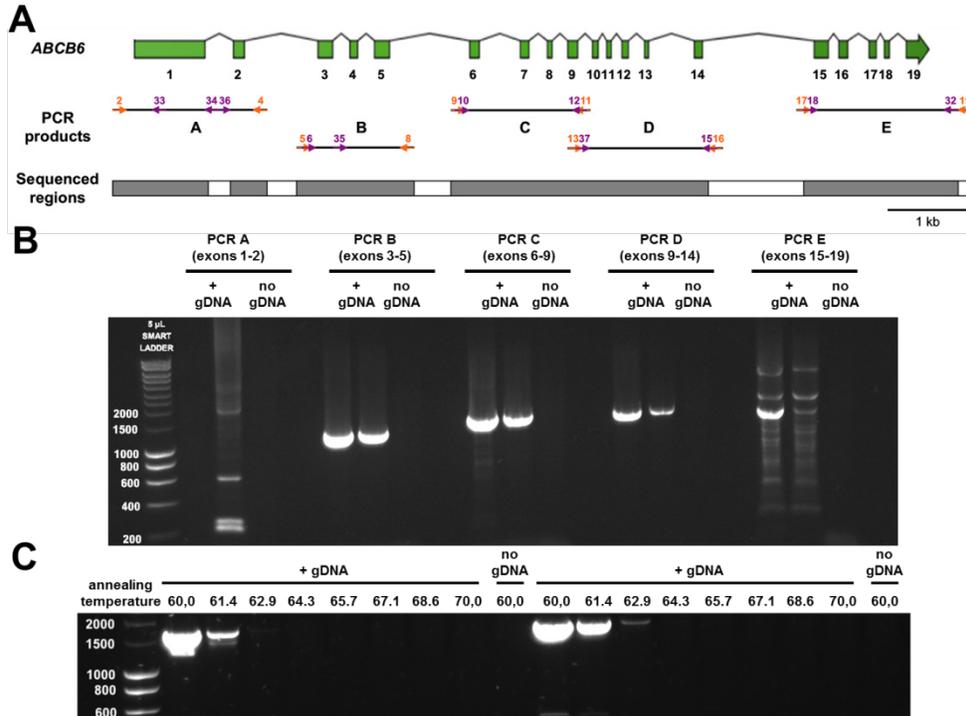


Supplemental Material – Farrell et al.



Supplemental Figure 1: Method French cohort gene amplification and sequencing.

A: Scheme of ABCB6 gene according to Helias et al.⁷. **B:** PCR (A, B, C, D and E) test using LA Taq polymerase (TaKaRa). The 20 uL reaction mix was loaded and migrated on 1% agarose gel containing SYBR Safe 1:10 000. Two different genomic DNA (gDNA) were used. Control (no gDNA) is the PCR mix without gDNA. **C:** PCR (A and E) test using Advantage® GC Genomic LA DNA polymerase (ThermoFisher). Eight different annealing temperature (from 60.0°C to 70.0°C) were tested. The 20 uL reaction mix was loaded and migrated on 1% agarose gel containing SYBR Safe 1:10 000. Two different genomic DNA (gDNA) were used. Control (no gDNA) is the PCR mix without gDNA.

Supplemental M&M

French cohort sequencing: Primers used to sequence all 19 exons of ABCB6 (Supplemental Figure 1) are described in Supplemental Table 1 and are based on the work from Helias et al.⁷. Since PCR conditions are not described in this article we have developed our own conditions for the five PCR reactions (A-E).

Amplification of fragment B, C and D were successfully done using LA Taq polymerase from Clontech (Supplemental 1B). PCR Condition for 1 sample reaction mix (20 uL final) : buffer 1X - dNTP 0.4 mM each, primer forward 0.2 uM, reverse primer 0.2 uM, 1 unit LA Taq polymerase, 1 uL genomic DNA. PCR program: 1 cycle (94°C for 1 min); 35 cycles (94°C for 30 sec; 60°C for 30 sec; 68°C for 2 min); 1 cycle (72°C for 10 min; 20°C for 1 min).

Amplification of fragment A and E were obtained using Advantage® GC Genomic LA DNA polymerase from Clontech (Supplemental Figure 1, C). PCR Condition for 1 sample reaction mix (20 uL final) : buffer 1X - dNTP 0.5 mM each, primer forward 0.5 uM, reverse primer 0.5 uM, 1 unit Advantage® GC Genomic LA DNA polymerase, 1 uL genomic DNA. PCR program: 1

cycle (94°C for 1 min); 35 cycles (94°C for 30 sec; 60°C for 30 sec; 68°C for 2 min); 1 cycle (72°C for 10 min; 20°C for 1 min). Sanger sequencing reactions were subcontracted by Eurofins MWG. We cannot rule out that some alleles were not amplified or sequenced due to loss of PCR amplification, known as allelic dropout.

Supplemental Table 1. Primers used to amplify ABCB6 for sequencing of the French cohort.

ID PRIMER	SEQUENCE (5' > 3')	POSITION ON NCBI REFERENCE SEQUENCE: NG_032110.1		USE	ARTICLE REFERENCE
		FROM	TO		
ABCB6-2	GGCCTGGTCGTCAGATTTTC	4848	4866	PCR A	PubMed PMID: 22246506
ABCB6-4	GTGTCATGCACCTGTAGTC	6465	6483	PCR A	PubMed PMID: 22246506
ABCB6-5	TCCCTGTCATCATTCCTTCC	6954	6973	PCR B	PubMed PMID: 22246506
ABCB6-8	CTGCCCGCATTACCTAAGT	8152	8170	PCR B	PubMed PMID: 22246506
ABCB6-9	TTTAGCCACCCACCTCTTG	8760	8778	PCR C	PubMed PMID: 22246506
ABCB6-11	GGCAATCAAGGAAGCAGAG	10302	10320	PCR C	PubMed PMID: 22246506
ABCB6-13	TTTGGAGTGGAAGTCGAGC	10042	10060	PCR D	PubMed PMID: 22246506
ABCB6-16	ACATCTCCTCTCTCTGGAC	11671	11689	PCR D	PubMed PMID: 22246506
ABCB6-17	ACAGAGAGGAGTGATTTGCC	12725	12744	PCR E	PubMed PMID: 22246506
ABCB6-19	ATGTGGAGTGGGCAGGAAG	14386	14404	PCR E	PubMed PMID: 22246506
ABCB6-33	CCAAGACAGCGAATCAGC	5492	5509	SEQUENCING OF A	PubMed PMID: 22246506
ABCB6-34	GCAGTGCTTTCTCCCAAG	5909	5926	SEQUENCING OF A	PubMed PMID: 22246506
ABCB6-36	TCCCGGCCCTATTATAAGTAG	6106	6127	SEQUENCING OF A	PubMed PMID: 22246506
ABCB6-6	AGTTTTTCTGACCCATCAAGG	7017	7038	SEQUENCING OF B	PubMed PMID: 22246506
ABCB6-35	TGCCTGAAGAGAGTCCTG	7471	7488	SEQUENCING OF B	PubMed PMID: 22246506
B-IRV	CAAGGTGGGAGCGGCAG	8090	8106	SEQUENCING OF B	This study
ABCB6-10	CCTAGAGGCAGGGTTGCT	8843	8860	SEQUENCING OF C	PubMed PMID: 22246506
ABCB6-12	AGGCCGCTCTTCTTGTC	10264	10282	SEQUENCING OF C	PubMed PMID: 22246506
C-IFW	CTACACATGAGACTTTGTGCAGG	9326	9348	SEQUENCING OF C	This study
ABCB6-37	GGATCTTGATGAGAGAGTGG	10174	10194	SEQUENCING OF D	PubMed PMID: 22246506
ABCB6-15	CAGCCAGCCCTTATCATTC	11652	11671	SEQUENCING OF D	PubMed PMID: 22246506
D-IFW	CACTGCCTTCTGCTTCTC	10794	10813	SEQUENCING OF D	This study
ABCB6-18	ACACGGCTAGGATATGGGG	12746	12764	SEQUENCING OF E	PubMed PMID: 22246506
ABCB6-32	GTTTCTCTCTCTCTAACCT	14311	14332	SEQUENCING OF E	PubMed PMID: 22246506
E-IFW	CTCAAGCCCAACAAGGCTC	13404	13423	SEQUENCING OF E	This study

American Cohort SNP typing:

Supplemental Table 2. Primers used to amplify ABCB6 for SNP typing of the American cohort.

ID Primer	Sequence (5' > 3')	Use	Region
ABCB1F	CCTGCACATGGGACAGACCG	pcr, seq	ex1-4, 3445bp
ABCB1R	TCCCGGCAGGACTCAGCC	seq	
ABCB2F	TAAGCCTGTAAGTGCTGAATGTGT	seq	
ABCB3F	CTGGGAGCTGTAACCCCATAG	seq	
ABCB4R	GTCAGCCCAGCACCAGGATG	pcr, seq	ex1-4, 3445bp
ABCB5F	AGGCATTCCCTCAGCATCCG	pcr, seq	ex5-13, 3426bp
ABCB5R	AGCGGCAGCTTCTCAGGC	seq	
ABCB6F	AGACTGGTTCCAGTCTGTTGCTTG	seq	
ABCB7R	CCTTCCTGAGTTCAAACATCACAC	seq	
ABCB8F	CTGTGGAATCTGGGTGTCCG	seq	
ABCB9R	GCTCTTCTTGTGTCACCCTGC	seq	
ABCB10F	CCCTGGCCTGGTAGAATTTTC	seq	
ABCB13F	CTTCCTGCTTCTCAGTGCTCTGAATC	pcr, seq	ex13-19, 3435bp
ABCB13R	TCCGGTTGCCTATCGTACTG	seq	
ABCB14R	CACCATACAAATCCTTAACATGAC	seq	
ABCB15F	GAAGCAGGAAGAGAAGATGGGG	seq	
ABCB16R	GAGCCTTGTTTGGGCTTGAG	seq	
ABCB17F	TTCCAGTTCTCAAGCCCAAAC	seq	
ABCB19R	GCCACAGTGATCCTCGCACAG	pcr, seq	ex13-19, 3435bp

Supplemental Table 3. Allele frequency in the population compared to the cohort.

	Fischers_ OR	Fischers_ _PVal	EXAC_ Alt	EXAC_Ref	EXAC_ MAF	Cohort_ Alt	Cohort_ Ref	Cohort_MAF
R192Q	2.134237	0.014172	338.0	66246.0	0.005076	12.0	1102.0	0.010772
R192W	4.245601	0.000014	199.0	66383.0	0.002989	14.0	1100.0	0.012567
R247C	16.208034	0.010513	6.0	54070.0	0.000111	2.0	1112.0	0.001795
R276W	0.477040	0.999571	494.0	16172.0	0.029641	16.0	1098.0	0.014363
A492T	0.940959	0.618299	635.0	65965.0	0.009535	10.0	1104.0	0.008977
T521S	2.612835	0.004736	253.0	66285.0	0.003802	11.0	1103.0	0.009874
G588S	2.289070	0.001582	475.0	66205.0	0.007124	18.0	1096.0	0.016158
A681T	0.000000	1.000000	27.0	66673.0	0.000405	0.0	1114.0	0.000000
G729S	14.675651	0.081056	4.0	65336.0	0.000061	1.0	1113.0	0.000898

Supplemental Table 4. SNPs identified in the current study with associated NCBI accession.

<u>local identifier</u>	<u>NCBI subsnp#</u>
p.Gly729Ser	2137544296
p.Ala681Thr	5505748203
p.Gly588Ser	5505748204
p.Thr521Ser	5505748205
p.Ala492Thr	5505748206
p.Arg343Gln	5505748207
p.Arg276Trp	5505748208
p.Arg247Cys	5505748209
p.Arg192Gln	5505748210
p.Arg192Trp	5505748211