Supplemental Data

Warfarin Genotyping with Hybridization-induced Aggregation on a Poly(ethylene terephthalate) Microdevice

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Contents:

Oligonucleotide		Sequence
PCR Primers	*CYP2C9* *2 Fwd	5'-GGATGGGGAAGAGGAcCATTGAGGgCt-3'
	CYP2C9 *2 Rev	5'-CCGCTTCACATaAGCTAACAACCAGaACTCAT-5'
	CYP2C9 *3 Fwd	5'-TGTGGTGCAgGAGGTCCAGAGATgCc-3'
	CYP2C9 *3 Rev	5'-CCAGACACTAGGACCTcTTACAAACCTTTATtGC-3'
	VKORC1 (1173C>T) Fwd	5'-ACTTAAGGTCTAAGATGAAAAGCAGGGCCTAC-3'
	VKORC1 (1173C>T) Rev	5'-TCCTCTGTTCCCCCACCTCCCATCCTtGTCCAAtA-3'
	KRAS Fwd	5'- GACTGAATATAAACTTGTGGTAGTTGGA -3'
	KRAS Rev	5'-CATATTCGTCCACAAAATGATTCTG-3'
HIA Probes	*CYP2C9* *2 5' Probe	5'-[Biotin-TEG]TTTTTTGGATGGGGAAGAGGACCATTGAGGGCT-3'
	CYP2C9 *2 3' Probe	5'-ATGAGTTCTGGTTGTTAGCTTATGTGAAGCGGTTTTTT[Biotin-TEG~Q]-3'
	CYP2C9 *3 5' Probe	5'-[Biotin-TEG]TTTTTTTGTGGTGCAGGAGGTCCAGAGATGCC-3'
	CYP2C9 *3 3' Probe	5'-GCAATAAAGGTTTGTAAGAGGTCCTAGTGTCTGGTTTTTT[Biotin-TEG~Q]-3'
	VKORC1 (1173C>T) 5' Probe	5'-[Biotin-TEG]TTTTTTACTTAAGGTCTAAGATGAAAAGCAGGGCCTAC-3'
	VKORC1 (1173C>T) 3' Probe	5'-TATTGGACAAGGATGGGAGGTGGGGGAACAGAGGATTTTTT[Biotin-TEG~Q]-3'
	KRAS 5' Probe	5'- [Biotin-TEG]TTTTTTCTGAATTAGCTGTATCGTCAAGGCACTC -3'
	KRAS 3' Probe	5'- CTACGCCtCCAGCTCTTTTTT[Biotin-TEG~Q] -3'

Supplemental Table 1. Oligonucleotide sequences. Lowercase letters in primer sequences indicate intentional mismatches versus the genomic template. Primer sequences for *2, *3, and 1173C>T products are described by Poe et al (28).

	KRAS	*CYP2C9 *2*			*CYP2C9 *3*			*VKORC1*		
Patient ID	**HIA result**	**HIA result**	**Mutation status (HIA)**	**Mutation status (conventional)**	**HIA result**	**Mutation status (HIA)**	**Mutation status (conventional)**	**HIA result**	**1173C>T Mutation status (HIA)**	**-1639G>A Mutation status (conventional)**
1A	70657	559447	WT	WT	56584	MUT	MUT (homo)	35306	MUT	MUT (homo)
1B	59939	527161	WT	WT	29631	MUT	MUT (het)	76512	MUT	MUT (homo)
1C	145516	571634	WT	WT	60587	MUT	MUT (het)	71060	MUT	MUT (het)
2A	67816	535747	WT	WT	527046	WT	WT	71181	MUT	MUT (het)
2B	193413	573865	WT	WT	580443	WT	WT	89230	MUT	MUT (homo)
2C	112646	553185	WT	WT	533363	WT	WT	49390	MUT	MUT (het)
3B	100677	561849	WT	WT	562184	WT	WT	518379	WT	WT
3C	68920	515978	WT	WT	516363	WT	WT	516439	WT	WT
4A	72431	58946	MUT	MUT (het)	76139	MUT	MUT (het)	63106	MUT	MUT (homo)
4B	127903	115143	MUT	MUT (het)	43257	MUT	MUT (het)	68826	MUT	MUT (het)
4C	84202	62729	MUT	MUT (het)	54498	MUT	MUT (het)	55290	MUT	MUT (het)
5A	42822	45726	MUT	MUT (het)	53879	MUT	MUT (het)	509143	WT	WT
5B	70629	54471	MUT	MUT (het)	33766	MUT	MUT (het)	572715	WT	WT
5C	51413	45738	MUT	MUT (het)	33885	MUT	MUT (het)	490905	WT	WT
6A	114753	81826	MUT	MUT (het)	597814	WT	WT	557388	WT	WT
6B	44249	60688	MUT	MUT (het)	523468	WT	WT	538828	WT	WT
6C	42121	39494	MUT	MUT (het)	545783	WT	WT	544827	WT	WT
7A	45143	47085	MUT	MUT (het)	528937	WT	WT	42426	MUT	MUT (het)
7B	119695	74811	MUT	MUT (het)	587639	WT	WT	115265	MUT	MUT (het)
7C	64450	58801	MUT	MUT (het)	553244	WT	WT	49164	MUT	MUT (homo)
8A	35135	580857	WT	WT	52540	MUT	MUT (homo)	580695	WT	WT
8B	67897	554474	WT	WT	40247	MUT	MUT (het)	583727	WT	WT
8C	66503	591540	WT	WT	45963	MUT	MUT (het)	593232	WT	WT

Supplemental Table 2. Genotyping results of 23 patient samples. All HIA results are reported in terms of saturation value. Using the threshold saturation value (310,000), genotypes were assigned as wild-type (WT; saturation<310,000) or mutant (MUT; saturation>310,000). The genotyping results as determined by the conventional test (WARFGENO, ARUP laboratories) are also reported. Mutant genotypes reported by the conventional test are distinguished as heterozygous (het) or homozygous (homo).

Supplemental Methods and Materials

Instrumentation for microdevice PCR

Custom built instrumentation comprising a pair of Peltier thermoelectric cooling modules (Laird Part 430446-503) clamped around the PCR reservoir was used for temperature cycling. Temperatures were measured with a T-type thermocouple and digitized before being sent to a Parallax Propeller microcontroller (Parallax Part 32150). These signals provided feedback so that temperatures inside the PCR chamber could be regulated with a Proportion-Intergral control algorithm run on the microcontroller. Peltier throttle control signals were generated by the microcontroller, converted to an analog signal with a digital to analog converter (Analog devices AD557), and amplified by a high current operational amplifier (Texas Instruments Part OPA548), before reaching the Peltier modules. User inputs related to thermocycling temperatures and duration were sent to the microcontroller via a text-based serial terminal interface.

HIA instrumentation

Motion profiles were created using a bipolar stepper motor (Sanmotion Type SS2422-5041). Rotation speeds were controlled in an open loop fashion by changing the frequency of step pulses sent to the motor. These pulses, produced by a Parallax Propeller microcontroller (Parallax Part 32150), were decoded and amplified by a Texas Instruments DRV8825 IC based microstepping breakout board (Pololu Part 2133) in full step mode, creating a step size of 1.8 degrees. User inputs concerning spin speeds and times were communicated to the microcontroller by a text-based serial terminal interface designed in house.

Microdevice fabrication

Microdevices used for HIA assays and chip PCR were fabricated with a poly(ethylene terephthalate) substrate in the form of overhead transparency sheets (TRANSNS; Filmsource, Maryland Heights, MO). Each device was composed of 5 layers. Layers 2 and 4 of the device were coated with toner (HPC-4127X black; Hewlett-Packard, Palo Alto, CA) by printing two layers of the toner on each side of the transparency at 600dpi, using a HPLaserJet 4000 printer (Hewlett-Packard, Palo Alto, CA). Layers 1 and 5 were treated with plasma oxidation (PE-75; Plasma Etch, Carson City, NV). The features of each layer of the microdevice were designed using CorelDraw software and cut from transparency sheets using a CO_2 laser cutter (VersaLASER VLS3.50; Universal Laser Systems, Scottsdale, AZ). The device was bonded using an office laminator (UltraLam 250B; Akiles, Mira Loma, CA), allowing the printer toner to serve as an adhesive agent. The layers were initially taped together to maintain alignment and subsequently inserted into the laminator several times to ensure sufficient bonding.