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Simultaneous Identification of Five Cyp2C9 Alleles using Multiplexed Sequencing Technology (MultiGEN)

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SUMMARY

We have developed a multiplexed sequencing technology, MultiGEN, that simultaneously detects allele profiles associated with Cyp29C mutations. A single electropherogram is generated that distinctively detects all five allele mutations (R144c, 1359L,1359T D360E and 818 del). We have tested 56 samples and found that 50 samples were wild type allele for all alleles. There were six patients with heterozygous allele status for variant 3.

INTRODUCTION

Warfarin is a drug that prevents the clotting of blood.(1,2) It is frequently used in patients with atrial fibrillation (an irregular heart rhythm), pulmonary embolism, and before surgery for orthopedic procedures. The ability to clot after treatment with Warfarin varies between patients, depending upon their genetic makeup, as mutations at specific alleles affect the rate of Warfarin catabolism(3,4). These variations are functionally measured by a test called the Prothrombin Time (PT). This manuscript describes an assay that identifies all three states (wild, heterozygous and mutated versions) of the five most common alleles that affect the rate of Warfarin catabolism.

MATERIALS AND METHODS

Anonymous patient samples were tested for mutations at all five alleles. Total DNA was extracted using BugsNBeads A (NorDiag, USA). Multiple PCR of target amplicons was performed in a 50-ul volume containing 5ul of extracted DNA. The amplified multiple targets were purified using Ampure (Agencourt, USA). Purified amplicons were sequenced using MultiGEN sequencing primers by cycle sequencing using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit version 1.1 (Applied Biosystems, USA) on a GeneAmp 2400 thermocycler (PE Applied Biosystems, USA). Unincorporated dye terminators were removed using Clean Seq (Agencourt USA). The samples were then dried in a speed vac (DNA 120, ThermoSavant, USA) and re-suspended in 20 ul of Hi-Di formamide. Samples were analyzed by capillary electrophoresis using the ABI PRISM Genetic Analyzer 3130.

REFERENCES:

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Fig. I SINGLE Electropherogram showing ALL FIVE alleles

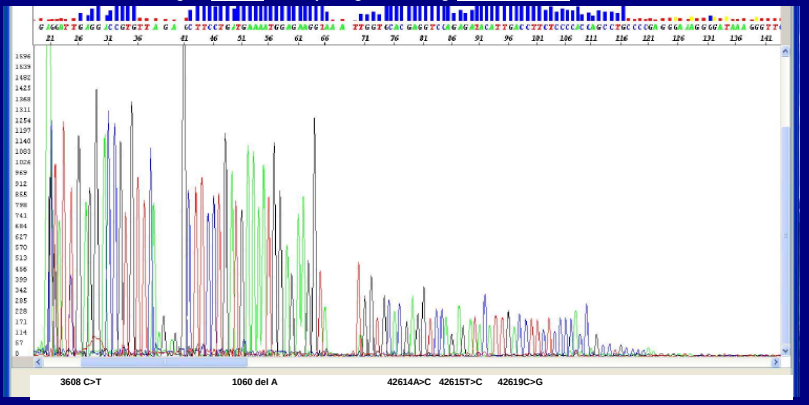

Table 2 Cyp2C9 Allele Profile

	Variant *2	Variant *3	Variant *4	Variant *5	Variant *6
Variant	(3608C>T)	(42614A>C)	(42615T>C)	(42619C>G)	(10601 del A)
Allele1	AGGACCGT	ATACATTGAC	ATACATTGAC	CATTGACCT	GAGAAGGT
Allele 2	AGGACCGT	ATACATTGAC	ATACATTGAC	CATTGACCT	GAGAAGGT
Allele1	AGGACCGT	ATACATTGAC	ATACATTGAC	CATTGACCT	GAGAAGGT
Allele 2	AGGATCGT	ATACCTTGAC	ATACACTGAC	CATTGAGCT	GAGAGGT
Allele1	AGGATCGT	ATACCTTGAC	ATACACTGAC	CATTGAGCT	GAGAGGT
Allele 2	AGGATCGT	ATACCTTGAC	ATACACTGAC	CATTGAGCT	GAGAGGT

Allele	No.Samples	Variant *2	Variant *3	Variant *4	Variant *5	Variant *6
Profile 1	50	ACC	CAT	ATT	ACC	GAA
Profile 2	6	ACC	CA/CT	ATT	ACC	GAA

RESULTS AND DISCUSSION

Out of the samples tested, 50 were the wild type allele for all of the five variants tested, and 6 samples were heterozygous for alleles 2, 4, 5 and 6. Although allele variation is common within the Caucasian population,we did not confirm this with our samples. Genetic mutations are mainly a single base change, and their accurate detection requires a method that can accurately identify the affected locus among the 3.1 billion nucleotides of the human genome. Sanger sequencing is the accepted method of choice in acheiving this task. However, as each allele region would have to be sequenced separately, traditional Sanger sequencing is not routinely used for this type of assay. MultiGEN is a novel modification of Sanger sequencing that allows the simultaneous sequencing of multiple loci.