Online-Only Supplement

Figure 1. Ancestry admixtures by imputed race/ethnicity in 15,000 patients. Each column represents a patient's genetic ancestry proportions. Based on genetic ancestry proportions, race/ethnicity imputation predicts how the patient would be expected to self-identify, indicated by the x-axis labels. Patients with no majority ancestry are grouped into an "Admixed" race/ethnicity group.

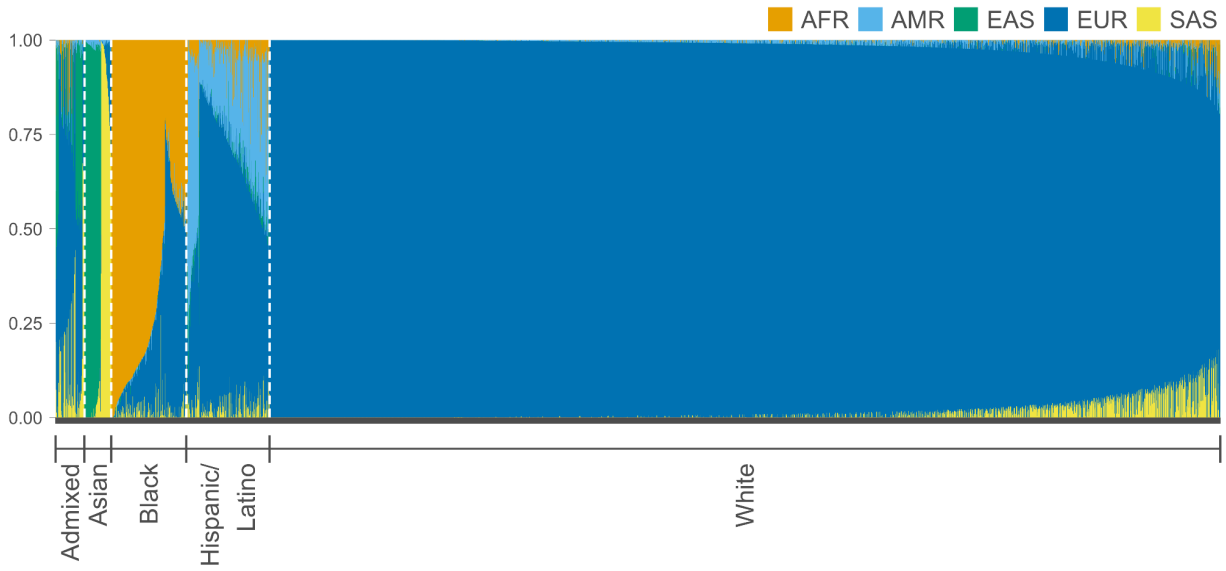

Table 1. Clinical actionability for medications attempted.

Medication	No. of patients	No. of patients actionable	Fraction actionable
escitalopram	556	182	33
citalopram	188	57	30
amitriptyline	62	43	69
doxepin	34	26	76
aripiprazole	377	23	6
venlafaxine	283	17	6
sertraline	670	16	2
dextroamphetamine	326	15	5
nortriptyline	27	15	56

clomipramine	16	14	88
paroxetine	132	10	8
vortioxetine	128	8	6
atomoxetine	119	7	6
imipramine	11	7	64
brexpiprazole	57	4	7
desipramine	4	3	75
fluvoxamine	49	1	2
valbenazine	4	1	25
clozapine	7	0	0
thioridazine	5	0	0
deutetrabenazine	2	0	0
clobazam	1	0	0
iloperidone	1	0	0

Table 2. Clinical actionability for medications considered at the time of PGx testing.

Medication	No. of patients	No. of patients actionable	Fraction actionable
escitalopram	425	134	32
citalopram	158	48	30
amitriptyline	41	27	66
dextroamphetamine	231	19	8
doxepin	37	18	49
sertraline	552	16	3
aripiprazole	319	13	4
venlafaxine	274	12	4

atomoxetine	127	12	9
paroxetine	126	11	9
vortioxetine	219	10	5
nortriptyline	30	10	33
clomipramine	21	10	48
brexpiprazole	112	4	4
fluvoxamine	73	4	5
imipramine	3	2	67
desipramine	2	2	100
clozapine	21	1	5
iloperidone	2	0	0
deutetrabenazine	1	0	0
valbenazine	1	0	0

Table 3. Clinical actionability for medication classes attempted at the time of PGx testing.

This is the same underlying data as shown in Table S1, but counts are added up for all medications in a given drug class.

Therapy Class	No. of attempted medications	No. of patients	Fraction of medications actionable
SSRI	1595	1190	.17
Second Generation	442	422	.06
Stimulant	326	326	.05
SNRI	283	283	.06
TCA	154	132	.70

Other Antidepressants	128	128	.06
Non-stimulant	119	119	.06
Other	6	6	.17
First Generation	5	5	0
Benzodiazepine	1	1	0

Table 4. Clinical actionability for medication classes considered at the time of PGx testing.

Therapy Class	No. of considered medications	No. of patients	Fraction actionable
SSRI	1334	990	0.16
Antipsychotic	454	423	0.04
SNRI	274	274	0.04
Stimulant	231	231	0.08
Other Antidepressants	219	219	0.05
TCA	134	123	0.52
Non-stimulant	127	127	0.09
Other	2	1	0

SSRI: selective serotonin reuptake inhibitor, SNRI: serotonin norepinephrine reuptake inhibitor, TCA: tricyclic antidepressants

Table 5. Phenoconversion of patients who have taken or are considering *CYP2D6* inhibitor medications with different strengths.

genetic metabolism	CYP2D6 inhibition	No. of patients	effective metabolism	is metabolism changed
Normal	strong	888	Poor	yes
Intermediate	strong	648	Poor	yes
Normal	moderate	126	Intermediate	yes
Intermediate	moderate	110	Intermediate	no
Poor	strong	120	Poor	no
Ultrarapid	strong	67	Poor	yes
Poor	moderate	18	Poor	no
Ultrarapid	moderate	5	Normal	yes

Table 6. Frequency of CNVs by imputed race/ethnicity.

Race/Ethnicity	No. of patients	No. with CNVs	Fraction w/ CNVs	No. with Allele-Assignment-Dependent	Fraction Allele-Assignment-Dependent
White	12,250	741	0.06	259	0.02
Hispanic/Latino	1,077	78	0.07	18	0.02
Black	964	122	0.13	44	0.05
Admixed	371	62	0.17	11	0.03
Asian	338	101	0.30	6	0.02

Table 7. Frequency of *CYP2C* TG haplotype actionability by imputed race/ethnicity. The TG haplotype may increase metabolism in patients with normal *CYP2C19* phenotypes, which would effectively increase metabolism of *CYP2C19* substrates to ‘rapid’ or ‘ultrarapid’, which corresponds to actionability changes for six medications.

Race/Ethnicity	No. of Patients	Percent with CYP2C-TG	Percent with *1/*1	Percent with altered Phenotypes
Hispanic/Latino	253	46	49	30
Admixed	85	44	38	21
White	3,484	37	39	21
Asian	80	35	30	19
Black	212	15	37	9

Table 8. Pairwise proportion tests for *CYP2C*-TG carrier frequency differences between imputed race/ethnicities. 5 non-significant comparisons omitted.

Race/Ethnicity 1	Race/Ethnicity 2	adjusted p-value
Hispanic/Latino	Black	3e-11
White	Black	3e-9
Black	Admixed	3e-6
Black	Asian	2e-3
Hispanic/Latino	White	3e-02

Table 9. Pairwise proportion tests for different rates of CYP2C-TG actionability change between imputed race/ethnicities. 6 non-significant comparisons omitted.

Race/Ethnicity 1	Race/Ethnicity 2	adjusted p-value
Hispanic/Latino	Black	9e-17
White	Black	1e-3
White	Hispanic/Latino	4e-3
Black	Admixed	8e-2

Hispanic/Latino	White	3e-02

Methods

Sequencing Equipment, Bioinformatic Processing, and Validation

Tempus nP includes a combination of targeted hybridization pull-down capture and NGS sequencing with Illumina 2x150bp paired end libraries in the NovaSeq 6000 (Illumina, San Diego California), and MassARRAY® (Agena Biosciences, San Diego California) based quantitative genotyping. The targeted sequencing capture assay includes a WES backbone with custom probes added to increase depth of coverage around the PGx genes of interest. Reads were aligned to the human reference genome (hg19) with the bioinformatics software BWA and variant calling was performed with GATK HaplotypeCaller. An average of 116,661 variants in exons or regions targeted by the assay are called; and the mean target exon read depth was 62x (interquartile range 52-72x; estimated with 100 random files).

Variant call data was processed into preliminary star allele and metabolizer phenotypes by in-house software. The PGx assay has been analytically validated for *CYP2C19* alleles *1, *2, *3, *4A, *4B, *5 *6, *7 ,*8, *9, *10, *17, and *35, and for *CYP2D6* alleles *1, *1xN, *2, *2xN, *3, *3xN, *4, *4xN, *5, *6, *6xN, *7, *8, *9, *9xN, *10, *10xN, *11, *12, *14, *15, *17,*17xN, *29, *29xN, *35, *35xN, *40, *41, and *41xN.

Haplotype Imputation

The imputation software, GLIMPSE,[1] leverages low-coverage NGS data with a genetic map and a haplotype reference panel in order to impute and phase genotypes for a patient sample at every polymorphic site in the reference panel. In this study, GLIMPSE utilized for imputation: 1)

low-coverage NGS sequencing data from nP off-target reads for each patient, 2) a genetic recombination rate map from HapMapII (data),[2] and 3) a panel of reference haplotypes from the 1000 Genomes Project (TGP) phase 3[3] (data). The NIST sample, HG001, was removed during subsequent performance benchmarking of imputation results (described below), but was kept for the genotype imputation of patient samples. The GLIMPSE imputation algorithm cannot properly utilize loci with >2 alleles (i.e., non-bi-allelic), therefore these sites were excluded from imputation. Further, from the patient off-target reads NGS data, GLIMPSE utilizes genotype likelihoods as the primary inputs. These likelihoods were generated using bcftools at each non-bi-allelic SNP position in the TGP reference panel.[4, 5] However, since bcftools generates poor genotype likelihoods for insertions/deletions, these were imputed using uniform genotype likelihoods. All TGP reference panel variants (minus non-bi-allelic sites) in the genomic region of interest, chr10:96375267-98547144 (build GRCh37), were imputed. An additional 200 kb of buffer bases were also added to the flanks of the region of interest in order to avoid low-quality edge effects.

To characterize the parameters that contribute to imputation performance, we also measured the number of sites in the TGP reference panel that were directly sequenced by nP NGS off-target reads and the sequencing coverage at those sites, which is known as the “effective coverage” [6].

Imputation commands

```
# compute SNP GT likelihoods of off-target reads with bcftools
# (example here for region of interest 10:96375267-98547144)
bcftools mpileup -f hs37d5.fa.gz -I -E -a 'FORMAT/DP' -T \
chr10_TGP_SNPs.vcf.gz -r 10 input_sample.bam -Ou | bcftools call -Aim -C \
alleles -T chr10_TGP_SNPs.tsv.gz -Oz -o chr10_TGP_SNPs_GT_likelihoods.vcf.gz

# run genotype imputation with GLIMPSE across region of interest
# (10:96375267-98547144)
# NOTE: input chr10_TGP_GT_likelihoods.vcf.gz includes indels with uniform GT #
likelihoods
GLIMPSE_phase --input chr10_TGP_GT_likelihoods.vcf.gz --reference \
```

```
chr10_TGP.bcf --map genetic_map_GRCh37_chr10.txt.gz --main 15 --input-region \
"10:96175228-98747177" --output-region "10:96375267-98547144" --output \
chr10_96375267_98547144_imputed_sample.bcf

# phase imputed genotypes with GLIMPSE and convert output to vcf.gz
GLIMPSE_sample --input chr10_96375267_98547144_imputed_sample.bcf --solve \
--output chr10_96375267_98547144_imputed_sample.phased.bcf
bcftools index -f chr10_96375267_98547144_imputed_sample.phased.bcf
bcftools view chr10_96375267_98547144_imputed_sample.phased.bcf -Oz -o \
chr10_96375267_98547144_imputed_sample.phased.vcf.gz
bcftools index -f -t chr10_96375267_98547144_imputed_sample.phased.vcf.gz
```

Haplotype Imputation Benchmarking

We performed haplotype imputation benchmarking using the HG001 reference human cell line (also known as Coriell Institute cell line NA12878) characterized by the NIST Genome-in-a-Bottle (GiaB) consortium. DNA from the HG001 lymphoblastoid cell line obtained from the Coriell Institute (Camden, NJ) is sequenced on every flow cell for the nP assay as a quality control standard. For HG001, there is also a high-quality variant truth data set released by GiaB that can be used for performance benchmarking (version 4.2.1; data: https://ftp.ncbi.nlm.nih.gov/ReferenceSamples/giab/release/NA12878_HG001/NISTv4.2.1/GRCh37/) [7]. We ran imputation on 8 nP HG001 sample replicates (1 reference patient specimen, 8 sequencing reactions/vials, 4 flow-cells) and checked prediction accuracy by comparing imputed genotypes to the GiaB high-quality benchmark using best practices put forward by the Global Alliance for Genomics and Health Benchmarking Team [8].

We first evaluated imputation performance on the 2 loci that contribute to the novel *CYP2C* haplotype: rs2860840 and rs11188059. We report the accuracy at each locus as the number of samples predicted to have the same genotypes as found in the GiaB high-quality benchmark.

We then measured imputation performance on millions of common variants across the whole genome. Specifically, common variants on chr1-22 (i.e., all bi-allelic variants found in the TGP reference panel for chr1-22; N=81,646,132 variant sites) were imputed for HG001 and the

results were compared to the GiaB high-quality benchmark. 3,891,440 sites in the HG001 GiaB high-quality benchmark are characterized as polymorphic on chr1-22. We then measured the genome-wide imputation performance by calculating precision and sensitivity for each imputed sample replicate and reported the mean and extreme performance observations across the samples.

Results

Imputation performance was evaluated using the HG001 high-quality benchmark. The HG001 high-quality benchmark has reference genotypes of 0/0 and 0/1 at rs2860840 and rs11188059, respectively, and the 8 imputed nP HG001 samples were all predicted to have 0/0 and 0/1 at rs2860840 and rs11188059 (100% accuracy, N=8 sequencing replicates). Whole-genome (chr1-22) imputation was used to predict genotypes at ~82 million sites in HG001 sequencing replicates, of which ~3.9 million are characterized as polymorphic in the high-quality benchmark. For the whole-genome results across the 8 sequencing replicates, the mean positive predictive value (PPV) for imputed genotypes was observed to be 85% (the worst and best replicates were 83% and 86%) and mean sensitivity was observed to be 75% (the worst and best replicates were 73% and 76%).

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