Pharmacogenomic Genotyping Performance Across Biological Specimens

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RPRD DIAGNOSTICS
— Right Patient | Right Drug —

Background

- Preemptive genotyping of relevant pharmacogenetic (PGx) genes and HLA typing for known associations with drug metabolism and hypersensitivity enables a clinician to provide a patient with an optimized treatment regimen by maximizing drug efficacy and limiting adverse reactions.
- There are over 300 actionable genetic variants with dosing guidelines on FDA-approved medications.[1]
- These drugs span a wide range of categories from pain management to cancer, impacting a significant percentage of prescription medication (e.g. codeine, warfarin, allopurinol).[1-3]
- High risk genotypes vary in frequency between different ethnicities , with certain high-risk alleles being common in some populations.[4]

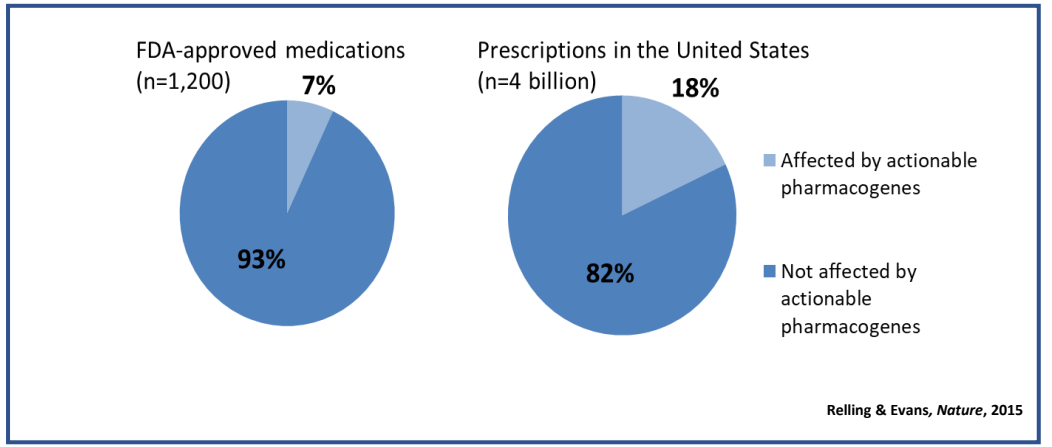

Figure 1. Percentage of medications and prescriptions affected by genotyping of actionable pharmacogenes.

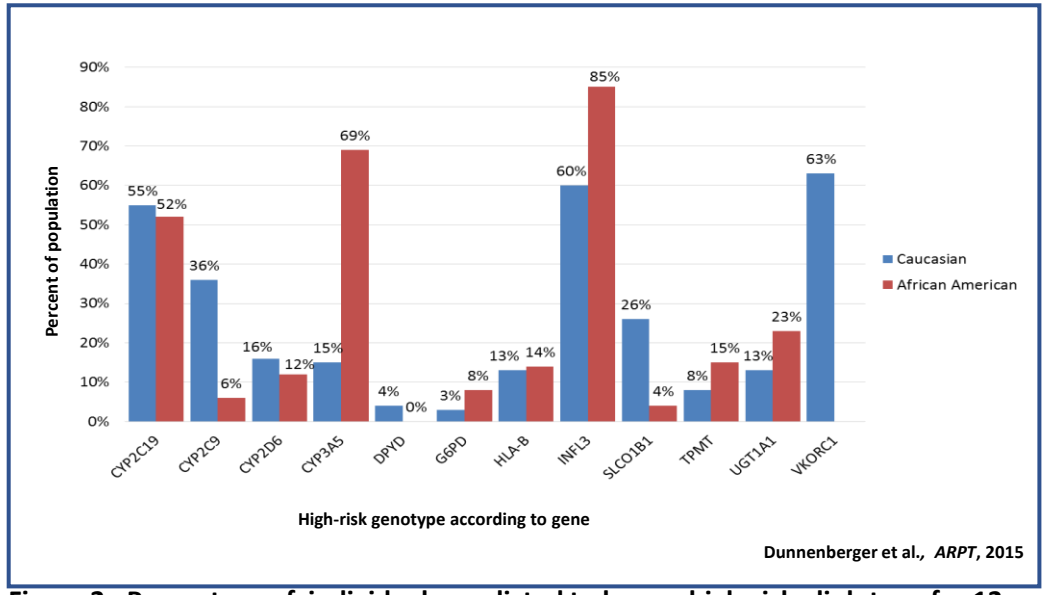

Figure 2. Percentage of individuals predicted to have a high-risk diplotype for 12 PGx relevant genes.

Methods

- Genomic DNA (gDNA) was acquired from Coriell (isolated from LCL cell lines)[5] or isolated from Liver, blood, buccal and saliva.
- Samples were batched (including mixed sample types per plate) and run using the PharmacoScan™ Assay Kit 24-Format (pScan), or the PharmacoFocus™ Assay Kit 96-Format (pFocus).
- Array analysis was done on the Axiom™ Analysis Suite and performance of the assay was determined looking at both inter- and intra-run specificity, sensitivity, and concordance.

CPIC Genes	Haplotype Calling Genes			Copy Number Genes
CFTR	CDA	POR	UGT2B17	CYP2A6
CYP2B6	CYP1A1	PTGIS	COMT	CYP2D6
CYP2C9	CYP1B1	SLC15A2	CYP1A2	GSTM1
CYP2C19	CYP2A13	SLC22A2	CYP2A6	GSTT1
CYP2D6	CYP2F1	SLCO2B1	CYP2C8	UGT2B17
CYP3A5	CYP2J2	SULT1A1	CYP2E1	
DPYD	CYP2S1	TBXAS1	CYP3A4	
G6PD	CYP3A43	UGT1A3	CYP3A7	
IFNL3	CYP4B1	UGT1A4	GSTM1	
SLCO1B1	CYP4F2	UGT1A6	GSTP1	
TPMT	CYP19A1	UGT1A7	NAT1	
UGT1A1	F5	UGT1A8	NAT2	
VKORC1	FMO1	UGT1A9	UGT2B7	
CACNA1S*	FMO2	UGT1A10		
NUDT15				
RYR1*				

Table 1. A subset of PGx genes genotyped on both Assays. *ACMG Gene

ThermoFisher Scientific

Sample Information

Samples run on PharmacoScan™:

- Coriell Institute (from LCL cell lines; n= 112); Isolated from liver tissue (n=161), blood (n=20), or saliva (n=20). Analysis was done on the Axiom™ Analysis Suite 4.1.
- Additionally, the blood, saliva and Coriell samples were run on the DMET™ Plus array and received CYP2D6 TaqMan-based CN testing.

Samples run on PharmacoFocus™:

- Coriell Institute (from LCL cell lines; n= 127), blood (n=27), or saliva (n=27), buccal (n=4). Analysis was done on the Axiom™ Analysis Suite 5.0.
- Additionally, the blood, saliva and Coriell samples were run on the PharmacoScan™ array and other platforms listed in the samples run on PharmacoScan section.

Results: pScan

All sample types interrogated had passing rates of >98% for all array quality control (QC) metrics.

- Genomic DNA samples from blood, saliva and Coriell demonstrated call rates of 99.85-99.99%, intra-run concordance of 99.65-99.99% and inter-run concordance of 98.88-100% (**Table 2**).
- Liver-derived gDNA samples had call rates of 99.67-99.93%.
- The Diagnostic sensitivity and specificity were determined.
    - As shown in **Tables 3** and **4**, the % False Positive and % False Negative range was 0.09-0.25%.

Table 2 : Intra-run and Inter-run Concordance for PharmacoScan™ Array for blood, saliva and gDNA (Coriell).

	Plates 1 & 2% inter-run Concordance	Plates 1 & 2% intra-run Concordance	Gender Match %	% Copy Number Concordance (9 CNV Locations, 24 Samples)
Average Concordance	99.838	99.986	100.000	99.537
Concordance Range	98.882-100	99.961-100	100.000	88.89-100
Average concordance with in -house extractions	99.918	99.975	100.000	100.000
Average concordance with Coriell controls	99.789	99.997	100.000	99.259

Table 3: Diagnostic Sensitivity

Plate/Sample information	% False Negative	%True Positive
Plate 1: Coriell Samples	0.09%	99.91%
Plate 2: Coriell Samples	0.00%	100%
Plate 1: In-house Extracted Samples	0.25%	99.75%
Plate 2: In-house Extracted Samples	0.00%	100%

Table 4: Diagnostic Specificity

Plate/Sample information	% False Positive	%True Negative
Plate 1-Coriell Samples	0.09%	99.91%
Plate 2-Coriell Samples	0.09%	99.91%
Plate 1-In-house extracted Samples	0.00%	100%
Plate 2- In-house extracted Samples	0.00%	100%

Results: pFocus

All sample types interrogated had passing rates of >98% for all array quality control (QC) metrics.

- **Plates 1 and 2 Analysis:**
  - 91 Genomic DNA samples from Coriell and 2 kit supplied controls.
  - Concordance between plate 1 vs plate 2 and pScan results vs pFocus results are shown in **Figure 3**. On average the concordance between all runs was >99.5%.

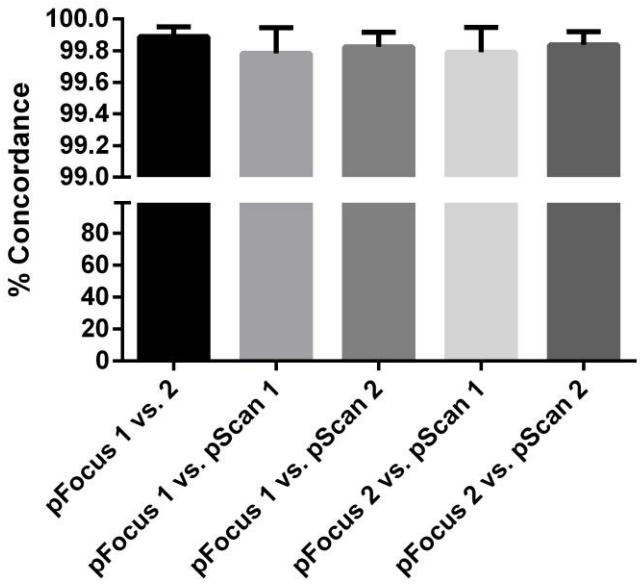

Figure 3. Probe Level Concordance. A probe level concordance was performed comparing results for the same samples run on pFocus plate1 vs plate 2 and pFocus vs pScan.

- **Plate 3 Analysis:**
  - 34 Genomic DNA samples from Coriell, 4 Buccal samples, 27 Blood, 29 Saliva and 2 kit supplied controls.
  - Buccal samples were collected utilizing 2 different extraction kits (Genteuri (1) and IsoHelix (2)). Samples from the same donors were also collected and extracted from saliva. Concordance results between the kits is shown in **Table 5**.

	QC Call Rate	vs Saliva	vs Kit (2)
Kit (1)	99.09-99.64	99.80-99.94	99.76-99.99
Kit (2)	99.48-99.53	99.80-99.95	N/A

Table 5: Buccal Performance. QC call rate and concordance of two buccal collection and extraction Methodologies compared to saliva collection and extraction.

Conclusions

- Regardless of gDNA origin, samples produced results that meet the array QC requirements for both assays.
- Comparison with 1000 Genomes and DMET results allowed for the determination of the accuracy of the blood, saliva and Coriell samples on pScan.
    - Of note, the array accurately detected *CYP2D6* CN variation and *CYP2D6* hybrid or gene conversion arrangements, which is integral for metabolizer status prediction.
- Downstream inclusion of this data in the EMR will enable clinicians to preemptively make the most informed drug choices and dosing decisions, providing cost effective and better individualized patient care.

References

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