Color Medication Response Genetic Test

Version 2.0 – Updated 07.11.2024

Executive summary

Pharmacogenomic testing

- Pharmacogenomic testing can provide individuals with information about how their body may respond to medications based on their genetic background.
- Up to 50% of spontaneously reported adverse drug reactions have identifiable causes, many of which can be partially explained by a genetic variant.[1]
- An estimated 90-99% of the general population has at least one actionable variant in an established pharmacogenomics (PGx) gene.[1,2]
- Of the FDA biomarker–drug pairs annotated with PGx levels, 59.7% of labels are associated with cancer drugs.[3]
- The impact and the management of adverse drug reactions is complex and may cost up to 30.1 billion dollars annually in the U.S.[2]

Color's Medication Response Test

- Color has developed a high-sensitivity, low-cost, next generation sequencing assay that identifies well-established alleles in 14 PGx-related genes.
- The test aligns with current standards of pharmacogenomic guidelines, including the Association for Molecular Pathology (AMP) pharmacogenomic working group, and includes a broad allele set that captures clinically actionable pharmacogenomic variation across diverse populations, as recently emphasized by the American Cancer Society (ACS).[4,5]
- The validation of the test demonstrated 100% concordance in diplotype calling and 99.98% accuracy in variant calling, confirming its reliability for clinical use.

Introduction

Prescription drug use is common, with more than 66% of adults in the United States (131 million people) taking these medications and 24% taking three or more in the last month. [1,6] However, it is estimated that only about half of patients respond favorably to their medications and may experience therapy failure and adverse drug reactions (ADRs).[7–9] It has been estimated that over 2 million serious ADRs occur each year, leading to 106,000 deaths, making it the fourth leading cause of death in the U.S..[10–13]

Pharmacogenomic (PGx) testing, which aims to tailor medication choices to an individual's genetic profile, has the potential to significantly reduce these ADRs and associated healthcare costs and maximize therapeutic benefit by ensuring that patients receive the most effective and safe treatments from the outset. Notably, between 90-99% of the general population are estimated to have one or more actionable variants in an established PGx gene, underscoring the widespread potential impact of this personalized approach to medicine.[1,2]

The Clinical Pharmacogenetics Implementation Consortium (CPIC) was established to help physicians understand how to utilize PGx information. CPIC built a standardized grading system to evaluate the levels of evidence linking genotypes to clinical phenotypes, outline prescribing recommendations based on genotype/phenotype, and provide a standardized method for assigning strength to each prescribing recommendation.[14–16] CPIC guidelines have been endorsed by the Association for Molecular Pathology (AMP), the American Society for Clinical Pharmacology and Therapeutics (ASCPT), and the American Society of Health-System Pharmacists (ASHP). Additionally, the FDA has issued guidance about how to use pharmacogenomic test results for over 264 drug labels and provides instruction on how to incorporate PGx data into new drug submissions.[17,18]

Color Medication Response Genetic Test

Color Medication Response Genetic Test is built upon Color's clinical grade, quality-controlled sequencing platform to analyze the most recognized drug-related genes. The test uses targeted sequencing to identify genotypes at predetermined positions, derives the most likely diplotype to explain the observed genotypes, converts the diplotype into an established phenotype, and then based on phenotype, provides clinical interpretations from sources such as FDA and CPIC.[19] Here, we describe the validation approach and data supporting Color's Medication Response Test.

Box 1. Key definitions

Genotype: The nucleotide sequence at a particular chromosomal position (i.e. AA, AG, or GG)
Haplotype: A large region of the genome inherited from one parent in a block. In this context, haplotype refers to a "version" of a gene or DNA sequence
Diplotype: A pair of haplotypes on homologous chromosomes
Star-allele: A previously described haplotype of a pharmacogenomic gene that has an established effect on the protein's enzymatic activity. For each gene, a person has two star-alleles, one for each haplotype
Key variant: Important function-altering variants with guideline-supported drug dosing, which may be present in multiple star alleles

Methods

Laboratory procedure

The Color Medication Response Genetic Test uses the same, proven enrichment and NGS sequencing technology utilized in the Color Hereditary Cancer Genetic Test and the Color Hereditary Heart Health Genetic Test. Our laboratory is certified by CLIA (05D2081492) and accredited by CAP (8975161).

Data Analysis - Calling Diplotypes from NGS

The bioinformatics pipeline aligns sequence data and calls variants using a suite of well established algorithms such as BWA-MEM, SAMtools, Picard, GATK and CNVkit. Diplotype calls are computed using a customized and validated bioinformatic pipeline that includes copy number analysis to support accurate analysis of the important CYP2D6 gene.

Clinical Interpretation of Results

Our current pharmacogenomic test covers 14 genes and can accurately characterize 146 alleles related to medication response. This comprehensive panel was designed to align with the American Cancer Society guidelines on pharmacogenomic equitability by supporting the detection of alleles that are more common in diverse populations.

The functional status of alleles (haplotypes) is established based on CPIC consensus terms and recommendations.[15] Alleles are then summarized into diplotypes and associated with the predicted

phenotype using data from CPIC, PharmGKB and PharmVar. Results are then interpreted according to CPIC guidelines, Dutch guidelines, FDA drug package inserts, and related literature. Reports are reviewed by a certified medical professional.

PGx analysis and reporting focuses on the identification of previously described haplotypes, called "star-alleles". Star-alleles are characterized by a set of observable genetic variants, some of which are functional and some of which are markers for a larger haplotype. *1 indicates that none of the interrogated alleles are present, and is assay and analysis dependent; *1 does not eliminate the possibility that an unanalyzed star-allele, or that a novel loss or gain of function variant is present.[22,23]

In addition to the star alleles, Color's test also reports on 12 "key variants" in 5 genes (Appendix 1) in addition to diplotypes for genes with incomplete or inconsistent allele definitions and guidance.

Box 2. Key phenotypes in drug-metabolizing genes [15,20,15,24]

- *Rapid or Ultra-Rapid Metabolizers:* People who have rapid or ultrarapid metabolizer results break down certain medications more quickly than average. For specific medications, this means they may not remain in the body at expected levels for enough time. The impact of a rapid or ultrarapid metabolizer result is different depending on the specific medication, and may be influenced by other factors.
- *Normal Metabolizers:* People who have normal metabolizer results are expected to break down certain medications at the average rate. For specific medications this means they may remain at typical levels in the body for the expected amount of time.
- *Poor or Intermediate Metabolizer:* People who have poor or intermediate metabolizer results may take longer to break down certain medications. This means that some medications may remain at higher or active levels in the body for longer than expected. The impact of a poor or intermediate metabolizer result is different depending on the specific medication, and may be influenced by other factors.

Note: alternate nomenclature conventions are used for certain genes.

Validation study

Sample Selection

To validate the sensitivity, specificity, and precision of the Color Medication Response Genetic Test, DNA derived from 426 cell line samples were compared to previously characterized results. The cell lines had consensus diplotypes reported by numerous studies.[25–28] The validation consisted of samples with a diplotype status falling into one of the following groups:

- Known "negative" or reference allele samples with a "normal" metabolizer status. e.g. (*1/*1)
- Known "positive" samples with reportable, non-normal diplotypes.

All samples were blinded to the operators and treated under identical experimental conditions

Data analysis

As described above, *1 indicates the absence of any tested allele, and is assay and analysis dependent; additionally, specific reporting of certain alleles depends on the inclusion or exclusion of other related refining alleles. It is therefore possible that analytically equivalent results can be reported as different diplotypes by different laboratories. Because published documentation for cell lines often only includes diplotypes without sufficient information about the set of tested alleles or the underlying genotypes, analysis of validation results followed a two-step process. Diplotype matches

were counted as concordant. In cases of discordance at the diplotype level, a comparison of underlying contributing genotypes was made. Cases where all overlapping underlying genotypes were consistent were also counted as concordant.

Results

The Color Medication Response Genetic Test showed 100% concordance across all genes in all tested samples. In this dataset, 5,936 diplotype results were compared, with no false positives called in any of the 426 samples. In addition, the PGx variant calling pipeline, used to call and annotate key PGx variants for reporting, was able to accurately identify 99.98% (5111/5112) variants from these 426 samples.

The acceptance criteria for the study were met (Table 1). For diplotype calling, the true positive rate was 100% with 424 out of 424 cases correctly identified, and the false positive rate was 0% with no incorrect identifications. Similarly, PGx variant calling showed a true positive rate of 99.98% with 5111 out of 5112 cases correctly identified, and a false positive rate of 0.02% with only 1 incorrect identification.

Component	Metric	Expected	Observed
Diplotype calling	True positive	100%	100% (424/424)
	False positive	0%	0% (0/424)
PGx variant calling	True positive	>99.5%	99.98% (5111/5112)val
	False positive	<0.5%	0.02% (1/5112)

Table 1. Validation results. *464 samples were validated for diplotype calls (star-alleles) and key PGx variants.*

CYP2D6 copy number

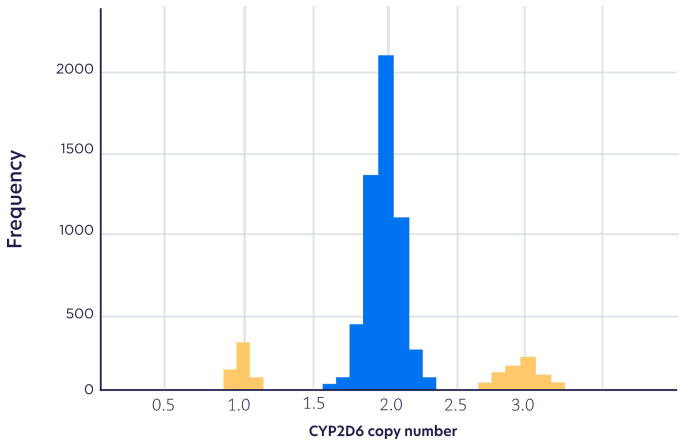

Figure 1. CYP2D6 mini-study. *A set of 6266 samples were examined for copy number in representative sites within CYP2D6. Distinct separation of copy numbers was observed.*

Correct analysis of the *CYP2D6* gene requires extra complexity. In addition to being adjacent to two highly homologous pseudogenes, *CYP2D7* and *CYP2D8*, it has over 100 reported alleles that vary in frequency by ethnicity.[29] These allelic variants are composed of single nucleotide polymorphisms (SNPs), insertions and deletions, copy number variants, larger rearrangements, and hybrid gene conversion events.[30]. In particular, copy number changes are quite common. An estimated 12.6% of the US population has zero, one, or three or more copies.[31]

To derive a clear signal amidst these homology complications, *CYP2D6* copy number is assessed by an analysis of exon 1, exon 6, and exon 9 (including flanking intronic regions). To confirm that homology does not confound copy number assessment, observed copy number across a set of 6266 samples was evaluated. A clear separation of integer copy numbers was observed. In addition, the validation set included 11 known copy number variants, and all were accurately detected.

Conclusions

Actionable PGx variants are common and it is likely that most people will be prescribed a medication with an established PGx interaction at some point in their lifetime. Color has developed an accurate and affordable preemptive PGx testing platform that helps healthcare providers select medications and make dose adjustments from the very first prescription, thus enhancing treatment efficacy and reducing the risk of ADRs.

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Appendix 1. PGx star alleles and risk variants included in the Color Medication Response Test

Gene	Star Alleles and Variants
CYP1A2	*1, *1F, *1K
CY2C9	*1, *2, *3, *4, *5, *6, *8, *9, *11, *12, *13, *14, *15, *16, *23, *24, *26, *29, *31, *33, *35, *39, *42, *43, *44, *45, *46, *55, *61
CYP2C19	*1, *2, *3, *4, *5, *6, *7, *8, *9, *10, *16, *17, *19, *22, *24, *25, *26, *35, *38
CYP2D6	*1, *2, *3, *4, *4N, *5, *6, *7, *8, *9, *10, *11, *12, *13, *14, *15, *17, *18, *19, *21, *29, *31, *32, *35, *36, *40, *41, *42, *45, *49, *54, *55, *56, *59, *68, *69, *114
CYP3A4	*1, *20, *22
CYP3A5	*1, *3, *6, *7
CYP4F2	*1, *2, *3, *4, rs2108622
DPYD	*1, *2A, *3, *4, *5, *6, *7, *8, *9A, *9B, *10, *11, *12, *13, HapB3, rs3918290, rs55886062.1, rs75017182, rs56038477, rs67376798, rs115232898
F5	rs6025
IFNL3	rs12979860
NUDT15	*1, *2, *3, *4, *6, *9, *14, rs116855232
SLCO1B1	*1, *5, *9, *14, *15, *20, *31, *46, *47, rs2306283, rs4149056
TPMT	*1, *2, *3A, *3B, *3C, *4, *8, *11, *14, *15, *23, *24, *29, *41, *42
VKORC1	rs9923231, rs72547529, rs61742245