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Analytical validity of a genotyping assay for use with personalized antihypertensive and chronic kidney disease therapy

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Abstract

Hypertension and chronic kidney disease are inextricably linked. Hypertension is a well-recognized contributor to chronic kidney disease progression and, in turn, renal disease potentiates hypertension. A generalized approach to drug selection and dosage has not proven effective in managing these conditions, in part, because patients with heterogeneous kidney disease and hypertension etiologies are frequently grouped according to functional or severity classifications. Genetic testing may serve as an important tool in the armamentarium of clinicians who embrace precision medicine. Increasing scientific evidence has supported the utilization of genomic information to select efficacious antihypertensive therapy and understand hereditary contributors to chronic kidney disease progression. Given the wide array of antihypertensive agents available and diversity of genetic renal disease predictors, a panel-based approach to genotyping may be an efficient and economic means of establishing an individualized blood pressure response profile for patients with various forms of chronic kidney disease and hypertension. In this manuscript, we discuss the validation process of a Clinical Laboratory Improvement Amendments (CLIA)-approved genetic test to relay information on 72 genetic variants associated with kidney disease progression and hypertension therapy. These genomic-based interventions, in addition to routine clinical data, may help inform physicians to provide personalized therapy.

Introduction

Hypertension and chronic kidney disease are intersecting diseases with enormous economic burdens. The design and implementation of genotype-guided care in clinics enables providers to identify drug-gene interactions to inform medication selection, improve treatment efficacy, and reduce adverse events and their associated medical costs. Genetic predictors of chronic kidney disease (CKD) progression may augment standard clinical prediction models, impacting prognosis, medication selection, dialysis initiation, and renal transplant organ allocation. We have designed a genotyping test to detect 72 genetic variants

in drug metabolizing enzymes and other genes to aid providers in the management of hypertension, chronic kidney disease, and other related conditions.

This communication focuses on the analytical validity of the renal precision medicine assay, including CLIA validation with the assessment of precision and accuracy, analytical sensitivity and specificity, variant reporting strategy, and confirmation of positive reference materials through sequencing. Our goal is to facilitate implementation for future investigators and health systems. A detailed discussion of each variant's clinical validity extends beyond the scope of this manuscript; however, content examples are provided in order to convey context and purpose.

Variant Selection

Variants in the renal precision medicine assay were selected after balancing their significance, effect size, minor allele frequency, and level of evidence. When applicable, drug-gene phenotypes were utilized from the Pharmacogene Variation Consortium (PharmVar), the Food and Drug Administration (FDA) drug label, Clinical Pharmacogenomics Implementation Consortium (CPIC) and Dutch Pharmacogenetics Working Group (DPWG) [1, 2]. The three groups of variants represented include predictors of 1) antihypertensive drug response, 2) drug response relevant to immunosuppression or cardiovascular disease, and 3) CKD progression. Antihypertensive pharmacogenomic variants were included for metoprolol (*CYP2D6*), other beta-blockers (*GRK4*, *ADRB1*), hydralazine (*NAT2*), losartan (*CYP2C9*), amlodipine (*F7*), thiazide diuretics (*YEATS4*, *FGF5*/*SH2B3*/*EBF1*), diuretics (*NEDD4L*), angiotensin-converting-enzyme inhibitors (*F7*), and angiotensin II receptor blockers (*NPHS1*) (Table 1) [3-11]. The clinical validity of these variants has been previously reviewed and their associated levels of evidence vary [12, 13]. For example, *CYP2D6*-guided dosing of metoprolol has been recommended by the DPWG. The FDA drug labels of hydralazine and losartan include NAT2 and CYP2C9 metabolizer status, respectively, as predictors of response. This assay employs genotype as a surrogate to establish metabolizer status for these drugs. Other antihypertensive response predictors included in the assay have lower levels of evidence for implementation. Although they have been replicated in clinical trials, their clinical use is still considered investigational.

A second group of pharmacogenomic variants found in the *CYP3A4* and *CYP3A5*, *TPMT*, *SLCO1B1*, *CYP2C9*, *VKORC1*, and *CYP2C19* genes have been widely studied with genotype-guided dosing recommendations from CPIC and DPWG for drugs such as tacrolimus, azathioprine, simvastatin, warfarin, and clopidogrel (Table 1) [14-18]. The immunosuppressant drug-gene pairs were selected to aid in glomerulonephritis and post-transplant care. The cardiovascular drug-gene pairs were included due to the preponderance of cardiovascular disease in patients with CKD.

A third group of variants have been identified as predictors of CKD progression (Table 2) [19-22]. Genes associated with progression were identified from large genome-wide association studies or prospective clinical cohorts and trials such as the Chronic Renal Insufficiency Cohort and African American Study of Kidney Disease and Hypertension [20]. These genes include *APOL1*, relevant to individuals with African ancestry, as well as other

genes identified from African-American, Asian and Caucasian populations, with or without diabetes mellitus. Their clinical validity has been reviewed elsewhere [23].

Variant Reporting

When feasible, genetic nomenclature and allele reporting was utilized from PharmVar and CPIC. Drug-gene pairs with lower levels of evidence often lack a consensus allelic structure. As a result, the *ADRB1*, *FGF5*/*SH2B3*/*EBF1*, and *GRK4* genes each include a multi-variant model to predict efficacy that is summarized by copies of amino acids or efficacy alleles. The *APOL1* gene is comprised of the *G1 (G and GM) and *G2 variant alleles. The *G1 allele is described as two missense variants and the *G2 allele is a 6 base pair deletion that is in high linkage disequilibrium with a single nucleotide polymorphism marker used in this assay. These variants are reported based on quantity of risk alleles. All other variants were classified based on the nucleotides detected. The effects of some variants were only studied or found to be significantly associated with phenotypes in certain populations. As such, some variants are only relevant in a single ethnicity or in individuals without diabetes. This distinction is made in the relevant population column of Table 1 and 2.

Genotyping Assay Validation

We designed a custom OpenArray in which Taqman allele discrimination was used for genotyping analysis for our selected variants. DNA samples were amplified in singlicate by real-time PCR on the LifeTech QuantStudio 12K Flex (software v1.2.2; Grand Island, NY) using commercially available reagents for the custom designed OpenArray according to the user manual (Life Technologies, Grand Island, NY). DNA samples used for analytical validation were obtained from the Coriell Cell Repository (Camden, NJ) and Icahn School of Medicine at Mount Sinai (New York, NY) [24]. In this paper, we describe the CLIA validation process for the variants provided in Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/FPC/B331 . The CYP450s, *SLCO1B1, TPMT*, and *VKORC1* variants have been previously validated as part of the INGENIOUS trial [25].

Since there were no known reference materials for most variants, selected DNA samples were Sanger sequenced with custom designed primers (Integrated DNA Technologies, Coralville, IA) for accuracy studies (Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/FPC/B331). PCR amplification was performed on the GeneAmp PCR System 2720 (Applied Biosystems). The targeted gene region was amplified using custom primers and PCR SuperMix (Invitrogen, Carlsbad, CA) at the following PCR amplification conditions: 94°C for 2 minutes, 35 cycles at 94°C for 15 seconds, 56°C for 30 seconds, 72°C for 1 minute and a final extension step at 72°C for 7 minutes. PCR purification was performed using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA) according to the user manual. Samples were prepared for sequencing using 10 μL of purified PCR amplicon (50 ng total) and 2 μL of the sequencing primer [1.7 μM] and Sanger sequenced by ACGT Inc. (Germantown, MD). For *ADRB1,* reference materials that were previously whole genome sequenced in the 1000 Genomes Project were obtained and genotyped. Variant information from the project was retrieved from https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/ to confirm the accuracy of the *ADRB1* analytical validation study.

The sequencing results were compared to the array genotyping results and were completely concordant for 100% accuracy. Based on the orthogonal method (e.g. DNA sequencing), the variant and reference allele used to calculate the assay's analytical sensitivity and specificity (Table 3 and Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/FPC/B332). The analytical sensitivity was 100% for the detection of variant alleles, with no reported false negatives. The analytical specificity was 100% for detection of reference alleles, with no false positive results reported (note at the 95th percentile, the confidence intervals varied based on variant frequencies). DNA samples were also used to assess intra- and inter-assay variation. In all, 14 samples obtained from Icahn School of Medicine at Mount Sinai were included in intra-assay validation. There were 18 samples included in the inter-assay validation which were comprised of the 14 Icahn School of Medicine at Mount Sinai samples plus four additional Coriell cell line samples that included the less frequent genotypes (NA07357, NA10847, NA18524, NA18563). In order to find these less frequent genotypes needed for validation, 189 total Coriell samples were genotyped successfully. These results are included in Supplemental Table 2 , Supplemental Digital Content 2, http://links.lww.com/FPC/B332, to serve as reference materials for laboratories that may validate the same variants. The intra- (within) assay variation studies showed that all three replicates of the 14 samples ran on the same plate, were concordant with expected results. The inter- (between) assay variation studies showed that the 18 samples consistently yielded the same result across three separate runs. The stability had been previously established for the following conditions: DNA isolated from whole blood at room temperature after 1 week, refrigerated for 2 weeks, and frozen for 4 weeks, saliva after 5 years at room temperature, frozen indefinitely, and extracted DNA refrigerated and frozen for 5 and 15 years, respectively.

Implementation

The implementation of genetic testing to aid in personalized therapy has become more widespread. Applications for the variants detected with this assay may extend beyond the clinical care of individuals with hypertension and CKD as illustrated by the implementation efforts of networks like the Implementing Genomics in Practice (IGNITE) and APOL1 Long-Term Kidney Transplantation Outcomes (APOLLO) consortia. Further pragmatic trials are needed to determine clinical validity and utility. Understanding the underlying genetic architecture which predisposes an individual to develop chronic kidney disease allows us to build upon existing functional CKD classifications, which are presently based on estimate glomerular filtration rates and proteinuria. The implementation of genetic testing into clinical practice has the potential to augment drug selection, dose adjustment, improve the classification of CKD in patients, and more accurately predict the risk of progression. This testing serves as a complement to, not a replacement for, effective clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Efficacy and Adverse Event Phenotype-Genotype Interactions

Gene	*Variant*	*Relevant Population**	*Genotyping Result*	*Predicted Phenotype*
ADRB1	rs1801252, rs1801253		0 copies of 49S-389R[a]	Reduced Beta-Blocker Efficacy
			1 copy of 49S-389R	Standard Beta Blocker Efficacy
			2 copies of 49S-389R	Increased Beta Blocker Efficacy
CYP2C19	rs4244285, rs4986893, rs28399504, rs72552267, rs41291556, rs6413438, rs12248560		*1/*1, *1/*17	Standard Clopidogrel Efficacy
			*1/*2, *3, *4, *6, *8, *2/*2, *3, *4, *6, *8, *17[b]	Reduced Clopidogrel Efficacy
			*17/*17	Increased Risk of Bleeding
CYP2C9	rs1799853, rs1057910, rs28371686, rs9332131, rs7900194, rs28371685		*1/*1	Standard Losartan Efficacy
			*1/*2, *3, *5, *6, *8, *11[b]	Reduced Losartan Efficacy
CYP2D6	rs16947, rs1135840, rs35742686, rs3892097, rs1065852, rs5030655, rs5030867, rs5030865(A), rs5030656, rs1065852, rs1135840, rs5030865(T), rs28371706, rs61736512, rs59421388, rs1135840, rs28371725		*1/*1, *2, *9, *10, *17, *2/*2, *9, *10, *17, *29, *41[b]	Standard Metoprolol Efficacy
			*1/*1xN, *1/*2xN, *2/*2xN[b]	Reduced Metoprolol Efficacy
			*1/*3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16[c]	Increased Bradycardia with Metoprolol
CYP3A4	rs55785340, rs35599367	CYP3A5 *3/*3, *6, *7 [b]	*1/*1, *1/*22	Standard Tacrolimus Dosing
			*22/*22	Reduced Tacrolimus Dosing
CYP3A5	rs776746, rs10264272, rs41303343		*3/*3, *6, *7[b]	Standard Tacrolimus Dosing
			*1/*1, *3, *6, *7	Increased Tacrolimus Dosing
F7	rs6046	African American	G/G	Standard Amlodipine Efficacy
			G/A	Reduced Amlodipine Efficacy
			A/A	Poor Amlodipine Efficacy
FGF5/ SH2B3/EBF1	rs1458038, rs3184504, rs4551053	Caucasian	0 efficacy alleles[c]	Reduced Thiazide Efficacy
			1 or 2 efficacy alleles	Standard Thiazide Efficacy
			3 or more efficacy alleles	Increased Thiazide Efficacy
GRK4	rs2960306, rs1024323		0 copies of 65L-142V[a]	Increased Beta-Blocker Efficacy
			1 copy of 65L-142V	Standard Beta Blocker Efficacy
			2 copies of 65L-142V	Reduced Beta Blocker Efficacy
NAT2	rs1801279, rs1801280, rs1799930, rs1799931		*4/*4	Standard Hydralazine Efficacy
			*4/*5, *6, *7, *14[b]	Increased Hydralazine Efficacy
NEDD4L	rs4149601	Caucasian	G/G	Increased Diuretic Efficacy
			G/A	Standard Diuretic Efficacy
			A/A	Reduced Diuretic Efficacy

Gene	*Variant*	*Relevant Population**	*Genotyping Result*	*Predicted Phenotype*
NPHS1	rs3814995	Caucasian	G/G	Standard ARB Efficacy
			G/A or A/A	Increased ARB Efficacy
SLCO1B1	rs4149056, rs4149015		*1/*1, *1/*21, *21/*21	Standard Simvastatin Dosing
			*1/*5, *1/*17, *5/*5,	Reduced Simvastatin Dosing
			*5/*17, *17/*17, *17/*21	Reduced Simvastatin Dosing
TPMT	rs1800462, rs1800460 and rs1142345, rs1800460, rs1142345, rs1800584		*1/*1	Standard Azathioprine Dosing
			*1/*2, *1/*3, *2/*2, *2/*3, *3/*3	Reduced Azathioprine Dosing
				Reduced Azathioprine Dosing
VASP	rs10995	Caucasian	A/A	Standard Thiazide Efficacy
			A/G or G/G	Increased Thiazide Efficacy
VKORC1	rs9923231		G/G	Standard Warfarin Sensitivity
			G/A or A/A	Increased Warfarin Sensitivity
YEATS4	rs7297610	African American	T/T or T/C C/C	Standard Thiazide Efficacy
			C/C	Increased Thiazide Efficacy

*Relevant population provided if results have only been identified in a specific population.

[a]2-variant model summarized by the number of copies of the amino acid pairs.

[b]An abbreviated list is provided due to the large number of possible genotyping results.

[c]3-variant model summarized by the number of efficacy alleles. Efficacy alleles are as follows: rs1458038 (C), rs3184504 (C), rs4551053 (G)

Table 2:

Genotype-Predicted Risks for Chronic Kidney Disease Progression

Gene	*Variant*	*Relevant Population* *	*Genotyping Result*	*Predicted CKD Progression Risk*
APOL1	rs73885319, rs60910145, rs71785313	African American	0 or 1 risk allele[a]	Standard Risk
			2 or more risk alleles	Increased Risk
LINC00923	rs653747	African American non-diabetic	C/C	Standard Risk
			C/T or T/T	Increased Risk
SHROOM3	rs17319721	Caucasian	G/G	Standard Risk
			G/A or A/A	Increased Risk
SHROOM3	rs4371638	African American	C/C	Standard Risk
			C/T or T/T	Increased Risk
SHROOM3	rs13146355	Asian	G/G	Standard Risk
			G/A or A/A	Increased Risk
SLC3A2	rs489381	Caucasian	C/C	Standard Risk
			C/A or A/A	Increased Risk
SLC3A2/LOC105369332	rs2282538	African American	G/G	Standard Risk
			G/A or A/A	Increased Risk
UMOD	rs4293393	African American	T/T or T/C	Standard Risk
			C/C	Reduced Risk
UMOD	rs12917707	Caucasian	C/C or C/A	Standard Risk
			A/A	Reduced Risk
UMOD/PDILT	rs11864909	Asian	G/G or G/A	Standard Risk
			A/A	Reduced Risk

*Relevant population provided if results have only been identified in a specific population.

[a]3-variant model summarized by the number of risk alleles, * G1(G), * G1(GM), and* G2.

Table 3:

Analytical Sensitivity, Specificity, and Accuracy of OpenArray

Gene	Variant	Analytical Sensitivity	Analytical Specificity	Accuracy
ADRB1	c.1165G>C (rs1801253)	100% (95% CI; 93-100)	100% (95% CI; 98-100)	100%
	c.145A>G (rs1801252)			
APOL1	c.1072A>G (rs73885319)	100% (95% CI; 91-100)	100% (95% CI; 98-100)	100%
	c.1200T>G (rs60910145)			
	c.1212_1217delTTATAA (rs71785313)			
EBF1	g.158411534G>A (rs4551053)	100% (95% CI; 57-100)	100% (95% CI; 74-100)	100%
FGF5	g.81164723C>T (rs1458038)	100% (95% CI; 51-100)	100% (95% CI; 61-100)	100%
GRK4	c.194G>T (rs2960306)	100% (95% CI: 82-100)	100% (95% CI; 85-100)	100%
	c.329C>T (rs1024323)			
LINC00923	g.97659878C>T (rs653747)	100% (95% CI; 79-100)	100% (95% CI; 72-100)	100%
NAT2	c.857G>A (rs1799931)	100% (95% CI; 97-100)	100% (95% CI; 95-100)	100%
	c.191G>A (rs1801279)			
	c.590G>A (rs1799930)			
	c.341T>C (rs1801280)			
NEDD4L	c.24G>A (rs4149601)	100% (95% CI; 57-100)	100% (95% CI; 83-100)	100%
NPHS1	g.35851310C>T (rs3814995)	100% (95% CI; 65-100)	100% (95% CI; 65-100)	100%
SHROOM3	c.168+11474G>A (rs17319721)	100% (95% CI; 57-100)	100% (95% CI; 65-100)	100%
	c.169-36495T>C (rs4371638)			
	c.168+54767G>A (rs13146355)			
SH2B3	c.784T>C (rs3184504)	100% (95% CI; 61-100)	100% (95% CI; 72-100)	100%
SLC3A2	c.1066-41A>G (rs489381)	100% (95% CI; 77-100)	100% (95% CI; 72-100)	100%
SLC3A2/LOC105369332	g.62832260C>T (rs2282538)	100% (95% CI; 34-100)	100% (95% CI; 84-100)	100%
UMOD	c-680T>C (rs4293393)	100% (95% CI; 68-100)	100% (95% CI; 90-100)	100%
	c.−214C>A (rs12917707)			
UMOD/PDILT	c.203-4666G>A (rs11864909)	100% (95% CI; 34-100)	100% (95% CI; 85-100)	100%
VASP	c.*719G>A (rs10995)	100% (95% CI; 70-100)	100% (95% CI; 57-100)	100%
YEATS4	g.69430244C>T (rs7297610)	100% (95% CI; 34-100)	100% (95% CI; 85-100)	100%