

# Supplementary Materials: Vitamin D-Related Gene Polymorphisms, Plasma 25-Hydroxy-Vitamin D, Cigarette Smoke and Non-Small Cell Lung Cancer (NSCLC) Risk

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**Table S1.** Characteristics of the single-nucleotide polymorphisms (SNPs) and primers, polymerase chain reaction (PCR) conditions used in the study.

SNP <sup>a</sup> Reference	Gene	Chr <sup>b</sup>	Position	Type	W/V <sup>c</sup>	MAF <sup>d</sup>	Primer Sequence	PCR Conditions
rs10735810	VDR ( <i>Fok1</i> )	12: (46559162)	No block (Exon 4)	Missense	C/T	0.41	F:5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3' R:5'-ATGGAAACACCTTGCTTCTTCCCTC-3'	95 °C for 5 min (95 °C for 30 s, 61 °C for 30 s, 72 °C for 30 s) ×40 cycles, 72 °C for 5 min
rs11568820	VDR ( <i>Cdx2</i> )	12: (46588812)	BlockC2 (Intron 2)	Intron	T/C	0.49	F:5'-AGTAAACTAGGTCACAATAAAAACTTATTTCCTT-3' R:5'-AGTAAACTAGGTCACAGTAAAAACTTATTTCCTT-3'	95 °C for 5 min (95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s) ×40 cycles, 72 °C for 5 min
rs1544410	VDR ( <i>Bsm1</i> )	12: (46526102)	Block B (Intron 2)	Intron	G/A	0.11	F:5'-CAGAGCATGGACAGGGAGCAA-3' R:5'-GCAACTCCTCATGGCTGAGGTCTC-3'	95 °C for 5 min (95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s) ×40 cycles, 72 °C for 5 min
rs7975232	VDR ( <i>Apa1</i> )	12: (46525104)	Block B (Intron 8)	Intron	C/A	0.44	F:5'-GCTCTCAGCTGGGCCCTCACTGCTCAAT-3' R:5'-GCTCTCAGCTGGGCACCTCACTGCTCAAT-3'	95 °C for 5 min (95 °C for 30 s, 70 °C for 30 s, 72 °C for 45 s) ×40 cycles, 72 °C for 5 min
rs731236	VDR ( <i>Taq1</i> )	12: (46525024)	Block B (Exon 11)	Synonymous	T/C	0.13	F:5'-CAGAGCATGGACAGGGAGCAA-3' R:5'-GCAACTCCTCATGGCTGAGGTCTC-3'	95 °C for 5 min (95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s) ×40 cycles, 72 °C for 5 min
rs6068816	<i>CYP24A1</i>	20: (52214498)	Block B (Exon 6)	Synonymous	C/T	0.48	F:5'-ACTTCGTCAGTAACGGACGAGTGA-3' R:5'-GAGTCGAGGTCATATCGTGAGTGA-3'	95 °C for 5 min (95 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s) ×40 cycles, 72 °C for 5 min
rs2244719	<i>CYP24A1</i>	20: (52216265)	Block C (Intron 4)	Intron	T/C	0.18	F:5'-ATAGGGTTTTTTTTCTTCCATTTCATCA-3' R:5'-ATAGGGTTTTTTTTCTTCCATTTCATCA-3'	95 °C for 5 min (95 °C for 30 s, 65 °C for 30 s, 72 °C for 45 s) ×40 cycles, 72 °C for 5 min
rs4809960	<i>CYP24A1</i>	20: (52219480)	Block D (Intron 4)	Intron	T/C	0.11	F:5'-ACTTCGTCAGTAACGGACGGCAGG-3' R:5'-GAGTCGAGGTCATATCGTGGCAGGT-3'	95 °C for 5 min (95 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s) ×40 cycles, 72 °C for 5 min

Table S1. Cont.

SNP <sup>a</sup> Reference	Gene	Chr <sup>b</sup>	Position	Type	W/V <sup>c</sup>	MAF <sup>d</sup>	Primer Sequence	PCR Conditions
rs2762939	CYP24A1	20: (52214658)	Block B (Intron 5)	Intron	G/C	0.40	F:5'-CTCTCTCTGCACCGGTCCTGGGAGCAATG-3' R:5'-CTCTCTCTGCACCGCTCCTGGGAGCAATG-3'	95 °C for 5 min (95 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s) ×40 cycles, 72 °C for 5 min
rs2181874	CYP24A1	20: (52217885)	No Block (Intron 4)	Intron	G/A	0.15	F:5'-GTAACCGGGAGCTGCGGGAATGTATTTCAG-3' R:5'-GTAACCGGGAGCTGTGGGAATGTATTTCAG-3'	95 °C for 5 min (95 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s) ×40 cycles, 72 °C for 5 min
rs2296241	CYP24A1	20: (52219626)	Block D (Exon 4)	Synonymous	G/A	0.49	F:5'-TTGAAGGTCTTGGCCGATTTTATGGGCAG-3' R:5'-TTGAAGGTCTTGGCTGATTTTATGGGCAG-3'	95 °C for 5 min (95 °C for 30 s, 65 °C for 30 s, 72 °C for 30 s) ×40 cycles, 72 °C for 5 min
rs10877012	CYP27B1	12: (56448352)	No block	downstream	G/T	0.41	F:5'-CTGGCACAGAGCCTCATAG-3' R:5'-CGTATGCCTGTAGTGCCTT-3'	95 °C for 5 min (95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s) ×35 cycles, 72 °C for 5 min
rs3782130	CYP27B1	12: (56448165)	Block A	downstream	C/G	0.39	F:5'-GGCCCTCTCTGTTCCTAG-3' R:5'-CAACACTTTGGGAGGCTAAGG-3'	95 °C for 5 min (95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s) ×35 cycles, 72 °C for 5 min
rs7041	GC	4: (72837198)	Block A (Exon 11)	Missense	T/G	0.36	F:5'-AAATAATGAGCAAATGAAAGAAGAC-3' R:5'-CAATAACAGCAAAGAAATGAGTAGA-3'	95 °C for 3min (95 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s) ×35 cycles, 72 °C for 7 min
rs4588	GC	4: (72837187)	Block A (Exon 11)	Missense	C/A	0.28	F:5'-AAATAATGAGCAAATGAAAGAAGAC-3' R:5'-CAATAACAGCAAAGAAATGAGTAG-3'	95 °C for 3min (95 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s) ×35 cycles, 72 °C for 7 min

<sup>a</sup> <http://hapmap.org>; <sup>b</sup> Chr (Chromosome); <sup>c</sup> W/V (Wild/Variation); <sup>d</sup> MAF (Minor Allele Frequency) among controls.

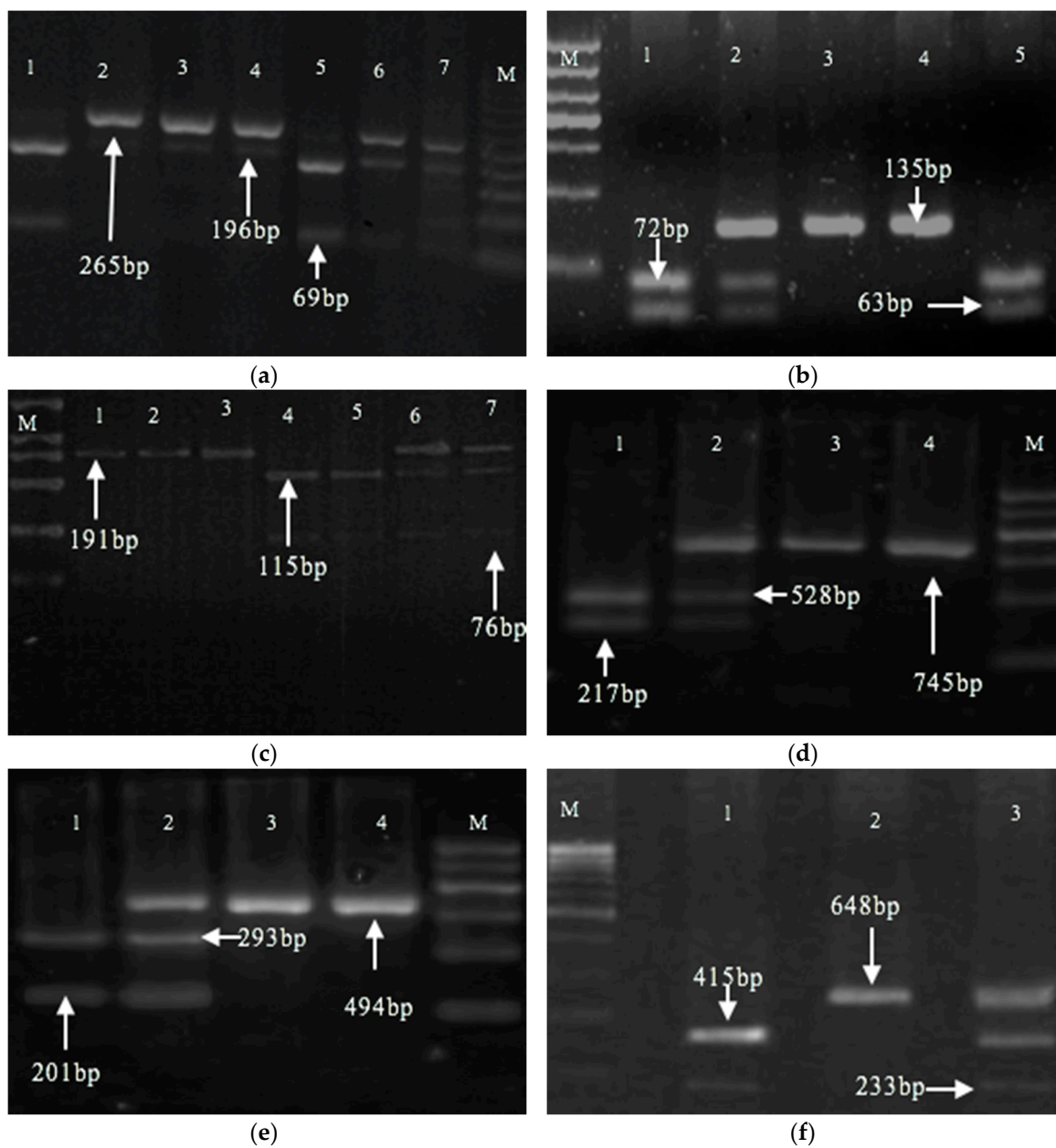


Figure S1. Cont.

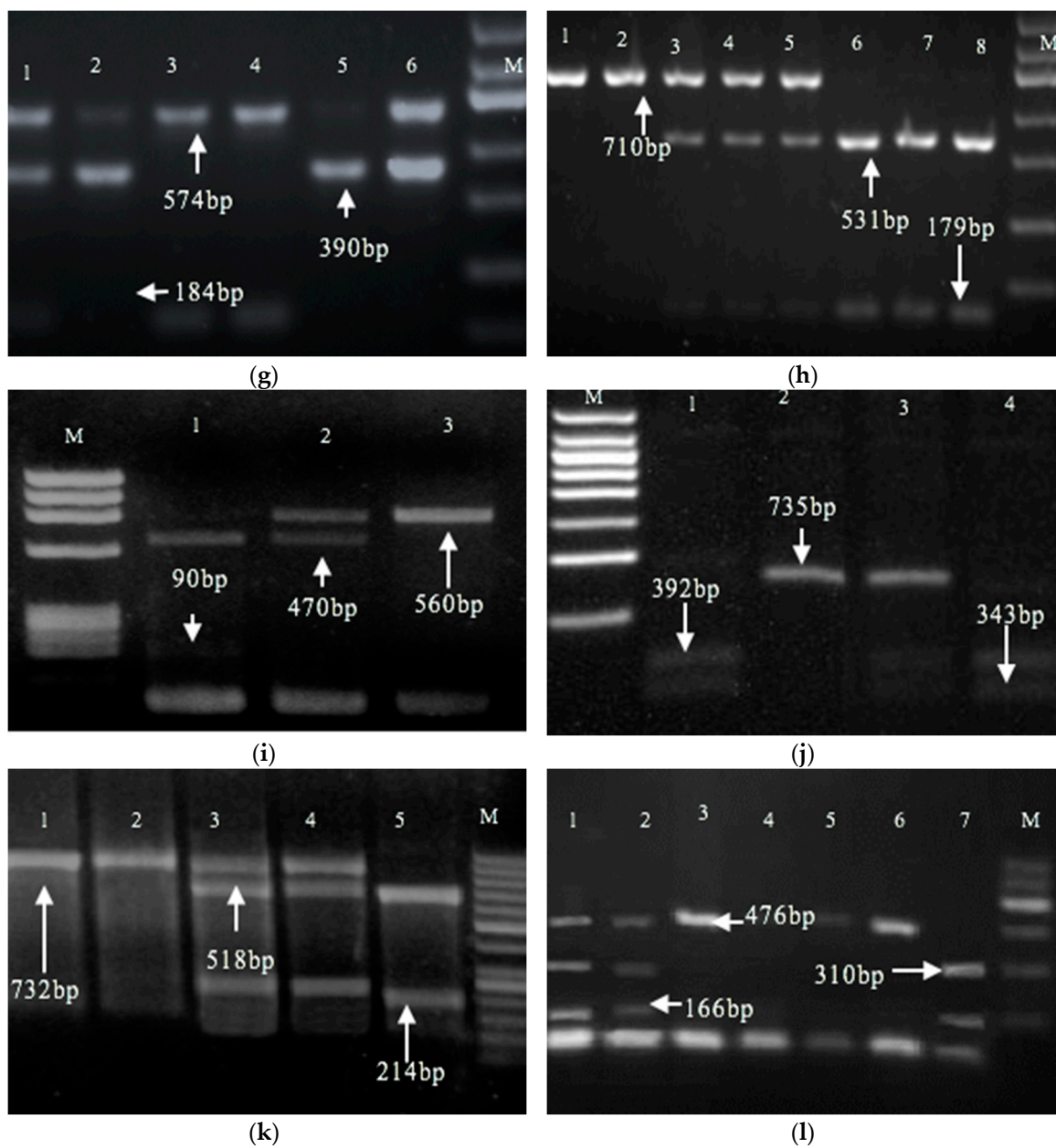
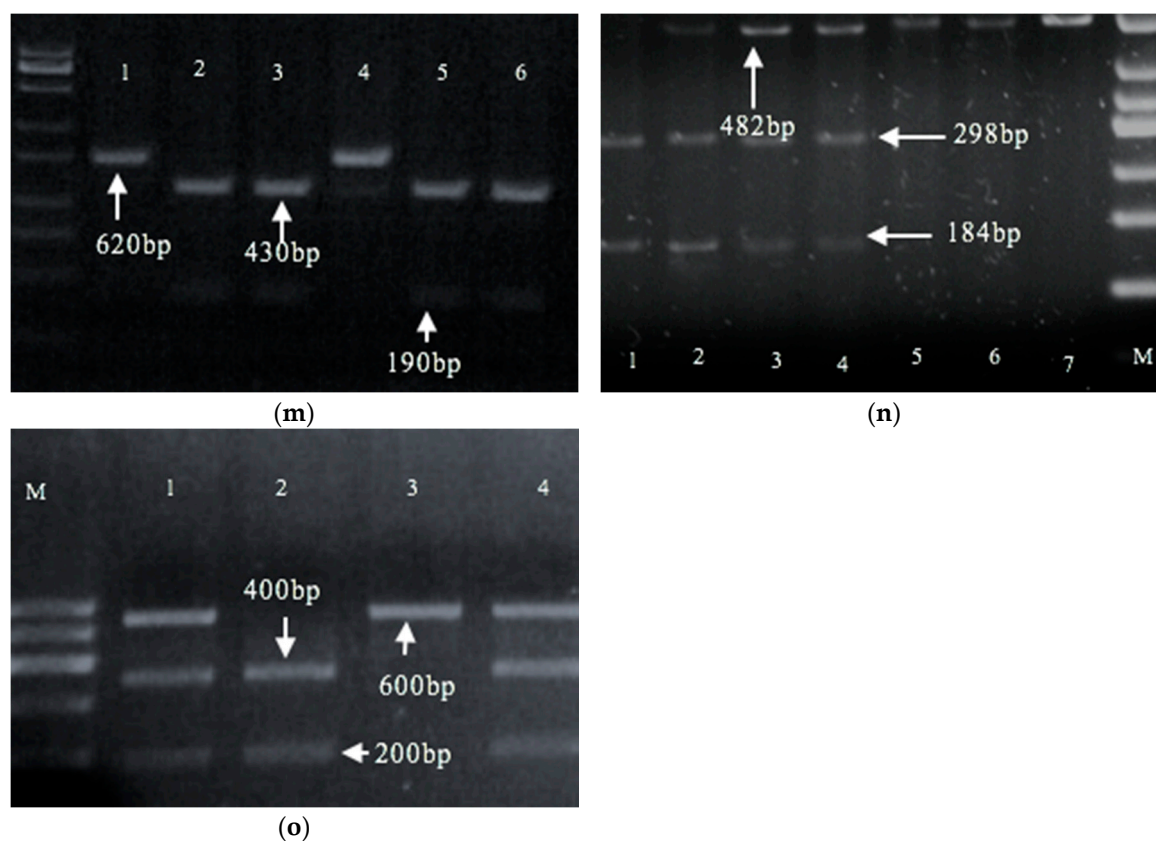


Figure S1. Cont.



**Figure S1.** Genotyping of the Vitamin D-related gene polymorphisms Polymorphism by PCR-RFLP. (a) Genotyping of the *VDR* rs10735810 (*FokI*) Polymorphism by PCR-RFLP. Lanes 2 and 3 CC (265 bp); Lanes 1 and 5 TT (196 bp and 69 bp); Lanes 4, 6 and 7 CT (265 bp, 196 bp and 69 bp); (b) Genotyping of the *VDR* rs11568820 (*Cdx2*) Polymorphism by PCR-RFLP. Lanes 3 and 4 TT (135 bp); Lanes 1 and 5 CC (72 bp and 63 bp); Lane 2 TC (135 bp, 72 bp and 63 bp); (c) Genotyping of the *VDR* rs1544410 (*BsmI*) Polymorphism by PCR-RFLP. Lanes 1, 2 and 3 GG (191 bp); Lanes 4 and 5 AA (115 and 76 bp); Lanes 6 and 7 GA (76, 115 and 191 bp); (d) Genotyping of the *VDR* rs7975232 (*ApaI*) Polymorphism by PCR-RFLP. Lanes 3 and 4 CC (745 bp); Lane 1 AA (528 and 217 bp); Lane 2 CA (745, 528 and 217 bp); (e) Genotyping of the *VDR* rs731236 (*TaqI*) Polymorphism by PCR-RFLP. Lanes 3 and 4 TT (494bp); Lane 1 CC (201 and 293 bp); Lane 2 TC (201, 293 and 494 bp); (f) Genotyping of the *CYP24A1* rs6068816 Polymorphism by PCR-RFLP. Lane 2 CC (648 bp); Lane 1 TT (415 and 233 bp); Lane 3 CT (648, 415 and 233 bp); (g) Genotyping of the *CYP24A1* rs2244719 Polymorphism by PCR-RFLP. Lanes 3 and 4 TT (574 bp); Lane 5 CC (390 and 184 bp); Lanes 1, 2 and 6 TC (574, 390 and 184 bp); (h) Genotyping of the *CYP24A1* rs4809960 Polymorphism by PCR-RFLP. Lanes 1 and 2 TT (710 bp); Lanes 6, 7 and 8 CC (531 and 179 bp); Lanes 9, 3, 4 and 5 TC (710, 531 and 179 bp); (i) Genotyping of the *CYP24A1* rs2762939 Polymorphism by PCR-RFLP. Lane 3 GG (560 bp); Lane 1 CC (470 and 90 bp); Lane 2 GC (560, 470 and 90 bp); (j) Genotyping of the *CYP24A1* rs2181874 Polymorphism by PCR-RFLP. Lane 2 GG (735 bp); Lanes 1 and 4 AA (392 and 343 bp); Lane 3 GA (735, 392 and 343 bp); (k) Genotyping of the *CYP24A1* rs2296241 Polymorphism by PCR-RFLP. Lanes 1 and 2 GG (732 bp); Lane 5 AA (518 and 214 bp); Lanes 3 and 4 GA (574, 390 and 184 bp); (l) Genotyping of the *CYP27B1* rs10877012 Polymorphism by PCR-RFLP. Lanes 3, 4, 5, 6 GG (476 bp); Lanes 7 TT (310 and 166 bp); Lanes 1, 2 GT (476, 310 and 166 bp); (m) Genotyping of the *CYP27B1* rs3782130 Polymorphism by PCR-RFLP. Lane 1 CC (620 bp); Lanes 2, 3, 5 and 6 GG (430 and 190 bp); Lane 4 CG (620, 430 and 190 bp); (n) Genotyping of the *GC* rs7041 Polymorphism by PCR-RFLP. Lanes 5, 6 and 7 TT (482 bp); Lanes 1 and 2 GG (298 and 184 bp); Lanes 3 and 4 TG (482, 298 and 184 bp); (o) Genotyping of the *GC* rs4588 Polymorphism by PCR-RFLP. Lane 3 CC (600 bp), Lane 2 AA (400 and 200 bp), Lanes 1 and 4 CA (600, 400 and 200 bp).