

Supplementary material

Table 1S: Primer sequences and PCR conditions for tri and tetra ARMS PCR assays of VDR, CYP2R1 and CYP27B1 genes polymorphisms

Gene	Primer name	Primer sequence (5'-3')	Product size	Annealing Temp.
<i>VDR</i>	rs7975232 (ApaI) C/A	FO = GCCACAGGCTGTCCTAGTCAGGAG FI = AAGGCACAGGAGCTCTCATCTGGACC RO = GTCTGGGCTACAGGGTAGAGTGTTGC RI = GGGGTGGTGGGATTGAGCAGTGAAGT	692bp (control band) 389bp (C allele) 329bp (A allele)	61 °C
	rs2228570 (FokI) A/G	FO = CACATGTAGGTGCTGAGGCTGAGGG FI = GTGGCCTGCTTGCTTCTTACAGGTAC RO = TGCAAGGGCTCCCCATGGAAACACCT RI = GAAGTGCTGGCCGCCATTGCCTACA	710bp (control band) 512bp (A allele) 252bp (G allele)	64 °C
CYP2R1	rs1074165 7 (A/G)	FO = GGACTTACTGGGTTTGGGACTCTTCTAAC FI = TGGTTGGGGAGATACTTTAGCAGTCA RO = AGAGATAATCAGAGAGACAGCGATGTTG RI = TCCTAATAAGACTTTCCTTGACAGCCATC	Control= 679bp A = 392 bp (Fi-Ro) G = 341 bp (Ri-Fo)	55 °C
	rs1076619 7 (G/A)	F = AATATGGCAGGAATATGAGAAGTCTGTGG R1 = GCTTGGTCCTTTCTGTATCTTGGAAT R2 = GCTTGGTCCTTTCTGTATCTTGGAAC	Tri arm Product size = 627bp	56 °C
CYP27B1	rs1087701 2 (G/T)	F = GGAGCTAAATGGATGTGGATGTTCTAATTCC R1 = GCAGAGAGGTAAACTGTGGGAGACTA R2 = GCAGAGAGGTAAACTGTGGGAGACTC	Tri arm Product size = 546 bp	58 °C

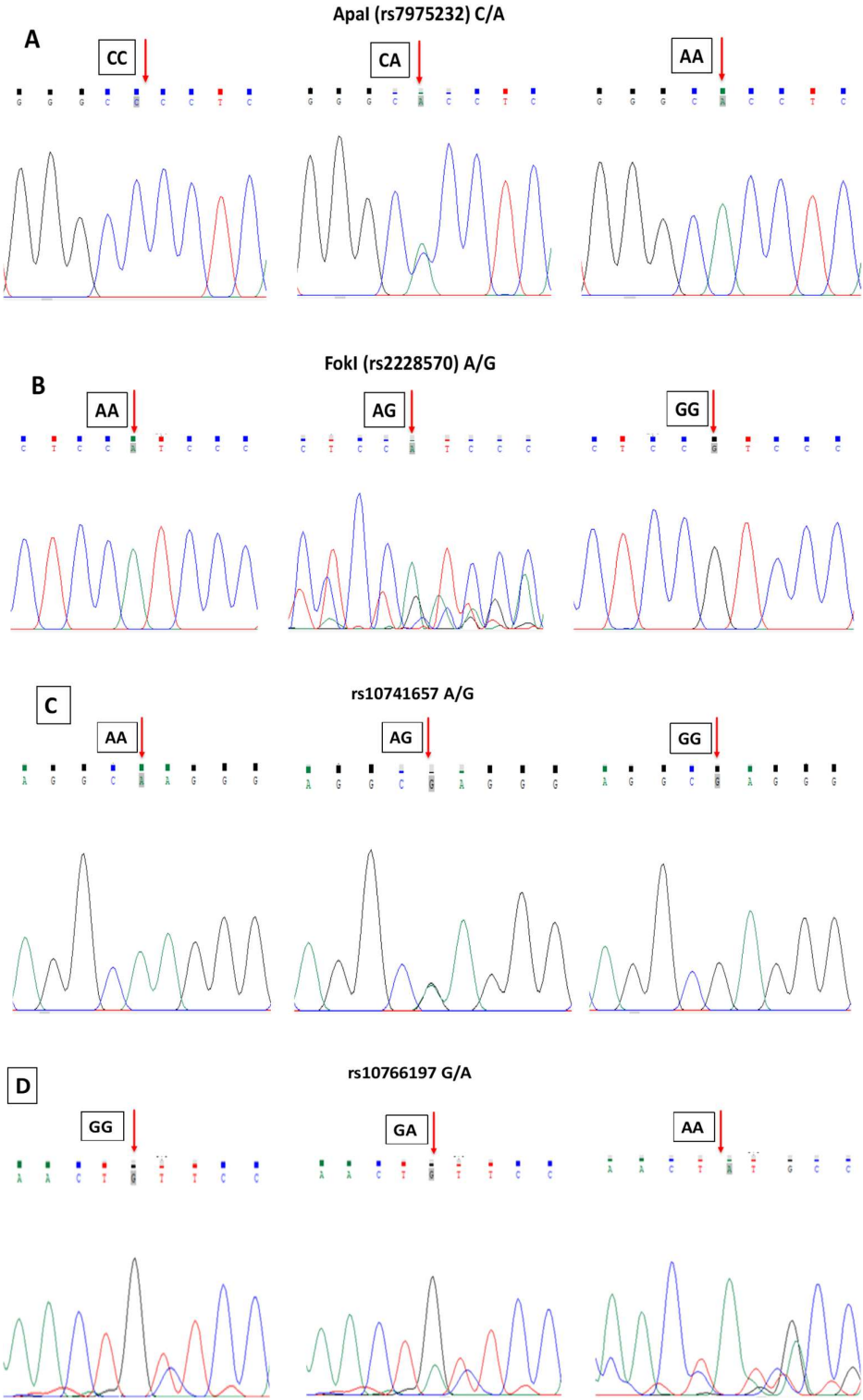
Table 2S: Tetra ARMS-PCR and Tri ARMS-PCR reagents and their volumes used for DNA amplification

Sr. No	Tetra ARMS-PCR Reagents	Reagents Volume (μL)			Tri ARMS-PCR reagents	Reagents Volume (μL)	
		rs7975232	rs2228570	rs10741657		rs10766197	rs10877012
1.	Sample DNA	02	02	02	Sample DNA	02	02
2.	Taq Buffer (10X)	03	03	03	Taq Buffer (10X)	03	03
3.	MgCl ₂ (25 mM)	1.8	1.8	1.8	MgCl ₂ (25mM)	1.4	1.8
4.	dNTPs (2 mM)	1.8	1.8	1.8	dNTPs (2mM)	1.8	1.8
5.	FO primer (10 pM/μL)	0.6	0.6	0.6	F primer (10 pM/μL)	0.6	0.8
6.	FI primer (10 pM/μL)	0.8	01	01	R1 primer / R2 primer (10 pM/μL)	0.8	0.6
7.	RO primer (10 pM/μL)	0.6	0.6	0.6	-----	-----	-----
8.	RI primer (10 pM/μL)	0.8	01	01	-----	-----	-----
9.	ddH ₂ O	18.2	17.8	17.8	ddH ₂ O	20	19.6
10.	Taq polymerase (5 U/μL)	0.4	0.4	0.4	Taq polymerase (5 U/μL)	0.4	0.4
	Total reaction volume	30 μL					

Table 3S: Genotype-wise values of biochemical and physiological parameters

		Patients			Controls		
SNPs	Genotype	Systolic BP (mmHg)	HDL-C (mg/dl)	LDL-C (mg/dl)	Systolic BP (mmHg)	Diastolic BP (mmHg)	LDL-C (mg/dl)
rs7975232	AA	144±25	49±11	72±12	119±18	80±12	82±12
	CA	143±28	47±10	75±11	117±15	77±12	84±10
	CC	143±27	51±10	72±11	120±15	80±10	87±13
rs2228570	GG	141±15	48±10	74±11	121±15	81±11	85±11
	AG	149±32	50±11	70±12	114±16	74±11	83±12
	AA	144±25	53±9	73±11	130±0	80±0	87±8

Supplementary Figure 1S: Validation of in-house developed tri and tetra ARMS PCR assays by Sanger DNA sequencing.



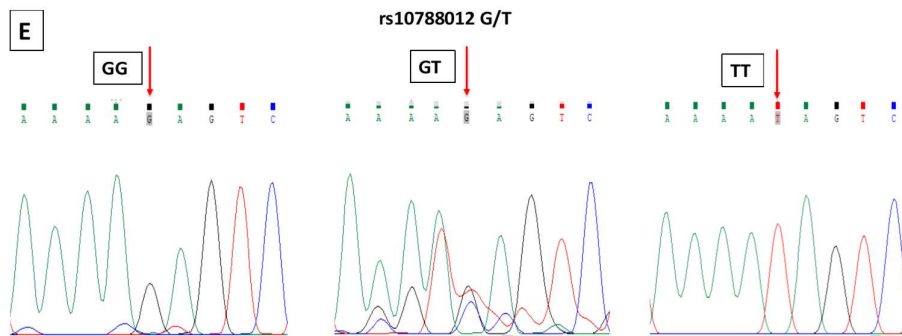


Figure 1S: Electropherograms of *VDR*, *CYP2R1* and *CYP27B1* genes polymorphisms

(A) CC shows wild type, CA shows heterozygous and AA shows altered genotype of rs7975232. (B) AA shows wild type, AG shows heterozygous and GG shows altered genotype of rs2228570. (C) AA shows wild type, AG shows heterozygous and GG shows altered genotype of rs10741657 respectively. (D) GG shows wild type genotype, GA and AA shows altered genotype of rs10766197 respectively. (E) GG shows wild type genotype, GT shows heterozygous and TT shows altered genotype of rs10877012 respectively.

Supplementary Figure 2S:

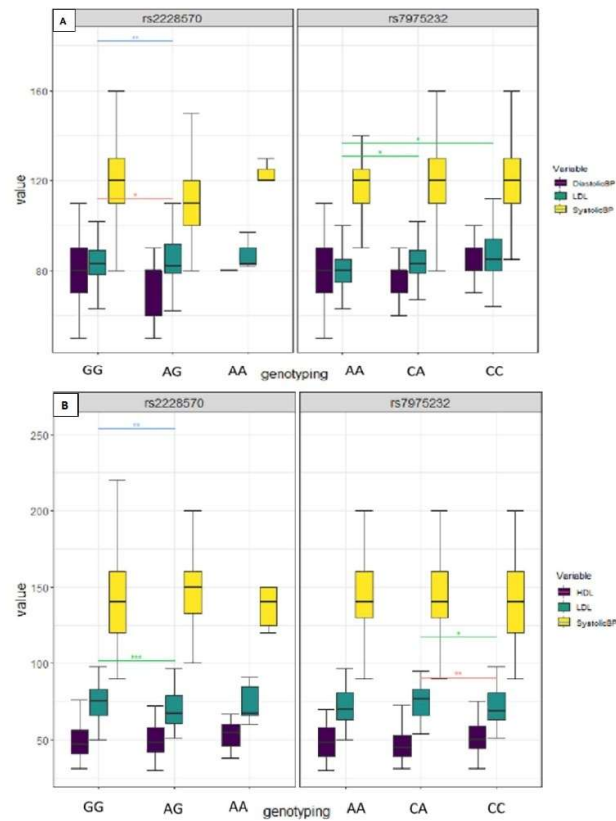


Figure 2S: (A) Association between biochemical parameters and rs2228570 A/G and rs7975232 C/A genotypes in control subjects. (B) Association between biochemical parameters and rs2228570 A/G and rs7975232 A/C genotypes in patients. *, ** & *** shows P value < 0.05, <0.01, <0.001.

Supplementary Figure 3S:

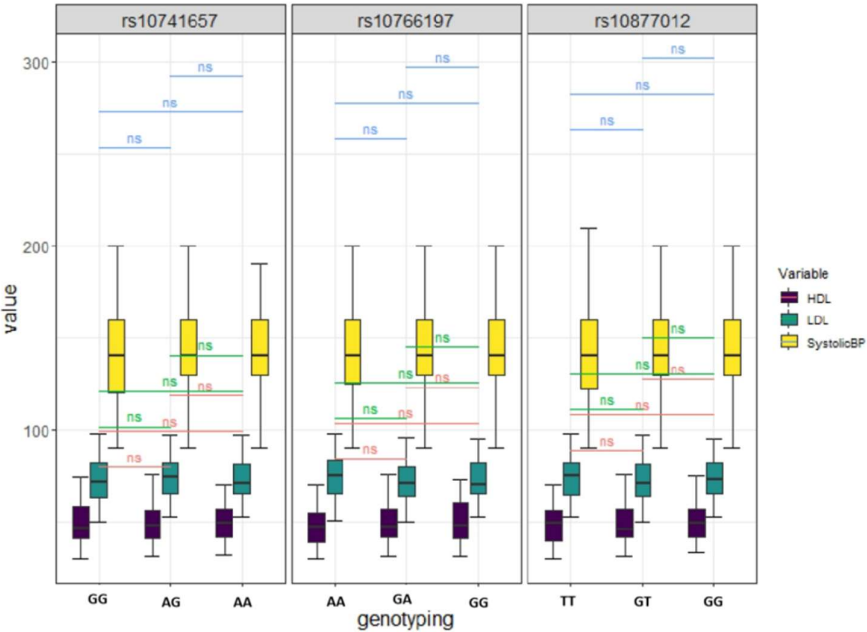


Figure 3S: Association between biochemical and physiological parameters and genotyping in patients
ns shows P value >0.05

Supplementary Figure 4S:

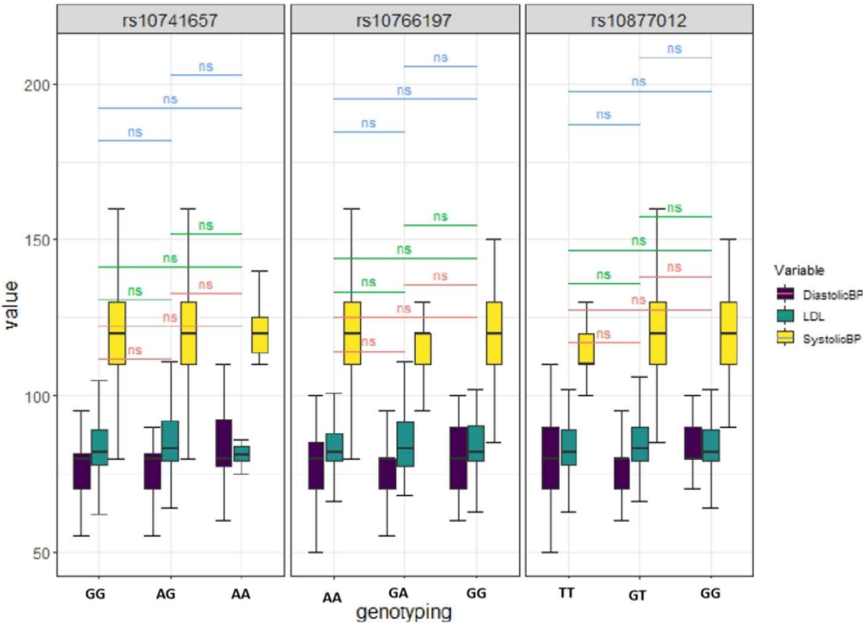


Figure 4S: Association between biochemical and physiological parameters and genotyping in controls
ns shows P value >0.05