

Table 4. Homocysteine levels among age groups.

677C > T				≤25	26 - 40	41 - 60	>60	P (Anova)
	CC	Male	N	(27) 9.061	35 9.403	104 9.912	68 11.53	<0.0001****
		Female	N	40 7.127	56 9.028	151 9.26	93 10.04	0.0003**
	CT	Male	N	24 9.143	45 10.23	101 11.02	59 12.26	0.0032**
		Female	N	40 7.769	102 8.531	157 9.588	92 9.892	<0.0001****
	TT	Male	N	4 9.03	7 12.6	23 10.85	7 13.89	0.2803
		Female	N	9 8.537	16 8.779	37 9.095	26 9.249	0.8584
1298A > C				≤25	26 - 40	41 - 60	>60	P (Anova)
	AA	Male	N	25 8.767	56 10.2	109 10.5	62 12.03	0.0009**
		Female	N	33 7.472	84 8.647	167 8.837	90 9.838	0.0002**
	AC	Male	N	24 9.247	56 9.728	101 10.69	55 12.22	0.0004**
		Female	N	45 7.647	95 8.596	157 9.346	71 10.29	<0.0001****
	CC	Male	N	6 9.852	6 9.393	20 10.04	11 11.7	0.1333
		Female	N	11 7.446	23 9.764	38 9.822	30 10.16	0.3227

levels significantly correlated with most serum cardiac markers except APOBAR, HSCRP, OXLDL, LPA, and PLAC in females (Table 5).

3.5. Distribution of Serum Cardiac Markers in Association with Gender and Age

In order to investigate the association of the MTHFR polymorphisms on serum cardiac markers with respect to gender and age, we evaluated the levels of all the serum cardiac markers across the three genotypes for rs1801133 and rs1801131. The complete data of all the serum cardiac markers with respect to age and gender for both the mutations are given in Supplementary Table S1. For the mutation, rs1801133, the markers, APOA ($P = 0.01$), APOB ($P = 0.0002$), APOBAR ($P = 0.0108$), CHOL ($P < 0.0001$), LDL ($P = 0.0006$) and LDLCAL ($P = 0.0004$) significantly differed with respect to age for wild-type males, while the markers, APOA ($P < 0.0001$), APOB ($P < 0.0001$), BNPNT ($P < 0.0001$), CHOL ($P <$

Table 5. Plasma Homocysteine correlation with serum cardiac markers.

	Male			Female		
Parameter	R	95% CI	P	r	95% CI	P
CHOL	0.03	(−0.07 - 0.12)	0.61	0.15	(0.076 - 0.2132)	<0.0001****
LDL	0.05	(−0.05 - 0.14)	0.31	0.11	(0.04 - 0.18)	0.002**
LDLCAL	0.02	(−0.07 - 0.12)	0.66	0.07	(0.0003 - 0.13)	0.04*
HDL	0.02	(−0.07 - 0.12)	0.63	0.10	(0.035 - 0.17)	0.003**
TRIG	−0.05	(−0.15 - 0.05)	0.3	0.15	(0.08 - 0.22)	<0.0001****
APOA	0.00	(−0.1 - 0.09)	0.92	0.12	(0.05 - 0.18)	0.001**
APOB	0.06	(−0.04 - 0.15)	0.26	0.15	(0.08 - 0.21)	<0.0001****
APOBAR	0.09	(−0.007 - 0.19)	0.06	0.05	(−0.02 - 0.11)	0.18
BNPNT	0.38	(0.29 - 0.46)	<0.0001****	0.20	(0.12 - 0.26)	<0.0001****
HSCRP	0.02	(−0.08 - 0.11)	0.74	0.07	(−0.001 - 0.14)	0.06
SDLDL	0.12	(0.019 - 0.21)	0.018*	0.14	(0.07 - 0.2)	0.0001***
OXLDL	0.01	(−0.09 - 0.11)	0.81	0.06	(−0.008 - 0.14)	0.08
LPA	−0.08	(−0.19 - 0.03)	0.15	0.02	(−0.06 - 0.1)	0.6
MPO	0.15	(0.05 - 0.24)	0.004**	0.11	(0.04 - 0.18)	0.002**
PLAC	0.07	(−0.032 - 0.16)	0.18	−0.01	(−0.08 - 0.05)	0.68

0.0001), LDL (P = 0.0004), LDLCAL (P < 0.0001), OXLDL (P = 0.0235) and SDLDL (P = 0.0005) showed a significant difference in wild-type females with respect to age. In wild type males, APOB, CHOL, LDL and LDLCAL increased up till 60 years after which a decline was seen whereas the markers, APOA and APOBAR showed periodic elevation and decline with age. In wild type females, APOA, APOB, BNPNT, CHOL, LDL, LDLCAL and OXLDL increased with increasing age whereas SDLDL levels showed periodic changes with age. The marker, LPA (P = 0.0022) significantly differed in heterozygous males with its levels periodically changing with age. For heterozygous females, the markers, APOA (P = 0.0033), APOB (P < 0.0001), BNPNT (P < 0.0001), CHOL (P < 0.0001), HDL (P = 0.0243), LDL (P < 0.0001) and SDLDL (P = 0.0019) showed a significant difference with respect to age. Of these markers, APOA, APOB, BNPNT and HDL were seen to increase with increase in age whereas SDLDL, CHOL and LDL increased till the age of 60 years post which the marker levels dropped in heterozygous females. The markers, APOB (P = 0.0024), BNPNT (P = 0.0037), CHOL (P = 0.0007), LDL (P = 0.0026) and LDLCAL (P = 0.0011) significantly increased with increase in age for mutant females wherein APOB, LDL, LDLCAL and CHOL levels increased up till 60 years after which the levels of these markers reduced, while BNPNT levels periodically changed with age. No significant difference was observed in the marker levels for mutant males with respect to age (Supplementary Table S1).

For the mutation rs1801131, the markers, APOA (P = 0.0096), APOB (P =

0.001), BNPNT (P = 0.0015), CHOL (P = 0.0034), LDL (P = 0.0148), LDLCAL (P = 0.0063), LPA (P = 0.0497), SDLDL (P = 0.0054) and TRIG (P = 0.0126) significantly differed with respect to age in wild type males while the markers, APOA (P < 0.0001), APOB (P < 0.0001), APOBAR (P = 0.0177), BNPNT (P < 0.0001), CHOL (P < 0.0001), HDL (P = 0.0209), LDL (P < 0.0001), LDLCAL (P < 0.0001), SDLDL (P < 0.0001) and TRIG (P = 0.0003) significantly differed with respect to age in wild type females. In wild type males, the levels of APOA, BNPNT, LPA and TRIG were seen to periodically change with age whereas the markers APOB, CHOL, LDL, LDLCAL, and SDLDL increased up till 60 years after which a decline was observed. For wild type females, the markers TRIG, HDL, and APOBAR showed a periodic change in association with age while SDLDL, LDLCAL, LDL, and APOB levels increased up till 60 years, post which their levels reduced. Additionally, CHOL, BNPNT and APOA levels increased with increasing age in wild type females. Age associated significant difference was seen in the levels of APOB (P = 0.0023), CHOL (P = 0.0009), LDL (P = 0.0024), LDLCAL (P = 0.0035), PLAC (P = 0.0998) and SDLDL (P = 0.0013) in heterozygous males, with the markers, SDLDL, LDLCAL, LDL, CHOL, and APOB periodically changing with age and PLAC levels increasing up till 60 years, followed by a decrease in its levels. Heterozygous females showed a significant difference in marker levels with respect to age for the markers, APOA (P = 0.0058), APOB (P < 0.0001), BNPNT (P < 0.0001), CHOL (P < 0.0001), HDL (P = 0.0268), LDL (P = 0.0004), LDLCAL (P < 0.0001), OXLDL (P = 0.042), PLAC (P = 0.0067) and SDLDL (P = 0.0047) OXLDL, LDLCAL, LDL, CHOL, BNPNT, APOA and APOB increased with increasing age whereas PLAC and HDL periodically changed in association with age in heterozygous females. At the same time, SDLDL levels increased up till 60 years and then decreased in heterozygous females. For mutant males, BNPNT (P = 0.0185) showed significant difference with respect to age and the marker levels were periodically altered with respect to age. No significant difference was seen in the marker levels for mutant females with respect to age (Supplementary Table S1).

4. Discussion

CVD is the principal cause of morbidity and mortality worldwide with inherited DNA sequence variants and environmental risk factors playing a role in conferring risk for the disease [13] [14]. MTHFR is a key regulatory enzyme in homocysteine metabolism as it catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. High homocysteine levels have been recognized as one of the emerging biomarker and independent risk factor for various diseases, including CVD, neurocognitive disorders, and osteoporotic fracture [15]. Several studies have proven that impairment in the methylation pathway and increased plasma total homocysteine concentrations are associated with atherosclerotic diseases and venous thrombosis [2]. The gene that codes for

the enzyme MTHFR which is associated with homocysteine metabolism has two alleles. Of the many polymorphisms coding for the enzyme, the rs1801133 and rs1801131 SNPs have been widely studied. In the current study, we evaluated the association of the two MTHFR mutations, rs1801133 and rs1801131 with 16 well-established serum cardiac markers. As age and gender are factors that can affect MTHFR mutations [10], we also examined the influence of age and gender on the association between the two MTHFR polymorphisms and serum cardiac marker levels.

Homocysteine's association with CVD might occur due to its thrombogenic effect, where homocysteine and its metabolites affect the expression of thrombomodulin, activation of protein C, increase platelet aggregation and thromboxane production [16] [17]. Additionally, the oxidation of homocysteine may result in the formation of hydrogen peroxide and free radicals, which can promote the oxidation of LDL and damage the endothelial lining, thus leading to further progression of atherosclerosis [18] [19]. In the current study, we observed that plasma tHcy levels did not differ significantly in males and females for both the mutations, rs1801133 (P = 0.08 and P = 0.91, respectively) and rs1801131 (P = 0.81 and P = 0.20, respectively) However, we observed that males had higher levels of tHcy (10.67 ± 3.6 μmol/L) than females (9.12 ± 3.17 μmol/L) (P < 0.0001) A study by Fonseca *et al*., 1999, hypothesizes that the differences in tHcy based on gender could occur due the interplay between hormones and endogenous sex hormones [20]. Another important factor that could affect tHcy level is vitamin B12. Vitamin B12 plays a crucial role in the remethylation of tHcy to methionine and its deficiency can impair this process leading to tHcy accumulation [9]. Moreover, vitamin B12 deficiency was found to be significantly associated with gender, with men having lower vitamin B12 levels than females [21]. This could explain the correlation of males having higher tHcy levels owing to a greater prevalence of vitamin B12 deficiency in males. Further studies can probe the association between vitamin B12 and circulating tHcy levels in association with gender. Additionally, a study byMunshi *et al*., 1996, proved that females had a greater tHcy flux through the trans-sulfuration pathway, which lowers tHcy levels [22]. The above-mentioned factors could therefore account for the gender-wise difference in plasma tHcy levels.

Age analysis revealed that increasing age correlated with a rise in tHcy levels. The increase in tHcy levels in association with age was observed in the wild and heterozygous genotypes for both the mutations, rs1801133 and rs1801131 (P < 0.05) This trendline is consistent with the findings of Jacques, et al, 1999, wherein they showed that tHcy concentrations increased with age throughout adulthood in white and black populations in the United States [23]. However, no significant difference was observed in the tHcy levels for the mutant genotypes of rs1801133 and rs1801131. Lack of significance in the mutant genotype could be a consequence of low sample numbers in mutant groups. Further studies with mutant groups could help warrant the association of tHcy with age for the two

mutations. Nevertheless, we did observe high levels of plasma tHcy in the younger age groups for the mutant genotypes of both the mutations. Thus, a lack of significance could also be a result of high tHcy levels present across all ages for the mutant genotype, implying that the mutation must be giving rise to high tHcy levels irrespective of age. Further studies must be carried out in order to fortify these findings. In line with previous studies, including, Zhang *et al*., 2020, and Yao *et al*., 2017, we suggest that gender-based increase in tHcy levels in males and increasing tHcy levels in association with age could be a risk factor contributing to CVD [24] [25]. Knowledge of general tHcy levels and its fluctuations in healthy populations with respect to age and gender can be clinically insightful as it can help clinicians correctly interpret tHcy results, particularly when used as a biomarker in the diagnosis, prognosis, or surveillance of CVD. This holds true for other cardiac biomarkers as well, thus, the current study evaluated the association of various serum cardiac markers with gender and age for rs1801133 and rs1801131.

In order to evaluate the effects of MTHFR polymorphisms on other serum cardiac markers, the differences in the levels of the markers between the wild and mutant genotypes were analyzed. We evaluated the levels of all the serum cardiac markers for rs1801133 and rs1801131 in association with gender and age in order to understand the influence of these factors on serum cardiac marker levels. We obtained significance for multiple markers with respect to gender and age for both the mutations. HDL showed significant difference only in females in correlation with age for the mutation rs1801133. These findings are in agreement with the study by Jousilahti *et al*., 1999, wherein HDL levels were higher in females compared to males [26]. We found that CHOL levels increased with increasing age for both genders. This observation was also made by Jousilahti *et al*., 1999, where they reported that the CHOL levels increased in both males and females but the levels dropped after the age of 45 years for males and 60 years for females. This observation is similar to our study except that we found a decrease in CHOL levels in males post 60 years of age and the levels increased for females with age. A study by Anagnostis *et al*., 2016, reported that CHOL levels were higher in post-menopausal women [27]. Thus, increase in CHOL in older women can be attributed to hormonal changes associated with age.

We also observed that APOA levels were found to be higher in females than males. Additionally, APOA levels increased with increasing age. APOB also increased with increase in age for both the sexes. Increase in APOA and APOB was seen in post-menopausal women [28]. Thus, the increase in APOA and APOB in females with respect to age could be associated with hormones. We observed that LDL marker levels showed periodic elevation and decline with increasing age for both the genders. According to Kim *et al*., 2019, the association between LDL and CVD risk was stronger in men than in women, which suggests that there are more atherogenic characteristics of LDL in men [29]. In the present study, BNPNT levels increased with age in both genders. It was also observed

that females had higher levels of BNPNT compared to males. Redfield *et al*., 2002, also showed that BNPNT levels were higher in females than males, and it increased with increasing age [29]. We did find significant associations for the markers, LDLCAL, SDLDL, APOBAR, OXLDL, LPA, TRIG, and PLAC with respect to gender and age for both the mutations. However, all these associations differed in trends and further studies will have to be conducted in order to understand these associations in correlation with age and gender for rs1801133 and rs1801131. The markers, HSCRP and MPO showed no significant correlation in this study. Although we did not observe any uniform trends in the marker levels, we stress on the importance of genetic testing to identify MTHFR polymorphisms at a young age. Genetic testing is easy to perform and the results are not influenced by environmental factors. Since, the polymorphisms are ascertained at birth, testing for it at a young age will help to take adequate measures to reduce/prevent the risk of developing CVDs later in life.

In conclusion, we aimed at understanding the association of 16 serum cardiac markers with respect to gender and age for the MTHFR polymorphisms, rs1801133 and rs1801131. To our knowledge, this is the first study that analyses the MTHFR polymorphisms against such a large set of serum cardiac markers. The study managed to establish the association between the MTHFR polymorphisms and serum cardiac markers with respect to age and gender. However, this study was accompanied with a few limitations. Firstly, we were unable to infer causality as this was a retrospective cross-sectional study. The study did not include aspects such as vitamin B12 and vitamin B9 that can influence tHcy levels and lifestyle factors such as smoking, diet, body mass index (BMI) that affect serum cardiac markers. Hormones are also important factors to be considered when conducting gender-based analysis. Additionally, the low number of mutant individuals presents after age and gender segregation could be affecting the current results. Thus, further studies can aim at including equal number of individuals of the three genotypes. In such cases, case-control studies with age and gender-matched cases and controls can be good prospects for fortifying the observations obtained in this study.

5. Conclusion

The present study reports the association of tHcy, HDL, LDL, CHOL, APOA, APOB, BNPNT, LDLCAL, SDLDL, APOBAR, OXLDL, LPA, TRIG, and PLAC with respect to age and gender for the mutations, rs1801133, and rs1801131. This was a comprehensive study that looked at the changing trends in serum cardiac marker levels for rs1801133 and rs1801131 with respect to gender and age. Previous studies have reported that homocysteine is an independent risk factor for CVDs. Since MTHFR has an important role in tHcy metabolism, genetic testing to identify MTHFR polymorphisms is recommended. Polymorphisms can be ascertained at birth and can be tested at a very young age which may help to prevent/delay the onset of CVDs via medication, diet, or lifestyle

changes. Moreover, environmental factors do not affect the polymorphisms. This study successfully indicated that there is an association between MTHFR polymorphisms and serum cardiac markers and that assessment of polymorphisms along with biomarker testing can be key in the early detection and prevention of CVDs. The present study found that high tHcy levels in males and increasing tHcy levels in association with age could be a risk factor contributing to CVD. We also observed high triglyceride levels in males having the mutant rs1801131 genotype. Frequent testing of triglyceride levels and lifestyle changes might help to reduce the risk of CVDs in these individuals. Although tHcy levels in females were not as high as in males, other serum cardiac markers were higher and can pose risk factors in females. Post-menopausal females with mutant rs1801133 allele showed high levels of cholesterol. Close monitoring of cholesterol levels of post-menopausal women is necessary to reduce or prevent the development of CVDs. Further analysis will be required to understand the pattern of association of the rest of the serum cardiac markers with age and gender for rs1801133 and rs180113. However, the inclusion of factors such as nutrient status (vitamin B12 and vitamin B9), hormones and lifestyle factors can be considered for further studies. In conclusion, early detection of MTHFR polymorphisms and lifestyle interventions may help prevent/reduce the risk of developing CVDs in susceptible individuals.

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Disclosure

This study is based on a retrospective analysis of de-identified laboratory data. The data and materials in this manuscript have not been published elsewhere and are not under consideration by another journal.

Authors' Contributions

UB, HKK, KK, and TW performed the research. HKK, JJR, and VJ designed the study. UB, HKK, KB and QS analysed the data. UB, MP, CS, and HKK wrote the article.

Availability of Data and Material

The data used to support the findings of this study can be acquired from Vibrant America LLC.

Ethics Approval and Consent to Participate

IRB exemption (work order #1-1098539-1) was determined by the Western Institutional Review Board (WIRB) for Vibrant America Biorepository to use de-linked and de-identified remnant human specimen and medical data for research purposes.

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Competing Interests

UB, MP, CS, and QS are paid employees of Vibrant America LLC. VJ, KK, TW, KB, JJR, and HKK are paid employees of Vibrant Sciences LLC.

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Abbreviations

TC - Total Cholesterol
LDL - Low Density Lipoprotein
HDL - High Density Lipoprotein
Apo A-1 - Apolipoprotein A-I
Apo B - Apolipoprotein B
ApoE - Apolipoprotein E
Lp(a) - Lipoprotein (A)
hs-CRP - High-Sensitivity C-Reactive Protein
NT-proBNP - N-terminal (NT)-pro hormone BNP
SdLDL - Small Density Low Density Lipoprotein
ox-LDL - Oxidised Low Density Lipoprotein
MPO - Myeloperoxidase
PLAC - Lipoprotein- Associated Phospholipase (Lp-PLA2) Test
SNP - Single Nucleotide Polymorphism
CVD - Cardio Vascular Disease
CAD - Coronary Artery Disease
MTHFR - 5,10-Methylenetetrahydrofolate Reductase

Supplemntary

Table S1. Serum marker levels with repect to genotypes of 677 C > t and 1298 A > C in age groups.

Genotype without gender

677	Mutant	≤25	26 - 40	40 - 60	>60	P
	CHOL	166.5	172.4	203.4	208.1	0.0003
	LDL	104.3	105	132.3	134	0.0012
	LDLCAL	97.08	97.03	124.1	125.9	0.0007
	HDL	55.23	58.09	56.87	62	0.4095
	TRIG	71.15	86.35	112.3	100.9	0.0692
	APOA	155.7	169.3	171.4	183	0.1706
	APOB	82.57	84.16	106	104.4	0.0005
	APOBAR	0.5498	0.5331	0.6103	0.5854	0.2983
	BNPNT	32.88	31.51	76.13	91.4	0.1934
	HOMOC	8.688	9.941	9.766	10.29	0.5769
	HSCRP	0.9692	4.547	3.343	1.812	0.4161
	SDLDL	22.28	27.35	34.15	30.11	0.0093
	OXLDL	43.16	43.61	48.47	47.62	0.7701
	LPA	29.61	35.22	32.71	32.38	0.9787
	MPO	800.8	1203	1081	1093	0.6967
	PLAC	147.8	151	168.7	139.9	0.0377

1298	Mutant	≤25	26 - 40	40 - 60	>60	P
	CHOL	173.6	187.5	207.5	199.9	0.5344
	LDL	110.8	118.5	134.4	127.6	0.876
	LDLCAL	99.72	105.4	127.8	118.2	0.9194
	HDL	54.47	59.45	59.81	60.15	0.7269
	TRIG	97	121.2	100.8	108	0.0952
	APOA	151.9	168	173.7	175.9	0.624
	APOB	86.06	92.65	104.9	100.6	0.8412
	APOBAR	0.6018	0.5822	0.6105	0.5818	0.4852
	BNPNT	41.08	88.28	67.37	145.1	0.0243
	HOMOC	8.295	9.688	9.896	10.57	0.7314
	HSCRP	3.699	2.741	2.146	2.162	0.8216
	SDLDL	29.88	29.56	34.39	31.07	0.9281
	OXLDL	42.61	44.09	50.39	49.82	0.1664
	LPA	44.59	40.06	36.13	44.3	0.5483
	MPO	1498	879.9	1022	1181	0.4024
	PLAC	154.5	148	166.5	162.6	0.4043

Genotype with gender 677

Male	677	Wild Male	≤25	20 - 40	40 - 60	>60	
			27	35	104	68	
		CHOL	159.6	187.9	201.5	175.9	<0.0001
		LDL	101.4	130.7	132.4	115.4	0.0006
		LDLCAL	92.21	121	124.1	106.5	0.0004
		HDL	49.89	46.63	51.2	48.48	0.2676
		TRIG	87.67	101.4	146.2	110.6	0.1451
		APOA	145.4	141.6	161	153.2	0.01
		APOB	77.41	100.4	104.6	95.45	0.0002
		APOBAR	0.532	0.7113	0.6501	0.639	0.0108
		BNPNT	28.98	28.69	41.73	291.4	0.1292
		HOMOC	9.061	9.403	9.912	11.53	<0.0001
		HSCRP	3.099	2.07	1.995	2.706	0.6748
		SDLDL	26.34	36.22	35.71	33.19	0.0429
		OXLDL	39.78	43.97	48.53	47.45	0.2999
		LPA	37.57	28.11	36.78	34.65	0.7275
		MPO	1141	1150	1150	920.6	0.5098
		PLAC	169.5	191	185.4	181	0.2282

1298

1298	Wild Male	≤25	20 - 40	40 - 60	>60	
		25	56	109	62	
	CHOL	163.2	193.1	197	182.6	0.0034
	LDL	108.2	125.9	132	118	0.0148
	LDLCAL	94.74	118.5	122.2	110.7	0.0063
	HDL	51.36	46.82	50.8	51.68	0.2208
	TRIG	85.28	198.5	116.3	105.7	0.0126
	APOA	149.9	141.5	158.8	159.5	0.0096
	APOB	81.23	97.39	105.2	96.81	0.001
	APOBAR	0.5702	0.6705	0.6564	0.6096	0.0741
	BNPNT	25.35	24.82	50.95	93.68	0.0015
	HOMOC	8.767	10.2	10.5	12.03	0.0009
	HSCRP	4.27	2.816	1.873	3.793	0.3467
	SDLDL	26.91	33.06	37.09	31.77	0.0054
	OXLDL	50.11	50	48.02	47.58	0.9074
	LPA	39.14	21.59	34.84	41.29	0.0497
	MPO	890	1066	1073	892.7	0.4637
	PLAC	167.6	191.6	187.2	181.2	0.1044

677	Hetero Male	≤25	20 - 40	40 - 60	>60	
		24	45	101	59	
	CHOL	173	197.8	197.1	190.6	0.1192
	LDL	113.6	129.9	132.9	125.8	0.1981
	LDLCAL	101.8	122.1	123.4	117.7	0.1132
	HDL	52.54	50.96	50.55	50.59	0.9477
	TRIG	93.33	123.6	113.8	111.6	0.382
	APOA	153.2	152.7	154.9	154.2	0.9837
	APOB	87.08	103.8	105.3	102.6	0.0589
	APOBAR	0.6133	0.6812	0.6751	0.6562	0.5919
	BNPNT	32.63	36.86	194.4	129.2	0.705
	HOMOC	9.143	10.23	11.02	12.26	0.0032
	HSCRP	5.067	1.493	2.528	3.822	0.2688
	SDLDL	29.57	37.73	37.25	33.76	0.1003
	OXLDL	58.18	49.49	48.3	44.77	0.0778
	LPA	46.14	21.16	30.36	48.27	0.0022
	MPO	1035	1010	1258	885.5	0.0884
	PLAC	167.1	191	193.5	185.1	0.0713

1298	Hetero Male	≤25	20 - 40	40 - 60	>60	
		24	56	101	55	
	CHOL	166.8	204.6	196.3	178.9	0.0009
	LDL	105.5	136.6	131.6	117.5	0.0024
	LDLCAL	99.17	128.9	122.4	108.7	0.0035
	HDL	50.92	50.18	51.29	48.31	0.6895
	TRIG	83.54	127.5	111.7	117.5	0.1092
	APOA	149.8	151.1	157.8	145.8	0.1983
	APOB	82.92	107.9	104.6	95.57	0.0023
	APOBAR	0.5649	0.7152	0.6677	0.6625	0.0597
	BNPNT	30.35	44.48	197.2	162.9	0.6608
	HOMOC	9.247	9.728	10.69	12.22	0.0004
	HSCRP	3.115	1.4	2.598	2.628	0.5091
	SDLDL	26.31	40.91	36.04	33.1	0.0013
	OXLDL	48.92	50.33	48.66	41.4	0.1457
	LPA	42.06	26.37	31.72	40.57	0.1915
	MPO	1183	1110	1311	991	0.3407
	PLAC	165.7	189.6	190.3	184.2	0.0998

Female 677	Mutant Male	≤25	20 - 40	40 - 60	>60	
		4	7	23	7	
	CHOL	163.5	154	184	175.9	0.1925
	LDL	113	103.6	123.7	120.1	0.519
	LDLCAL	103.2	94.29	113.4	108.5	0.5368
	HDL	46	44.57	50.57	51.43	0.6678
	TRIG	71.5	75.71	100.2	79.57	0.3016
	APOA	142.3	134.9	155	151.5	0.6177
	APOB	88.98	81.56	101.1	94.78	0.2496
	APOBAR	0.6505	0.6128	0.6423	0.6318	0.9797
	BNPNT	10.66	18.85	110.7	84.57	0.6615
	HOMOC	9.03	12.6	10.85	13.89	0.2803
	HSCRP	0.6875	10.35	2.948	0.7257	0.2844
	SDLDL	22.58	28.94	33.93	30.14	0.2386
	OXLDL	47.27	46.95	47.09	39.26	0.8894
	LPA	35.04	23.86	29.32	22.37	0.8951
	MPO	602.8	1212	1002	1099	0.6707
	PLAC	162.5	175.7	183	181.1	0.8063

1298	Mutant Male	≤25	20 - 40	40 - 60	>60	
		6	6	20	11	
	CHOL	172.5	180.7	205.9	183.1	0.3769
	LDL	113	130.7	139.2	133.4	0.6792
	LDLCAL	99.63	113.2	131.6	118.6	0.4069
	HDL	47.67	40.33	50.75	45.55	0.2501
	TRIG	126	135.8	122.2	94.73	0.4699
	APOA	138.2	129.6	159.3	142.4	0.1134
	APOB	85.83	101.5	110.2	104.6	0.4329
	APOBAR	0.6507	0.7985	0.692	0.6993	0.6875
	BNPNT	38.91	39.78	34.67	211.3	0.0185
	HOMOC	9.852	9.393	10.04	11.7	0.1333
	HSCRP	4.1	7.393	2.987	1.91	0.5272
	SDLDL	34.5	45.73	36.72	36.35	0.7644
	OXLDL	44.71	38.97	51	47.81	0.7209
	LPA	46.99	26.37	27.49	31.27	0.6579
	MPO	1245	797.8	950.9	881	0.7899
	PLAC	178.7	184.2	185.5	190.5	0.9431

677	Wild Female	≤25	20 - 40	40 - 60	>60	
		40	56	151	93	
	CHOL	169.3	182.4	204.2	208.3	<0.0001
	LDL	104.2	113.3	128.4	130.5	0.0004
	LDLCAL	95.04	103.9	119.8	123.4	<0.0001
	HDL	56.5	61.07	63.19	63.21	0.1111
	TRIG	88.95	89.13	106.1	108.4	0.0754
	APOA	160.2	172.3	183.1	188.8	<0.0001
	APOB	81.14	88.1	101.6	103.7	<0.0001
	APOBAR	0.5367	0.5122	0.5661	0.5603	0.3126
	BNPNT	33.05	62.76	72.63	105.3	<0.0001
	HOMOC	7.127	9.028	9.26	10.04	0.0003
	HSCRP	2.766	1.848	2.414	3.315	0.2232
	SDLDL	26.07	24.95	32.01	32.33	0.0005
	OXLDL	40.65	44.52	49.41	51.83	0.0235
	LPA	45.64	30.68	39	47.62	0.154
	MPO	1155	1277	1099	1443	0.149
	PLAC	135.6	152	149.1	151.2	0.154

1298	Wild Female	≤25	20 - 40	40 - 60	>60	
		33	84	167	90	
	CHOL	164.6	179.5	206.9	207.1	<0.0001
	LDL	99.73	109.1	131.8	126.3	<0.0001
	LDLCAL	92.32	102.2	123	122.2	<0.0001
	HDL	55.7	61.73	61.47	65.32	0.0209
	TRIG	82.82	79.93	112.1	101.3	0.0003
	APOA	158.6	174.8	181.2	192.3	<0.0001
	APOB	78.54	86.1	103.5	100.9	<0.0001
	APOBAR	0.5079	0.5016	0.5746	0.5431	0.0177
	BNPNT	35.92	46.93	52.82	105.5	<0.0001
	HOMOC	7.472	8.647	8.837	9.838	0.0002
	HSCRP	2.535	3.175	3.44	2.067	0.5856
	SDLDL	23.42	25.19	32.95	30.86	<0.0001
	OXLDL	47.54	42.3	47.95	48.44	0.1815
	LPA	30.35	30.57	35.76	41.36	0.3421
	MPO	986.2	1189	1074	1232	0.5825
	PLAC	137.4	148.5	150.9	147.5	0.2847

677	Hetero Female	≤25	20 - 40	40 - 60	>60	
		40	102	157	92	
	CHOL	170.8	184.1	207.8	206.8	<0.0001
	LDL	106.7	115.1	134.2	127.3	<0.0001
	LDLCAL	97.89	106.8	125.3	123.4	<0.0001
	HDL	57.03	58.91	62.24	64.16	0.0243
	TRIG	79.58	90.08	101.5	100	0.1319
	APOA	163.4	168.3	175.4	184.2	0.0033
	APOB	82.95	88.88	99.79	102.3	<0.0001
	APOBAR	0.5293	0.5358	0.573	0.566	0.2885
	BNPNT	46.59	56.09	80.19	145.5	<0.0001
	HOMOC	7.769	8.531	9.588	9.892	<0.0001
	HSCRP	2.176	3.156	2.859	1.972	0.6674
	SDLDL	23.94	26.78	32.15	29.02	0.0019
	OXLDL	44.9	43.52	42.63	46.64	0.4949
	LPA	34.92	34.4	29.1	33.73	0.663
	MPO	1067	1027	990.2	1104	0.8414
	PLAC	136.1	151.2	149.2	151.1	0.0703

1298	Hetero Female	≤25	20 - 40	40 - 60	>60	
		45	95	157	71	
	CHOL	172.7	180.7	203.4	208.5	<0.0001
	LDL	107.6	115.2	129.5	131.6	0.0004
	LDLCAL	98.27	105.2	121	125.6	<0.0001
	HDL	57.71	56.8	62.1	62.85	0.0268
	TRIG	83.42	90.29	101.7	100.1	0.1721
	APOA	164.7	166.5	177.1	182.8	0.0058
	APOB	83.13	89.78	101.3	105.9	<0.0001
	APOBAR	0.5355	0.534	0.5663	0.5901	0.2059
	BNPNT	42.46	57.01	87.02	154.8	<0.0001
	HOMOC	7.647	8.596	9.346	10.29	<0.0001
	HSCRP	1.911	1.847	2.573	3.177	0.3134
	SDLDL	25.02	25.79	30.29	30.15	0.0047
	OXLDL	39.67	43.44	43.91	49.84	0.0421
	LPA	45.71	36.02	31.95	38.36	0.2495
	MPO	1020	1093	1081	1279	0.4408
	PLAC	134.4	157.9	150.5	153.9	0.0067

677	Mutant Female	≤25	20 - 40	40 - 60	>60	
		9	16	37	26	
	CHOL	9	16	37	26	
	LDL	167.9	180.4	215.5	215.2	0.0007
	LDLCAL	100.4	105.6	137.6	136.5	0.0026
	HDL	94.36	98.24	130.8	129.4	0.0011
	TRIG	59.33	64	60.78	64.74	0.6389
	APOA	71	91	119.8	105.5	0.1615
	APOB	161.6	184.4	181.7	190.6	0.2012
	APOBAR	79.73	85.29	109.1	106.1	0.0024
	BNPNT	0.505	0.5003	0.5905	0.5699	0.269
	HOMOC	42.75	37.05	55.59	91.37	0.0037
	HSCRP	8.537	8.779	9.095	9.249	0.8584
	SDLDL	1.11	2.008	3.596	2.077	0.5141
	OXLDL	22.16	26.66	34.28	29.79	0.0611
	LPA	40.81	42.05	49.41	49.35	0.5739
	MPO	26.9	38.06	34.63	35.11	0.9192
	PLAC	899.7	1199	1132	1083	0.9187

1298	Mutant Female	≤25	20 - 40	40 - 60	>60	
		11	23	38	30	
	CHOL	174.2	189.3	208.3	206.1	0.0715
	LDL	109.5	115.3	131.9	125.5	0.2568
	LDLCAL	99.76	103.2	125.8	118	0.1087
	HDL	58.18	64.43	64.58	65.5	0.651
	TRIG	81.18	117.4	89.61	112.8	0.193
	APOA	159.3	178	181.2	188.2	0.0891
	APOB	86.19	90.35	102.2	99.13	0.1468
	APOBAR	0.5752	0.5257	0.5686	0.5426	0.796
	BNPNT	42.27	100.9	84.59	123	0.0442
	HOMOC	7.446	9.764	9.822	10.16	0.3227
	HSCRP	3.458	1.528	1.689	2.26	0.1769
	SDLDL	27.36	25.34	33.17	29.13	0.1379
	OXLDL	41.55	45.89	50.07	50.61	0.6385
	LPA	42.87	43	40.28	50.17	0.856
	MPO	1666	847.9	1056	1284	0.2169
	PLAC	141.3	138.6	156.5	152.3	0.2939

Age No gender

677	Mutant	≤25	26 - 40	40 - 60	>60	P
	CHOL	166.5	172.4	203.4	208.1	0.0003
	LDL	104.3	105	132.3	134	0.0012
	LDLCAL	97.08	97.03	124.1	125.9	0.0007
	HDL	55.23	58.09	56.87	62	0.4095
	TRIG	71.15	86.35	112.3	100.9	0.0692
	APOA	155.7	169.3	171.4	183	0.1706
	APOB	82.57	84.16	106	104.4	0.0005
	APOBAR	0.5498	0.5331	0.6103	0.5854	0.2983
	BNPNT	32.88	31.51	76.13	91.4	0.1934
	HOMOC	8.688	9.941	9.766	10.29	0.5769
	HSCRP	0.9692	4.547	3.343	1.812	0.4161
	SDLDL	22.28	27.35	34.15	30.11	0.0093
	OXLDL	43.16	43.61	48.47	47.62	0.7701
	LPA	29.61	35.22	32.71	32.38	0.9787
	MPO	800.8	1203	1081	1093	0.6967
	PLAC	147.8	151	168.7	139.9	0.0377

1298	Mutant	≤25	26 - 40	40 - 60	>60	P
	CHOL	173.6	187.5	207.5	199.9	0.5344
	LDL	110.8	118.5	134.4	127.6	0.876
	LDLCAL	99.72	105.4	127.8	118.2	0.9194
	HDL	54.47	59.45	59.81	60.15	0.7269
	TRIG	97	121.2	100.8	108	0.0952
	APOA	151.9	168	173.7	175.9	0.624
	APOB	86.06	92.65	104.9	100.6	0.8412
	APOBAR	0.6018	0.5822	0.6105	0.5818	0.4852
	BNPNT	41.08	88.28	67.37	145.1	0.0243
	HOMOC	8.295	9.688	9.896	10.57	0.7314
	HSCRP	3.699	2.741	2.146	2.162	0.8216
	SDLDL	29.88	29.56	34.39	31.07	0.9281
	OXLDL	42.61	44.09	50.39	49.82	0.1664
	LPA	44.59	40.06	36.13	44.3	0.5483
	MPO	1498	879.9	1022	1181	0.4024
	PLAC	154.5	148	166.5	162.6	0.4043