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Supplemental Data

Harmonizing Clinical Sequencing and Interpretation for the eMERGE III Network

The eMERGE Consortium

Figure S1.

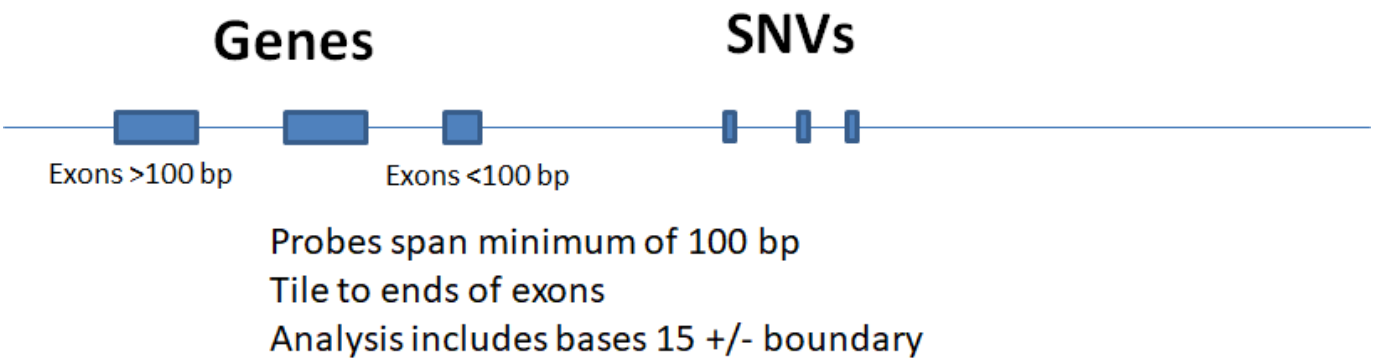

Figure S1. Capture design strategies. Probes were designed to capture targeted regions of the eMERGESeq panel according to the certain criteria that include but are not limited to those shown in the figure.

Figure S2.

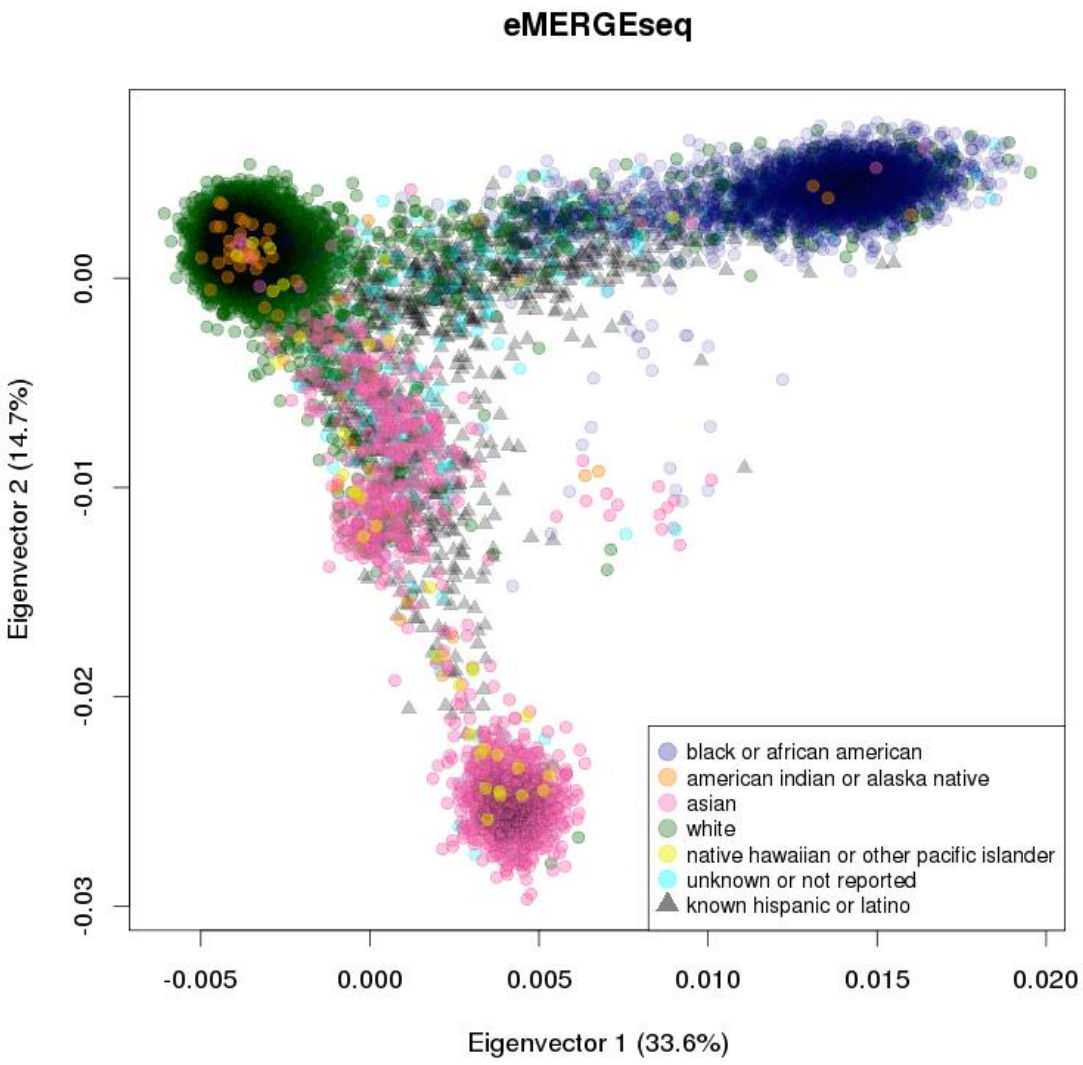

Figure S2. PCA plot of genetic ancestry for the eMERGEseq cohort.

Table S2. Capture design performance comparison between both sequencing centers before and after design optimization (Version 1 vs. Validated Panel).

	Broad/LMM		**Baylor**	
	Version 1 Panel	Validated Panel (v2)	Version 1 Panel	Validated Panel (v2)
% of Bases ≥20X	99.30%	99.80%	99.80%	99.90%
Number of bases <20X in ≥10% of samples	3,364	732	1,332	475
Gene_Exon with bases <20X in validated panel (% of bases in that exon <20X)	n/a	*CACNA1A*_Exon42 (100%) *TGFBR1*_Exon01 (100%) *KCNQ1*_Exon01 (44.5%) *RYR1*_Exon91 (22.4%) *CACNA1B*_Exon01 (19.4%) *CACNA1B*_Exon19 (7.6%) *RB1*_Exon09 (2.6%)	n/a	*SDHD*_Exon04 (100%) *CHEK2*_Exon14 (100%) *TGFBR1*_Exon01 (39.8%) *PKP2*_Exon09 (14.3%) *COL5A1*_Exon01 (11.8%) *RYR1*_Exon91 (10.6%) *APOB*_Exon29 (8.4%) *CACNA1B*_Exon19 (6.4%)
SNV with <20X	n/a	rs25531 rs25532 rs1135840 rs1702294	n/a	rs657452 rs6002655 rs8066602 rs8066731 rs35445101
SNVs determined by eMerge Annotation group to be clinically actionable (14) that have low or no coverage	n/a	None	n/a	
ClinVar Pathogenic/Likely pathogenic variants with low/missing coverage	n/a	rs199473441[a] rs397508096[c] rs794728563[a] rs794728544[a] rs199472884[a,b]	n/a	None

[a]single submitter for 'pathogenic' classification; [b] multiple submitters for 'likely pathogenic'; [c] multiple submitters for 'pathogenic'

Table S2. Comparison before and after design optimization (Version 1 vs. Validated Panel). While each design has unique strengths and deficiencies, the percent bases ≥ 20X coverage is greater than 99% for both sequencing sites.

Table S3. Summary of PGx report contents from sequencing centers to sites

Genes	# Variants Genotyped	Haplotypes or *Alleles Reported	Phenotypes Reported	Associated Drugs	Reporting differences between SCs	CPIC Publications
TPMT	4	*1, *2, *3A, *3B, *3C, *4	TPMT Normal, Intermediate, or Poor Metabolizer	Azathioprine, Mercaptopurine, Thioguanine (thiopurines, immunosuppressant)	None	Relling et al. 2011 PMID 21270794; Relling et al. 2013 PMID 23422873
CYP2C9	2	*1, *2, *3	CYP2C9 Normal, Intermediate, or Poor Metabolizer	Warfarin (anticoagulant), Phenytoin/fosphenytoin (anticonvulsant)	BCM-HGS reported CYP2C9 and VKORC1 together for warfarin only given that the phenytoin dosage guidelines rely on the HLA-B allele, which is not assessed in this assay.	Johnson et al. 2011 PMID: 21900891, Johnson et al. 2017 PMID: 28198005; Caudle et al. 2014 PMID: 25099164
VKORC1	1	c.-1639G>A (rs9923231)	VKORC1 Normal, Intermediate or Low Expression	Warfarin (anticoagulant)	None	Johnson et al. 2011 PMID: 21900891, Johnson et al. 2017 PMID: 28198005
IFNL3	1	c.-3180G>A (rs12979860)	IFNL3 Favorable or Unfavorable	PEG-IFN-α, Ribavirin (antiviral)	None	Muir et al. 2014 PMID: 24096968

			Response			
DPYD	3	*1, *2A, *13, c.2846A>T	DPYD Normal, Intermediate, or Poor Metabolizer	5-Fluorouracil, Capecitabine, Tegafur[a] (fluoropyrimidines, antineoplastic)	None	Caudle et al. 2013 PMID: 23988873
SLCO1B1	1	*1A, *5 (c.521T>C; rs4149056)	SLCO1B1 Normal, Decreased, or Poor Function	Simvastatin (cholesterol reduction)	None	Wilke et al. 2012 PMID: 22617227, Ramsey et al. 2014 PMID: 24918167
CYP2C19	8	*1, *2, *3, *4A, *4B, *5, *6, *7, *8, *17	CYP2C19 Ultrarapid, Rapid, Normal, Intermediate, or Poor Metabolizer	Clopidogrel (antiplatelet); Voriconazole (antifungal); Amitriptyline, Clomipramine, Doxepin, Imipramine, Trimipramine (tricyclic antidepressants or TCAs); Citalopram, Escitalopram, Sertraline (selective serotonin reuptake inhibitors or SSRIs)	Clomipramine, Doxepin, Imipramine, Trimipramine, Sertraline are CPIC level B; BCM-HGSC only reported CPIC level A gene/drug combinations.	Scott et al. 2011 PMID: 21716271, Scott et al. 2013 PMID: 23698643; Moriyama et al. 2016 PMID: 27981572; Hicks et al. 2013 PMID: 23486447, Hicks et al. 2016 PMID: 27997040; Hicks et al. 2015 PMID: 25974703

[a]Tegafur/DPYD had a CPIC level A when PGx report was implemented. It was since lowered to a level C

Table S4. The 39 preferred indication terms and codes for the eMERGE III network.

Preferred indication term	**Code**
Abnormal sex determination	EMERGE-GIS-LOCAL 10102-6 (abnormal sex determination)
Abnormality of pain sensation	EMERGE-GIS-LOCAL 10103-5 (abnormality of pain sensation)
Abnormality of the heart valves	EMERGE-GIS-LOCAL 10104-4 (abnormality of the heart valves)
Adult Migraine	EMERGE-GIS-LOCAL 10099-1 (adult migraine)
Amyloidosis, Hereditary, Transthyretin-Related	MIM 105210 (transthyretin amyloidosis)
Arrhythmia	MESH D001145 (arrhythmias, cardiac)
Ascending aortic dilation / Aneurysm	EMERGE-GIS-LOCAL 10106-2 (ascending aortic dilation / aneurysm)
Asthma	DOID 2841 (asthma)
Atopic dermatitis	DOID 3310 (atopic dermatitis)
Autistic behavior	EMERGE-GIS-LOCAL 10109-9 (autistic behavior)
Autoimmunity	EMERGE-GIS-LOCAL 10110-7 (autoimmunity)
Bipolar affective disorder	DOID 3312 (bipolar disorder)
Breast carcinoma	DOID 3459 (breast carcinoma)
Cardiomyopathy	ORPHA 167848 (cardiomyopathy)
Chronic kidney disease	MESH D051436 (renal insufficiency, chronic)
Chronic sinusitis	EMERGE-GIS-LOCAL 10111-6 (chronic sinusitis)
Cirrhosis	DOID 5082 (liver cirrhosis)
Colorectal Cancer / Polyps	EMERGE-GIS-LOCAL 10126-0 (colorectal cancer / polyps)
Congestive heart failure	DOID 6000 (congestive heart failure)
Coronary artery disease	DOID 3393 (coronary artery disease)
Depression	EMERGE-GIS-LOCAL 10128-8 (depression)
Ehlers-Danlos Syndrome	ORPHA 98249 (ehlers-danlos syndrome)
Familial hypercholesterolemia	EMERGE-GIS-LOCAL 10134-1 (familial hypercholesterolemia) MIM 143890 (familial hypercholesterolemia)
Healthy	EMERGE-GIS-LOCAL 10094-6 (healthy)
Hyperammonemia due to ornithine transcarbamylase deficiency	MIM 311250 (ornithine carbamoyl transferase deficiency)
Hyperlipidemia	MESH D006949 (hyperlipidemias)
Hypertriglyceridemia	MESH D015228 (hypertriglyceridemia)
Intellectual disability	DOID 1059 (intellectual disability)
Not selected for trait	EMERGE-GIS-LOCAL 10093-7 (not selected for trait)
Obesity	DOID 9970 (obesity)
Opioid dependence, Neonatal abstinence	EMERGE-GIS-LOCAL 10100-8 (opioid dependence, neonatal abstinence)
Ovarian Cancer, epithelial included	ORPHA 213500 (ovarian cancer)
Pediatric Migraine	EMERGE-GIS-LOCAL 10101-7 (pediatric migraine)
Pulmonary Hypertension	ORPHA 422 (primary pulmonary hypertension)
Rheumatoid arthritis	DOID 7148 (rheumatoid arthritis)
Schizophrenia	DOID 5419 (schizophrenia)
Seizures	EMERGE-GIS-LOCAL 10118-9 (seizures)
Stroke	MESH D020521 (stroke)
Tuberous sclerosis type 1	EMERGE-GIS-LOCAL 10135-0 (tuberous sclerosis type 1) MIM 191100 (tuberous sclerosis type 1)

Table S5. eMERGE III Clinical cohort description

Site	Total participants	eMERGESeq Cohort summary	Any phenotype enrichment?
Vanderbilt	2,452	biobank - prior PGx testing or interest in research	N
UW/KPW	2,500	biobank – Colorectal cancer/Polyps diagnosis or Asian ancestry	Y
Columbia	2,582	biobank & prospective, some specific clinics and studies	Y
Mayo	3,025	CRC/P & Hyperlipidemia cohorts	Y
Northwestern	2,985	prospective recruitment across clinics, some specialty	Y
Geisinger	2,500	biobank - suspicious genotype	Y
Harvard	2,500	biobank - unselected	N
CCHMC	3,000	biobank & adolescent prospective	N
CHOP	2,976	biobank - enriched for neuro phenotypes	Y
Meharry	495	Breast, prostate, colorectal, lung cancer or high risk for developing these cancers	Y

Table S6. Demographic information for participants for each site in the eMERGEIII network

Site	Sex		Total	Caucasian	African American	Asian	Hispanic	Native American	Other	Unknown
	Male	Female								
UW/KPW	984	1516	2500	1296	55	1008	40	50	27	24
Geisinger	809	1691	2500	2350	68	9	59	4	7	3
CCHMC[a]	1504	1496	3000	1773	1133	26	42	3	3	20
Harvard	1083	1417	2497	2043	147	75	137	0	0	98
Northwestern	1148	1837	2985	2257	405	132	167	4	3	17
Mayo[b]	1066	1497	3025	2391	14	19	114	2	0	485
CHOP	2045	931	2976	1533	1164	38	122	3	3	113
Columbia	1163	1419	2582	829	205	109	358	4	22	1055
Vanderbilt[c]	1257	1105	2452	2156	102	24	26	4	0	140
Meharry	275	220	495	0	495	0	0	0	0	0
Total	10352	11890	22242	15845	2573	1377	967	53	20	1407

[a]1 individual had no sex information; [b]457 individuals had no sex information; [c]90 individuals had no sex information

Table S9. Participants with actionable PGx results from the eMERGE III cohort

Drug	Gene	Recommended adjustment to standard dosing or alternate drug use based on genotype									
		UW/KPW (n=2500)	CCHMC (n=3000)	Geisinger (n=2500)	Harvard (n=2500)	Vanderbilt (n=2452)	Columbia (n=2582)	Mayo (n=3025)	North-western (n=2001)	CHOP (n=2976)	Meharry (n=495)
Thiopurines	*TPMT*	177 **(7%)**	296 **(10%)**	223 **(9%)**	186 **(7%)**	241 **(10%)**	223 **(9%)**	308 **(10%)**	254 **(9%)**	248 **(8%)**	52 **(11%)**
Warfarin[a]	*CYP2C9/ VKORC1*	1189 **(48%)**	740 **(25%)**	855 **(34%)**	944 **(38%)**	747 **(30%)**	856 **(33%)**	982 **(32%)**	962 **(32%)**	673 **(23%)**	14 **(3%)**
Phenytoin/ fosphenytoin		579 **(23%)**	730 **(24%)**	858 **(34%)**	844 **(34%)**	849 **(35%)**	716 **(28%)**	993 **(33%)**	912 **(31%)**	724 **(24%)**	40 **(8%)**
PEG-IFN-α, Ribavirin	*IFNL3*	991 **(40%)**	1940 **(65%)**	1412 **(56%)**	1348 **(54%)**	0[b] (n/a)	1512 **(59%)**	1651 **(55%)**	1692 **(57%)**	1952 **(66%)**	0[b] (n/a)
Fluoropyrimidines	*DPYD*	35 **(1%)**	55 **(2%)**	57 **(2%)**	52 **(2%)**	0[b] (n/a)	31 **(1%)**	51 **(2%)**	46 **(2%)**	52 **(2%)**	0[b] (n/a)
Simvastatin	*SLCO1B1*	589 **(24%)**	630 **(21%)**	706 **(28%)**	735 **(29%)**	653 **(27%)**	667 **(26%)**	876 **(29%)**	793 **(27%)**	597 **(20%)**	30 **(6%)**
Clopidogrel	*CYP2C19*	1012 **(41%)**	911 **(30%)**	702 **(28%)**	741 **(30%)**	699 **(29%)**	792 **(31%)**	890 **(29%)**	898 **(30%)**	881 **(30%)**	172 **(35%)**
Voriconazole		661 **(26%)**	1018 **(34%)**	873 **(35%)**	850 **(34%)**	856 **(35%)**	781 **(30%)**	970 **(32%)**	1002 **(34%)**	1030 **(35%)**	154 **(31%)**
Tricyclic antidepressants		661 **(26%)**	1018 **(34%)**	873 **(35%)**	850 **(34%)**	856 **(35%)**	781 **(30%)**	970 **(32%)**	1002 **(34%)**	1030 **(35%)**	154 **(31%)**
Citalopram, Escitalopram		661 **(26%)**	1018 **(34%)**	873 **(35%)**	850 **(34%)**	856 **(35%)**	781 **(30%)**	970 **(32%)**	1002 **(34%)**	1030 **(35%)**	154 **(31%)**
Sertraline		661 **(26%)**	1018 **(34%)**	873 **(35%)**	850 **(34%)**	856 **(35%)**	781 **(30%)**	970 **(32%)**	1002 **(34%)**	1030 **(35%)**	154 **(31%)**

[a]Warfarin dosing algorithms use both genetic and nongenetic factors such as age, sex, smoking status etc. to predict appropriate dose. Follow up with physician is recommended for all individuals

[b]this site elected to not receive this PGx result

Supplemental methods:

Proficiency Testing across clinical sequencing sites.

Both BCM and Broad clinical labs are accredited by the College of American Pathologists (CAP) and are therefore required to perform biannual proficiency testing (PT) on every clinical test offered. There are several acceptable means to perform PT on a sequencing-based assay including enrollment in CAP's PT program. As part of the PT program CAP sends out reference samples with known events for the clinical lab to prepare and sequence and results are submitted to CAP for scoring. The eMERGEseq panel presented a unique opportunity for an alternate PT program - lab exchange. Since both laboratories are running the same clinical test, both could perform PT by sending previously tested clinical samples to the other lab. Results from end-to-end processing were compared for concordance. This approach has the added benefit of ensuring both laboratories remain technically harmonized throughout the duration of the project. For this program, BCM performs both the CAP PT program in conjunction with the alternate PT program described above.

Results of mid-2017 PT sample from BCM run at Broad: passing variant calls 100% concordant with BCM variants.

Results of mid-2017 PT sample from Broad run at BCM: passing variant calls 100% concordant with Broad variants.

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Preliminary Report

goo.gl/cLdKB8

Patient Name:		Sample Collected Date:	
Patient ID:		Sample Received Date:	03/16/2016
Age:		Report Date:	
DOB:		Sample Type:	
Sex:		Indication for Testing:	
Patient Sample ID:		Ordering Physician Name:	
Accession #:			

emerge network
ELECTRONIC MEDICAL RECORDS AND GENOMICS

Preliminary eMERGE-Seq Panel Sequencing Report

HGSC-CL

This test interrogates the protein-coding and exon-splicing regions of 109 genes as well as 1551 single-nucleotide polymorphisms that may impact human health and disease. Clinical interpretation and reporting are provided for pathogenic and likely pathogenic variants for genes and single nucleotide polymorphisms as described in the methodology section.

PATHOGENIC AND/OR LIKELY PATHOGENIC VARIANTS DETECTED

A homozygous c.350G>A (p.R117H) pathogenic variant in the CFTR (NM_000492.3) gene was detected in this individual. Defects in CFTR are the cause of cystic fibrosis (CF) [MIM 219700], an autosomal recessive common generalized disorder of the exocrine glands which impairs clearance of secretions in a variety of organs. It is characterized by the triad of chronic bronchopulmonary disease (with recurrent respiratory infections), pancreatic insufficiency (which leads to malabsorption and growth retardation), and elevated sweat electrolytes. Defects in CFTR are also the cause of congenital bilateral absence of the vas deferens (CBAVD) [MIM 277180], an important cause of sterility in men and could represent an incomplete form of cystic fibrosis, as the majority of men suffering from cystic fibrosis lack the vas deferens.

Table 1: Details of Pathogenic and Likely Pathogenic Variants

Disease	Inh.	Gene	Position (NCBI 37)	Variant	Zyg.	Notes	Interpretation
Cystic fibrosis [MIM 219700]; Congenital bilateral absence of the vas deferens [MIM 277180]	AR	CFTR	chr7 g.117171029G>A	c.350G>A p.R117H	Homozygous	PMID 2344617, 23420618, 21228398, 21594800, 10103316, 24440181, 21507732, 22366207, 7506096, 22975760, 23951356, 12767731, 22658665, 20619026, 26324139, 23974870, 19880712, 23891399, 15246977, 26846474, 22332135, 20021716, 21520337, 23378603, 20797923, 20923678, 18778819, 19885835, 22572128, 23751316; rs78655421; [5T]	Pathogenic

Table 2: Details of Copy Number Variants:

No CNVs found for this sample.

Table 3: Details of Pharmacogenomic Variants

Pharmacogenomics variants are returned for the following genes: CYP2C19, DPYD, INFL3, SLCO1B1, TPMT, CYP2C9/VKORC1. Star alleles are determined based on the variants detected by this assay. Star alleles may not be accurately defined due to the limitations of this assay which include: 1) The presence of additional variants defining functional and non functional alleles in a patient, not detected by this assay, and 2) the lack of ability to determine the phase of the variants when a star allele is defined by multiple variants. Additionally, undetected genetic and/or non genetic factors such as drug-drug interactions, may also impact the phenotype. This pharmacogenomic report is limited to CPIC level A alleles and drug recommendations. Additional (level B and lower) drugs may be metabolized by these

reported enzymes; and additional enzymes, not reported here, may affect the metabolism of a reported drug. Refer to the current recommendation for dosage guidelines. See Methodology for details.

Gene	Drug	Diplotype	Phenotype	Recommendation
CYP2C19	clopidogrel	*1/*1	Normal metabolizer	https://cpicpgx.org/guidelines/guideline-for-clopidogrel-and-cyp2c19/
	voriconazole			https://cpicpgx.org/guidelines/guideline-for-voriconazole-and-cyp2c19/
	citalopram, escitalopram			https://cpicpgx.org/guidelines/guideline-for-selective-serotonin-reuptake-inhibitors-and-cyp2d6-and-cyp2c19/
	amitriptyline			https://cpicpgx.org/guidelines/guideline-for-tricyclic-antidepressants-and-cyp2d6-and-cyp2c19/
DPYD	capecitabine	*1/*1	Normal DPD activity and "normal" risk for fluoropyrimidine toxicity	https://cpicpgx.org/guidelines/guideline-for-fluoropyrimidines-and-dpyd/
	fluorouracil			
	tegafur			
IFNL3	peginterferon alfa-2a	rs12979860 C/C	Favorable response genotype	https://cpicpgx.org/guidelines/guideline-for-peg-interferon-alpha-based-regimens-and-ifnl3/
	peginterferon alfa-2b			
	ribavirin			
SLCO1B1	simvastatin	rs4149056 T/C	Intermediate function, Intermediate simvastatin induced myopathy risk	https://cpicpgx.org/guidelines/guideline-for-simvastatin-and-slco1b1/
TPMT	azathioprine	*1/*1	High activity	https://cpicpgx.org/guidelines/guideline-for-thiopurines-and-tpmt/
	mercaptopurine			
	thioguanine			
CYP2C9	warfarin	*1/*3	Intermediate metabolizer	https://cpicpgx.org/guidelines/guideline-for-warfarin-and-cyp2c9-and-vkorc1/
VKORC1		T/T		

Interpretation of Pharmacogenomic Variants:

This individual is homozygous for the wild type allele of the CYP2C19 gene. Based on the genotype result, this patient is predicted to have a CYP2C19 normal metabolizer phenotype. This genotype information can be used by patients and clinicians as part of the shared decision-making process for several drugs metabolized by CYP2C19 including clopidogrel, voriconazole, amitriptyline, citalopram and escitalopram. For clopidogrel, individuals with this diplotype are expected to have normal platelet inhibition and normal residual platelet aggregation in response to clopidogrel. Label recommended dosage and administration are recommended. Refer to current guidelines for dosage and recommendations at https://cpicpgx.org/guidelines/guideline-for-clopidogrel-and-cyp2c19/. For voriconazole, normal voriconazole metabolism is expected in individuals with this genotype. Initiate therapy with recommended standard of care dosing. Refer to current guidelines for dosage and recommendations at https://cpicpgx.org/guidelines/guideline-for-voriconazole-and-cyp2c19/. For citalopram and escitalopram, initiate therapy with recommended starting dose. Refer to current guidelines for dosage and recommendations at https://cpicpgx.org/guidelines/guideline-for-selective-serotonin-reuptake-inhibitors-and-cyp2d6-and-cyp2c19/. For amitriptyline, initiate therapy with recommended starting dose. Refer to current guidelines for dosage and recommendations at https://cpicpgx.org/guidelines/guideline-for-tricyclic-antidepressants-and-cyp2d6-and-cyp2c19/. For citalopram, escitalopram and amitriptyline, if CYP2D6 genotyping is available, refer to the current guidelines for dosing recommendations.

This individual is homozygous for the functional allele of the DPYD gene. This genotype information can be used by patients and clinicians as part of the shared decision-making process for fluoropyrimidines (capecitabine, fluorouracil, tegafur). Based on the genotype result, this patient is predicted to have a normal DPD activity phenotype. Individuals with this diplotype are expected to have "normal" risk for fluoropyrimidine toxicity. Recommendations include the use of label recommended dosage and administration. Refer to current guidelines for dosage and recommendations at https://cpicpgx.org/guidelines/guideline-for-fluoropyrimidines-and-dpyd/.

This individual is homozygous for the rs12979860 C/C allele in the IFNL3 gene. This variant is the strongest baseline predictor of response to peginterferon alfa and ribavirin therapy in previously untreated patients and can be used by patients and clinicians as part of the shared decision-making process for initiating treatment for hepatitis C virus infection. Based on the genotype result, this patient is predicted to have an increased likelihood of response (higher sustained virologic response rate) to peginterferon alfa and ribavirin therapy as compared with patients with unfavorable response genotype. Refer to current guidelines for dosage and recommendations at https://cpicpgx.org/guidelines/guideline-for-peg-interferon-alpha-based-regimens-and-ifnl3/.

This individual is heterozygous for the rs4149056 T/C allele in the SLCO1B1 gene. This genotype information can be used by patients and clinicians as part of the shared decision-making process for simvastatin and other drugs affected by SLCO1B1. Based on the genotype result, this patient is predicted to have intermediate SLCO1B1 function. This patient may be at risk for an adverse response to medications that are affected by SLCO1B1. To avoid an untoward drug response, dose adjustments may be necessary for medications affected by SLCO1B1. If simvastatin is prescribed to a patient with intermediate SLCO1B1 function, there is an increased risk for developing simvastatin-associated myopathy; such patients may need a lower starting dose of simvastatin or an alternate statin agent. Refer to current guidelines for dosage and recommendations at https://cpicpgx.org/guidelines/guideline-for-simvastatin-and-slco1b1/.

This individual is homozygous for the normal high activity allele of the TPMT gene. Decreased TPMT gene activity is associated with toxicity and myelosuppression in response to thiopurines, and this genotype information can be used by patients and clinicians as part of the shared decision-making process for initiating treatment. Based on the genotype result, this patient is predicted to have normal TPMT function. Individuals with this diplotype are expected to have a normal response to mercaptopurine, azathioprine and thioguanine. A normal dose of thiopurine and adjustment following the disease-specific guidelines is recommended. Refer to current guidelines for dosage and recommendations for each specific thiopurine drug at https://cpicpgx.org/guidelines/guideline-for-thiopurines-and-tpmt/.

This individual is heterozygous for the low function allele in the CYP2C9 gene. Based on the genotype result, this patient is predicted to have intermediate CYP2C9 function.This individual is also homozygous for the variant allele for the VKORC1 gene. Expression level of the VKORC1 gene is associated with warfarin sensitivity. Based on the genotype result, this patient is predicted to have high sensitivity to warfarin.

Comments & Recommendations:

It is recommended that correlation of these findings with the clinical phenotype be performed. Genetic counseling for the patient and at-risk family members is recommended.

This is a preliminary report because the variant has not yet been confirmed by Sanger sequencing.

Gene Coverage:

All genes have 100% of targeted bases sequenced to redundant coverage of 20x or greater with the following exceptions: APOB (99.39%), CACNA1B (96.45%), COL5A1 (98.03%), GRM5 (99.92%), KCNQ1 (94.28%), PKP2 (98.76%), PRKAG2 (99.83%), RYR1 (98.69%), TGFBR1 (93.56%). Further information, including specific coverage for this patient's sample, is available in the ExCID report.

Methodology:

1. eMERGE-Seq Version 2 NGS Panel: for the paired-end pre-capture library procedure, genome DNA is fragmented by sonicating genome DNA and ligating to the Illumina multiplexing PE adapters (reference 1). The adapter-ligated DNA is then PCR amplified using primers with sequencing barcodes (indexes). For target enrichment capture procedure, the pre-capture library is enriched by hybridizing to biotin labeled in-solution probes (reference 2) at 56°C for 16 - 19 hours. For massively parallel sequencing, the post-capture library DNA is subjected to sequence analysis on Illumina HiSeq platform for 100 bp paired-end reads. The following quality control metrics of the sequencing data are generally achieved: >70% of reads aligned to target, >99% target base covered at >20X, >98% target base covered at >40X, average coverage of target bases >200X. SNP concordance to SNPTrace genotype array: >99%. This test may not provide detection of certain genes or portions of certain genes due to local sequence characteristics or the presence of closely related pseudogenes. Gross deletions or duplications, changes from repetitive sequences may not be accurately identified by this methodology. Genomic rearrangements cannot be detected by this assay.

2. As a quality control measure, the individual's DNA is also analyzed by a SNP-array (Fluidigm SNPTrace panel (reference 3)). The SNP data are compared with the NGS panel data to ensure correct sample identification and to assess sequencing quality.

3. Data are analyzed by the Mercury 3.4 (reference 4) pipeline. The output data from Illumina HiSeq are converted from bcl file to FastQ file by Illumina bcl2fastq 1.8.3 software, and mapped to the hg19 human genome reference by the BWA program (reference 5). The variant calls are performed using Atlas-SNP and Atlas-indel developed in-house by BCM HGSC. Copy number variants were detected using Atlas-pcnv v0, developed in-house by the BCM HGSC. Variant annotations are performed using the Cassandra tool, developed in-house. Neptune version $VERSION was used to match variants against curated variants in the VIP database version [/hgsccl/next-gen/neptune/vip/vip.2016-11-07] and generate this report.**

4. The variants were interpreted according to ACMG guidelines (reference 6) and patient phenotypes. Synonymous variants, intronic variants not affecting splicing site, and common benign variants are excluded from interpretation unless they were previously reported as pathogenic variants. Reviewed variants are added to the VIP database for inclusion on future reports. It should be noted that the interpretation of the data is based on our current understanding of genes and variants at the time of reporting.

Clinical interpretation and reporting are provided for pathogenic and likely pathogenic variants as requested by BMGL for the following 68 medically actionable genes: ACTA2, ACTC1, APC, APOB, BMPR1A, BRCA1, BRCA2, CACNA1A, CACNA1S, COL3A1, COL5A1, DSC2, DSG2, DSP, FBN1, GLA, HNF1A, HNF1B, KCNE1, KCNH2, KCNJ2, KCNQ1, LDLR, LMNA, MEN1, MLH1, MSH2, MSH6, MUTYH, MYBPC3, MYH11, MYH7, MYL2, MYL3, MYLK, NF2, OTC, PALB2, PCSK9, PKP2, PMS2, POLD1, POLE, PRKAG2, PTEN, RB1, RET, RYR1, RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TMEM43, TNNI3, TNNT2, TP53, TPM1, TSC1, TSC2, VHL, WT1, the following non medically actionable genes: ANK2, ATM, ATP1A2, BMPR2, CACNA1C, CFH, CFTR, CHEK2, FLG, MC4R, MTHFR, NTRK1, SCN1A, SCN9A, SERPINA1, SLC2A10, TCF4, TCIRG1, TTR, TYK2, UMOD, VDR, the following medically actionable SNPs: rs77931234, rs387906225, rs79761867, rs386834233, rs113993962, rs397509431, rs6467, rs6025, rs80338898, rs1801175, rs1800562, rs28940579, rs61752717, rs193922376; and non medically actionable SNPs: rs151344623, rs76151636, rs111033258, rs786205104, rs786205103, rs147394623, rs121964990, rs121965064, rs121965063, rs104886456, rs201227603, rs74315447, rs61755320, rs724159981. For autosomal recessive disorders, only homozygous or biallelic variants will be returned. Variants in exon 3 of the FLG gene are not reported.

5. Variants related to patient phenotypes are confirmed by Sanger sequencing if the variant has been observed and confirmed fewer than 5 times by our laboratory or the Baylor Genetics Laboratory. Sanger confirmation is noted in the 'Notes' section of the tables if performed.

6. For the pharmogenomic variants, the star alleles are determined based on the variants detected by this assay. Alleles reported for TPMT are limited to *1, *2, *3A, *3B, *3C and *4. Alleles reported for CYP2C19 are limited to *1, *2, *4A, *4B, *5, *6, *7, *8, *17. If reported, alleles for DPD are limited to *1, *2A , *13 and rs67376798. Alleles reported for CYP2C9 are limited to *1, *2 and *3; and rs9923231 for VKORC1. Additional rare star alleles have been reported with reduced or no function for TPMT, CYP2C19 and DPD; however, the variants defining these additional star alleles are not detected with this assay. For SLCO1B1, this assay only detects rs4149056. The minor C allele at rs4149056 defines the SLCO1B1*5 (rs4149056 alone) but also tags the *15 and *17 alleles. Thus a *5 allele may represent a *15 or *17 allele. However, the magnitude of the phenotypic effect is similar for *5, *15, and *17 alleles.

** The VIP variant database was developed in conjunction with Baylor Genetics and the Partners Healthcare Laboratory for Molecular Medicine.

Sample clinical report (Partners/Broad)

PARTNERS HEALTHCARE | PERSONALIZED MEDICINE

MASSACHUSETTS GENERAL HOSPITAL | BRIGHAM AND WOMEN'S HOSPITAL

Laboratory for Molecular Medicine

65 Landsdowne Street, Cambridge MA 02139

Phone: (617) 768-8500 Fax: (617) 768-8513

www.partners.org/personalizedmedicine/lmm

Unit Number(s):

Lab Accession: **PM-16-A07001**

Patient Name: **68282000, 10038000**

Birth Date: **1/1/1800**

Age Sex: **215 Year old Female**

MOLECULAR DIAGNOSTICS REPORT

Specimen Type:	DNA, Isolated - Blood, Peripheral (edit)	**Received Date:**	9/1/2016
Related Accession(s):		**Referring Facility:**	HARVARD
Referring Physician:	EMERGE-CLINIC-TEST	**Referring Fac. MRN:**	
Copies To:	GENEINSIGHT EMERGE-HUB GENEINSIGHT	**Lab Control Number:** **Family Number:**	10038000_68282000-0_SM-B3ZZZ FAB123

TEST DESCRIPTION - Copy Number Variation Analysis
Sequence Confirmation Test eMERGE III Sequencing Panel

TEST PERFORMED - CNV-a; SeqConV2; EMERGE-pnlC

INDICATION FOR TEST - Not selected for trait

RESULTS

DNA VARIANTS:
Heterozygous c.338C>A (p.Ser113X), Exon 4, PMS2, Pathogenic

INTERPRETATION:
Positive. DNA sequencing of the coding regions and splice sites of 97 genes (see methodology section below) identified the variants listed above. Copy number analysis using NGS could not be completed because data did not meet quality standards for CNV detection. For a list of exons that are incompletely covered please see "Additional notes and disclaimers" section below.

SUMMARY:
This individual carries a Pathogenic variant in the PMS2 gene. The available information on this variant is described below. Disease-causing variants in the PMS2 gene are strongly associated with Lynch syndrome and this individual may be at risk for developing colorectal cancer / polyps.

ADDITIONAL NOTES AND DISCLAIMERS:
Disease penetrance and severity can vary due to modifier genes and/or environmental factors. The significance of a variant should therefore be interpreted in the context of the individual's clinical manifestations

DETAILED VARIANT INTERPRETATIONS:
p.Ser113X, c.338C>A (PMS2; NM_000535.5; Chr7g.6043336G>T; GRCh37):
The p.Ser113X variant in PMS2 has not been previously reported in individuals with Lynch syndrome and was absent from large population studies. This nonsense variant

MOLECULAR DIAGNOSTICS REPORT

leads to a premature termination codon at position 113, which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the PMS2 gene is an established disease mechanism in Lynch syndrome (http://www.ncbi.nlm.nih.gov/books/NBK1211/). In summary, this variant meets our criteria to be classified as pathogenic for Lynch syndrome (http://www.partners.org/personalizedmedicine/LMM) based upon predicted impact to the protein and absence in controls.

RECOMMENDATION:
Genetic counseling is recommended for this individual and their relatives. Familial variant testing is available for other relatives if desired. For assistance in locating genetic counseling services or disease specialists, please call the laboratory at 617-768-8500 or email at LMM@partners.org.

Please note that variant classifications may change over time if more information becomes available. Please contact us at 617-768-8500 or LMM@partners.org.

TEST INFORMATION

BACKGROUND:
The eMERGE (electronic MEdical Records and GEnomics) network combines DNA biorepositories with electronic health record (EHR) systems for large-scale discovery and clinical implementation research in genomic medicine. A main goal is the return of genomic testing results to patients in a clinical care setting. In phase III, participating sites are sequencing 109 clinically relevant genes in ~25,000 participants using a custom next generation sequencing panel.

METHODOLOGY:
Test content (target region): This test includes 109 genes (including the ACMG56 genes; PMID: 23788249, and additional genomic positions for known variants. For reference sequences exons/positions covered see http://personalizedmedicine.partners.org/Laboratory-For-Molecular-Medicine/).

Note that this test may not detect variants in regions with difficult sequence contexts (e.g. high or low GC content) and is generally not designed to detect deep intronic variants as well as variants in the 5' and 3'UTR. Regions with high sequence homology are only included in this test if a unique Sanger sequencing assay can be designed to rule out false positive calls.

Sample preparation, sequencing, variant calling and confirmation: This test is performed by next generation sequencing using sonicated genomic DNA (Covaris) followed by target enrichment (Illumina Rapid Capture Custom Kit), Illumina HiSeq sequencing (76 bp paired-end reads) and alignment/variant calling (BWA/GATK). A custom script is used to generate calls for the individual genomic positions. Sample identity is confirmed by comparing NGS derived genotypes of a custom set of SNPs to results generated for the same specimen using a fingerprinting genotype array. Samples with ≤95% of the target region covered at ≥20X are failed and repeated. Copy number variants (CNVs) of ≥3 exons are detected by an in-house developed tool (VisCap, PMID: 26681316). This assay is 99.33% sensitive to detect single nucleotide variants (95% CI = 96.30-99.88%), 100% sensitive to detect indels (95% CI = 79.61-100.00%) and 100% sensitive to detect CNVs (n=4). All variants included on this report are confirmed (SNVs and indels: Sanger sequencing, CNVs: ddPCR).

Sample clinical report (Partners/Broad)

Laboratory for Molecular Medicine
Partners HealthCare Personalized Medicine

Accession: **PM-16-A07001**
Patient Name: **68282000, 10038000**

MOLECULAR DIAGNOSTICS REPORT

Variant annotation and filtration: All variants within the coding sequence of the included genes (default: exons +/- 5 bp) are subjected to the following process: Variant annotations are derived from ExAC (vs 0.3), ClinVar (April 2016 release), HGMD (2016.1), 1000 Genomes (Phase 3), Alamut Batch (vs 1.4.4), (dbnsfp vs 3.1), and LMM's GeneInsight knowledge base (vs 5.3.2). The following variant types are further analyzed: a) Loss of function variants with a minor allele frequency (MAF)<1%, b) Variants previously classified as pathogenic or likely pathogenic regardless of MAF, c) ClinVar pathogenic or likely pathogenic and HGMD DM variants with a MAF<5%.

Variant interpretation and clinical reporting: Variants assessment is based on in-house developed expert criteria and the most recent ACMG classification framework (PMID: 25741868) with disease and gene-specific modifications when applicable. Please note that variant classifications can change over time. Reporting is restricted to pathogenic and likely pathogenic variants in a subset of eMERGE network genes and variants consisting of 62 genes and 1 variant in an additional gene: ACTA2, ACTC1, APC, APOB, BRCA1, BRCA2, CACNA1C, CACNA1S, COL3A1, DSC2, DSG2, DSP, FBN1, GLA, HNF1A, KCNE1, KCNH2, KCNJ2, KCNQ1, LDLR, LMNA, MEN1, MLH1, MSH2, MSH6, MUTYH, MYBPC3, MYH11, MYH7, MYL2, MYL3, MYLK, NF2, OTC, PCSK9, PKP2, PMS2, PRKAG2, PTEN, RB1, RET, RYR1, RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TMEM43,
TNNI3, TNNT2, TP53, TPM1, TSC1, TSC2, VHL, WT1 and HFE (rs1800562). Carrier status for autosomal recessive conditions will not be reported.

This test was developed and its performance characteristics determined by the Laboratory for Molecular Medicine at Partners HealthCare Personalized Medicine (LMM, 65 Landsdowne St, Cambridge, MA 02139; 617-768-8500; CLIA#22D1005307). It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

REFERENCES:

Hendriks YM, Jagmohan-Changur S, van der Klift HM, Morreau H, van Puijenbroek M, Tops C, van Os T, Wagner A, Ausems MG, Gomez E, Breuning MH, Bröcker-Vriends AH, Vasen HF, Wijnen JT. 2006. Heterozygous mutations in PMS2 cause hereditary nonpolyposis colorectal carcinoma (Lynch syndrome). Gastroenterology. 130(2):312-22.

REPORT by Matthew Lebo Ph.D., on Friday September 09, 2016 at 04:22:23PM
Final Diagnosis by **Matthew Lebo Ph.D.**, Electronically signed on Monday September 12, 2016 at 10:56:03AM