

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Schwarz UI, Ritchie MD, Bradford Y, et al. Genetic determinants of response to warfarin during initial anticoagulation. *N Engl J Med* 2008;358:999-1008.

Online Supplement Table 1. Methods, Primers and Reaction Conditions of the Fluorescent Allele-specific Oligonucleotide Ligation Assay (OLA) used to assess *CYP2C9* Genotype.

Nucleotide Change (rs number)	Major allele primer*	Minor allele primer*	Ligation primer**
	5'-FAM/TAMARA - tagged	5'-HEX/ROX - tagged	
<i>CYP2C9</i> *2 430C>T (1799853)	5'-FAM- AATGATCGACGAGACACTCTCGC CAAGAGGAGCATTGAGGACC-3'	5'-HEX- AAACGAGCTGCCGCGCAAGATAAGA GGAGCATTGAGGACT-3'	5'-P-GTGTTC AAGAGGA-3'
<i>CYP2C9</i> *3 1075A>C (1057910)	5'-TAMARA- ACATTCGCGATCGCCGCCCGCTTT ACGAGGTCCAGAGATACA-3'	5'-ROX- TCGCGACCGCATCCCAATCTCGAGGT CCAGAGATACC-3'	5'-P-TTGACCTTCTCC-3'
<i>CYP2C9</i> *5 1080C>G (28371686)	5'-FAM- ACATGACATTCGCGATCGCCGCC GCTTTGGTCCAGAGATACATTGAC -3'	5'-HEX- ATACGGTATCGCGACCGCATCCCAAT CTGGTCCAGAGATACATTGAG-3'	5'-P-CTTCTCCCCAC-3'

*5'-fluorescently tagged allele-specific primers with FAM or TAMARA for the major allele, and HEX or ROX for the minor allele.

The 3' - modifications at the polymorphic site are shown in bold.

**Ligation primers with 5'-phosphate (P) modification.

Methods: Two 5'-fluorescently labeled allele-specific primers that differ in sequence at the polymorphic site (3') and one ligation primer were used for each allele. Allele discrimination occurred by 3' match or mismatch of the allele-specific primer at the polymorphic site with subsequent ligation with the ligation primer or lack of ligation, respectively. Then, capillary electrophoresis of ligation products (automated sequencer, Applied Biosystems 3730), followed by laser excitation coupled with a CCD camera was carried out. Genotypes were discriminated based upon fluorescence as well as product size using ABI GeneMapper 3.5 software (Applied Biosystems). Assay validation was achieved by DNA sequencing of amplicons representing all possible allele combinations.

Ligase chain reaction conditions: initial denaturation at 96°C for 2 min, then 30 cycles at 94°C for 15 seconds and 1 min at 37°C in a 10 µl reaction volume. Reaction solution contained 10-20ng PCR-amplified target sequence (amplicons were Sephadex column purified if needed), 0.1 pmol of each allele-specific primer, 15 pmol of ligation primer, 15 units of *Taq* ligase (NEB), and a buffer (20 mM Tris-HCl, 25 mM potassium acetate, 10 mM magnesium acetate, and 10 mM DTT, 1 mM NAD, 0.1% Triton X-100; pH of 7.6 at 25°C).

Online Supplement Table 2. Custom and pre-designed (validated or functionally tested) TaqMan[®] SNP Genotyping Assays used to Assess *VKORC1* Genotype (7900 HT Sequence Detection System, Applied Biosystems, Foster City, CA, USA).

<i>VKORC1</i> variant	rs number	Primer	Primer Sequence (5' - 3')	Reporter Sequence (5' - 3')	Reporter Dye	Assay Type (ID)
3673	rs9923231	Forward	GGCCTCCCAAATGCTAGGATTATA	ATTGGCCGGGTGCG	VIC	Custom
		Reverse	AAGTCAAGCAAGAGAAGACCTGAAA	ATTGGCCAGGTGCG	FAM	
5808	rs2884737	Forward	GCGGTAGAGATTGACGATGGT	CCCCTTCCCCTGCGC	VIC	Custom
		Reverse	GCAGCCATCGCCAACAC	CCCCTTCACCTGCGC	FAM	
6484	rs9934438	Forward	CACCTGGGCTATCCTCTGTTC	CCTAGTCCAAGAGTCGAT	VIC	Custom
		Reverse	CCCGGTGCCAGGAGATC	CTAGTCCAAGGGTCGAT	FAM	
6853	rs8050894	Forward	NA	NA	NA	Pre-designed
		Reverse	NA	NA	NA	(C_2847860_10)
7566	rs2359612	Forward	NA	NA	NA	Pre-designed
		Reverse	NA	NA	NA	(C_26291751_10)

Footnote: NA - not available for pre-designed TaqMan[®] assays.
PharmGKB Accession IDs for the CYP2C9 data are PA130477740-PA130478731.

Online Supplement Table 3. Bleeding Events

Bleeding Event	Patient ID	CYP2C9	VKORC1	Time After Warfarin Initiation (Days)	Warfarin Dose (mg/day)	INR	Warfarin Dose at event (mg/day)	INR at Event	Event Description
Major	6	*1/*1	NonA/NonA	21	5.0	1.3	6.1		Intracranial hemorrhage, fatal
	63	*2/*2	A/NonA	82	2.7	2.4	2.0	2.2	Gastrointestinal bleeding with cardiogenic shock, fatal
	129	*1/*3	NonA/NonA	85	6.6	2.4	6.8	2.7	Intracranial hemorrhage, fatal
	8	*1/*1	A/A	8	1.5	2.1	2.5	3.2	Gastrointestinal bleeding
	170	*1/*1	A/NonA	3	5.4	1.6	5.0	1.9	Unexplained decrease in hemoglobin
	221	*1/*1	A/NonA	24	3.0	1.5	3.8	1.7	Gastrointestinal bleeding
	281	*1/*1	A/NonA	10	2.3	3.0	5.0	6.3	Gastrointestinal bleeding
	302	*1/*3	A/NonA	27	2.2	4.2	5	4.7	Hematoma
Minor	302	*1/*3	A/NonA	13	2.2	4.2	4.3	12.3	Anemia
	52	*1/*1	A/NonA	3	4.6	2.1	5.0	4.9	Epitaxis
	138	*1/*1	NonA/NonA	61	5.2	2.3	5.0	3.4	Hematemesis
	165	*1/*2	A/A	11	3.2	2.5	2.5	4	Hematoma
	297	*2/*2	A/NonA	96	4.0	2.4	3.9	4	Gross Hematuria