Reverse-Hybridization-Based Genetic Testing for the Prediction of Anticoagulant Dose Requirement

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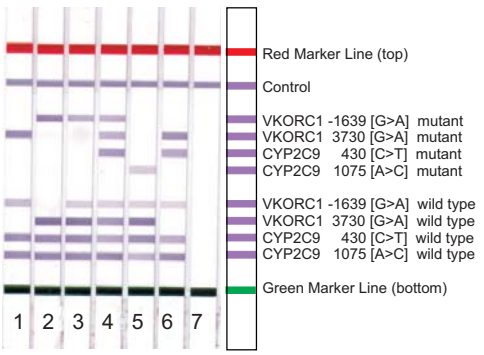
INTRODUCTION

Coumarin derivatives, such as warfarin, phenprocoumon or acenocoumarol, are the most widespread oral anticoagulant drugs for the prevention and treatment of arterial and venous thromboembolic disorders. However, these vitamin K antagonists have a narrow therapeutic range and a wide inter-individual variability in dose requirement. Despite adjustment for clinical variables adverse events (delay in achieving a stable maintenance dose or bleeding complications) are frequently encountered during the initial phase of therapy. Genetic polymorphisms in the drug-targeted vitamin K epoxide reductase complex 1 (VKORC1) and in the drug metabolizing cytochrome P450 isozyme CYP2C9 have been reported to account for the majority of variations in the therapeutic response to warfarin.[1 2 3]

TEST DESIGN

Mutations covered by the PGX-Thrombo StripAssay:

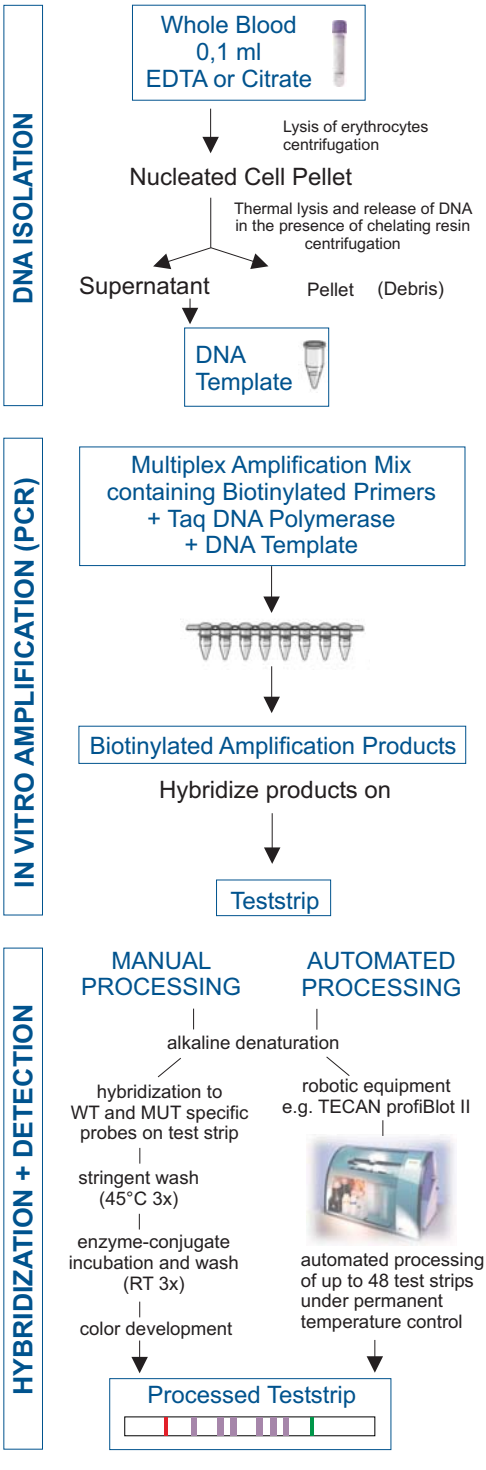
Gene	Poly-morphism	Geno-type	Coumarin sensitivity
VKORC1	-1639 G>A	GG	low
		GA	intermediate
		AA	high
	3730 G>A	GG	high
		GA	intermediate
		AA	low
			Metabolic status
CYP2C9	430 C>T (*2)	CC	extensive
		CT	intermediate
		TT	poor
	1075 A>C (*3)	AA	extensive
		AC	intermediate



Sample #	1	2	3	4	5	6	7
VKORC1 -1639 G>A	GG	AA	GA	GA	GG	GG	--
VKORC1 3730 G>A	AA	GG	GG	GA	GG	AA	--
CYP2C9	*1*1	*1*1	*1*1	*1*2	*1*3	*1*2	--

Figure 1: Image of different staining patterns obtained with the PGX-Thrombo StripAssay.
Strip # 1-6: Patient samples; strip 7: no template control

ASSAY PROCEDURE



References:
1. D'Andrea et al. 2005, Hemost Thromb Vasc Biol 105, 645-9.
2. Rieder et al. 2005, NEJM 352, 2285-33.
3. Sconce et al. 2005, Hemost Thromb Vasc Biol 106, 2329-33.

RESULTS

Fifty-three patients on phenprocoumon (Marcumar®) therapy were retrospectively genotyped for VKORC1 and CYP2C9 variants. The VKORC1 -1639 G>A polymorphism is clearly associated with higher phenprocoumon sensitivity, whereas the 3730 G>A variant is associated with lower sensitivity.

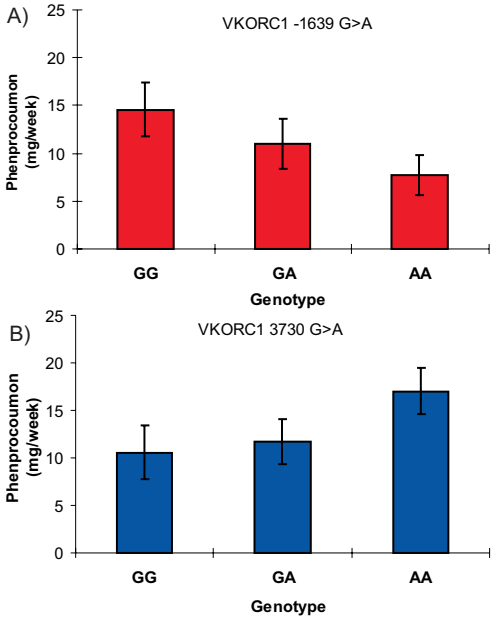


Figure 2: Effect of VKORC1 genotypes on phenprocoumon dose requirement.
Weekly phenprocoumon dose related to A) -1639 G>A genotypes (GG n=19; GA n=23; AA n=11) and B) 3730 G>A genotypes (GG n=25; GA n=21; AA n=7). Bars represent means +/- SD.

Due to the small number of *2 (n= 12) and *3 (n=7) CYP2C9 variants in our present patient cohort, their effect on phenprocoumon dose requirement will be assessed from a larger sample size in our ongoing study.

SUMMARY

- **OBJECTIVE:** to develop a diagnostic tool for predicting the response of patients to coumarin derivatives; results will assist clinicians to achieve a more individualized anticoagulant therapy
- **SPEED:** short total assay time (~5h)
- **CONVENIENCE:** ready-to-use reagents and prefabricated teststrips; proprietary software (Evaluator) available for automated scanning of teststrips and interpretation of band patterns
- **SAMPLE SIZE:** single multiplex PCR; only approx. 50 ng DNA needed
- **SIMPLICITY:** simple and straightforward protocol; inexpensive equipment (thermocycler, waterbath, shaker); easy and clear interpretation of results
- **EFFICIENCY:** accessible to automation using robotic equipment
- **FLEXIBILITY:** new mutations readily integrated

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