

Appendix 1. Primers for PCR-RFLP genotyping of PNPLA3 rs738409 and TM6SF2 rs58542926 polymorphisms

Polymorphism	Genomic location	Primer Name	Sequence	GRCh38.p13 primary assembly No.	TM (°C) ^a	PCR product size (bp)
PNPLA3 rs738409	22q13.31	PNP-F	5'-CGGGTAGCCTGGAAATAGGG-3'	43929066-43929047	59.6	291
		PNP-R	5'-TTAAAAGGGTGCTCTCGCCT-3'	43928776-43928795	59.3	
TM6SF2 rs58542926	19p13.11	TM-F	5'-AAAGCAACTGACACGGGGAA-3'	19268564-19268583	60.1	249
		TM-R	5'-AACAGATGTCCAGCAGGGTT-3'	19268812-19268793	59.2	

Abbreviations: TM, Melting Temperature; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; bp, base pair.

^aTM calculation of primers was performed using the SantaLucia formula.

Appendix 2. Assessment of Hardy–Weinberg equilibrium for the studied polymorphisms

Polymorphism	P value for Hardy–Weinberg equilibrium			
	Control (N=110)	Population-based NAFLD (N=108)	Clinic-based NAFLD (N=73)	Total (N= 291)
PNPLA3 rs738409	0.59	0.51	0.07	0.20
TM6SF2 rs58542926	0.65	0.58	0.53	0.35

Abbreviations: NAFLD, non-alcoholic fatty liver disease