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GLOBAL RESEARCH NETWORK

PGRN-ASHG 2021 Symposium

Advancements in Global Pharmacogenomics Through Collaboration

18 Oct, 2021

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Monday, October 18, 2021

Welcome to the 2nd Annual Meeting of the Pharmacogenomics Global Research Network (PGRN). The theme for this meeting is ***"Advancements in Global Pharmacogenomics through Collaboration"***. This reflects the exciting prospects of our growing society as we come together to form global and inter-disciplinary partnerships. The Program and Communication Committees have developed a wonderful program. Don't miss out on the opportunity to showcase your work at this meeting and network with fellow PGx enthusiasts!

The meeting will begin with a session focusing on advancing PGx discovery through industry-academic partnership, which will feature presentations and panel discussions about new resources from Regeneron, PacBio, 23andMe, LabCorp and Arup among others.

Next there will be a session on global precision medicine initiatives, including presentations from the International HundredK+ Cohorts Consortium (IHCC), SG100K, Human Heredity and Health in Africa (H3A) and All of Us Research Program. This session will highlight the current research infrastructure and capabilities that are available around the globe, with a specific focus on minority populations. We hope this session will provide our members with new ideas for collaboration in diverse patient populations across around the world.

These sessions will be followed by virtual poster sessions, covering all aspects of pharmacogenomics from discovery to clinical implementation, from presenters across the globe.

Finally, we will end the meeting with a Social Hour, in which trainee award winners will be announced, and we will enjoy fun networking activities. I am looking forward to seeing all of you at our PGRN meeting!

Andrew A. Monte, MD, PhD
President

Zoom Meeting Link:
https://us06web.zoom.us/j/81880866600?pwd=ZE1Td3VLV3hVTlpsa2M5RURFdGpiQT09

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IMPORTANT LINKS

PGRN 2021 Virtual Meeting Survey
https://www.surveymonkey.com/r/7ZVTC8K

PGRN Member Needs Assessment Survey
https://www.surveymonkey.com/r/D9TS7PW

PGRN/CPIC 2022/2023 Meeting Survey
https://www.surveymonkey.com/r/BGH3M2X

PGRN Research Collaboration Grant
https://www.pgrn.org/PGRN-Collaboration-Grant

PGRN Cohort Resource List
https://docs.google.com/spreadsheets/d/1nXzuan64f5DAPn-b77t1rhbmtBavqBvJYYWlV3cnrtQ/edit?usp=sharing

PGRN Virtual Meeting
Zoom: https://us06web.zoom.us/j/81880866600?pwd=ZE1Td3VLV3hVTlpsa2M5RURFdGpiQT09

Posters in Spatial Chat: https://spatial.chat/s/PGRN-ASHG-2021
Group A – 10:30 am–11:15 am PT / 1:30–2:15 pm ET / 7:30 pm–8:15 CET
Group B – 11:15 am–12:00 pm PT / 2:15–3:00 pm ET / 8:15 pm–9:00 CET

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9:00 am – 2:00 pm PT / 12:00 – 5:00 pm ET / 6:00 – 11:00 pm CET

Zoom: https://us06web.zoom.us/j/81880866600?pwd=ZE1Td3VLV3hVTlpsa2M5RURFdGpiQT09

AGENDA

9:00 - 9:10am PT **Introduction** – Andrew Monte, MD, PhD, PGRN President

9:10 - 10:10am PT **Advancing PGx Discovery Through Industry-Academic Partnership: Perspectives from industry**
Moderator: Uli Broeckel

- "Pharmacogenomics at the Regeneron Genetics Center"
  Charles Paulding PhD, Regeneron
- "Spectrum of Pharmacogenomics Collaborations: The Labcorp Experience"
  Annette Taylor MS, PhD, Labcorp
- "Pharmacogenetics in a direct-to-consumer database"
  Shirley Wu PhD, 23andMe
- "Long-read HiFi Sequencing for Haplotyping and Phasing of PGx Alleles"
  Nina Gonzaludo MS PhD, Pacific Biosciences
- Panel discussion with Speakers joined by Gwen McMillin, PhD (ARUP)

10:10 – 10:15am PT **Spatial Chat live demo –** Stephanie Huang **(Zoom)**

10:15 - 10:30am PT **Break** (poster rooms open in Spatial Chat)

10:30 - 11:15 am PT **Poster Session A - Spatial Chat:** https://spatial.chat/s/PGRN-ASHG-2021

11:15 – 12:00pm PT **Poster Session B - Spatial Chat:** https://spatial.chat/s/PGRN-ASHG-2021

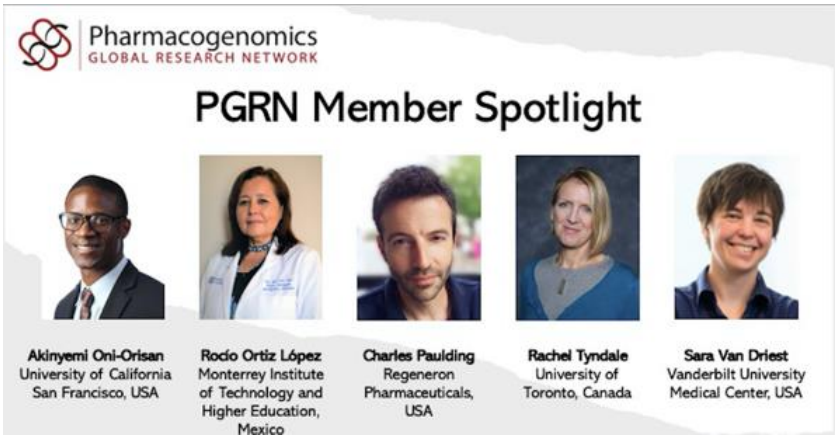
12:00 - 12:30pm PT **Break** (poster rooms open in Spatial Chat)

12:30 - 1:20pm PT **Global Pharmacogenomics Resources and Collaborative Opportunities (Zoom)**
Moderators: Folefac Aminkeng and Jun Yang

- International HundredK+ Cohorts Consortium (IHCC): *Ricardo Verdugo PhD*
- SG100K Precision Health Research Singapore: *John Chambers PhD*
- Pharmacogenomics: Germany, Europe and New York City: *Erwin Bottinger MD*
- All of Us Research Program: *Andrea Ramirez MD, MS*
- Human Heredity and Health in Africa (H3Africa): *Moses Joloba MS, PhD*

1:20 - 2:00 pm PT **Poster Awards and Networking (Zoom)**

- Breakout rooms:
  - IHCC
  - H3 Africa
  - PGx in Germany, Europe and NYC
  - All of Us
  - PGRN Mentoring Program
  - PGRN Member Spotlight and Networking



- Poster Awards, PGRN Annual Trivia Contest, Closing remarks
  Andrew Monte, President

EXCEL SPREADSHEET OF POSTERS IS POSTED ON THE PGRN.ORG WEBSITE:

https://www.pgrn.org/Posters-2021

A01

Association of *NAT2* Polymorphism with Antituberculosis InducedHepatotoxicity in a case control study

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**corresponding author: Prof. Dato' Dr.Mohd Zaki Salleh, Director iPROMISE, UiTM*

Abstract

Antituberculosis induced hepatotoxicity (ATDIH) is life-threatening and fatal which affects the outcome of tuberculosis (TB) treatment. N-acetyltransferase 2 (*NAT2*) polymorphism has been associated with ATDIH especially in South East Asian country, however there is lack of data among the patients in Malaysia. Our objective is to evaluate the association of *NAT2* polymorphism with ATDIH among the patients in Malaysia. We performed a case-control study comprised of 33 patients with ATDIH and 100 TB patients without ATDIH admitted to the tertiary hospitals in Malaysia. Seven single nucleotide polymorphisms (SNPs) of *NAT2* gene were determined through polymerase chain reaction and Sanger sequencing. The frequencies of the genotypes and alleles between the 2 groups were compared and analysed using chi-squared test. Odd ratio (OR) with 95% confidence interval (CI) were used to evaluate the strength of association between NAT2 gene polymorphism and ATDIH. We found that *NAT2*13* (rs1041983), *NAT2*6* (rs1799930) and NAT2 slow acetylators (SA) were significantly associated with ATDIH (OR (95% CI) = 3.09 (1.37-6.95), 2.20 (1.25-3.86) and 3.39 (1.43-8.04), respectively. *Conclusion*

NAT2 slow acetylator status, predominantly *NAT2*13* (rs1041983) and *NAT2*6* (rs1799930) significantly increased the risk of ATDIH among patients in Malaysia.

Acknowledgments: Funded by Ministry of Higher Education Malaysia (Grant numbers 600-IRMI/LRGS 5/3 (0003/2016)) and Ministry of Health Malaysia.

Keywords: Tuberculosis, NAT2 polymorphism, antituberculosis, hepatotoxicity, Malaysia.

A02

Variation in a nicotine and nitrosamine metabolism gene associates with lung diseases: A Phenome-wide Association and Mendelian Randomization study in the UK biobank

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Background: CYP2A6 is a genetically variable enzyme that metabolically *inactivates* nicotine and *activates* carcinogens (e.g. nitrosamines). Genetic variation in *CYP2A6* influences smoking behaviours and the risk for tobacco-related diseases. We previously developed a weighted genetic risk score (wGRS) incorporating seven *CYP2A6* variants that predicted smoking behaviors and clinical outcomes, including cessation, and explained ~30% of variation in the *in vivo* phenotypic biomarker of CYP2A6 activity such that higher wGRS conferred faster CYP2A6 metabolism. This Phenome-Wide Association Study (PheWAS) examined potential associations of *CYP2A6* wGRS with diseases in the UK Biobank. The top associations were then evaluated in Mendelian Randomization (MR) analyses to estimate causal effects of *CYP2A6* variation on these diseases.

Methods: *CYP2A6* wGRS was constructed for participants in the UK Biobank after extracting raw or imputed genotypes for each of the *CYP2A6* variants in the wGRS. Proxy variants (r^2>0.8) were used whenever a variant was not available except one structural variant (*4) that was omitted for lack of proxies. The phenome consisted of mapped disease codes from the UK biobank (ICD-9 & ICD-10) and used as phecodes. Associations between the *CYP2A6* wGRS and phecodes were tested using generalized linear models adjusting for relevant covariates. Two-sample MR was then performed on the top PheWAS hits.

Results: Higher *CYP2A6* wGRS (i.e. faster CYP2A6 metabolism) was positively associated with respiratory diseases. Lung cancer (165.1 Cancer of bronchus; lung) was the top hit in the total sample (N=409,307; Beta=0.017; P=$7.76x10^{-07}$) and in current smokers (N=41,340; Beta=0.036; P=$2.94x10^{-09}$). Further stratifying current smokers by sex indicated higher estimates for lung cancer risk in females (N=19,141; Beta=0.041; P=$6.18x10^{-06}$) compared to males (N=22,198; Beta=0.031; P=$8.64x10^{-05}$). Other phenome-wide hits in both the total group and in current smokers included cancer within the respiratory system, and emphysema. In current smokers, obstructive chronic bronchitis, chronic bronchitis, and chronic airway obstruction also reached phenome-wide significance (P<0.05/# of phecodes tested). No phenome-wide hits were detected in former and never smokers. MR estimates for the effect of CYP2A6 activity on the six PheWAS hits in current smokers were significant (P<0.001),

providing additional evidence supporting causal association.

Conclusions: Using a hypothesis-free approach we identified lung-related diseases associated with CYP2A6 metabolism, with faster CYP2A6 metabolizers at higher risk for developing respiratory cancersand obstructive lung diseases. Whether the effect is direct via nitrosamine activation or is mediated by increased smoking will be investigated next through mediation analyses. Along with prior candidate gene and genome-wide association study findings, these PheWAS and MR data provide additional support for the role of genetic variation in CYP2A6 as a risk factor for respiratory diseases. This studyalso sheds light on the utility of genetic risk scores and omics tools in assessing disease risk.

A03

Does genetic variation in a bitter taste receptor gene alter early smoking behaviors in youth?

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Introduction: Variation in the *TAS2R38* taste receptor gene alters the ability to taste bitter compounds. Three common missense variants in *TAS2R38* produce two common haplotypes: PAV and AVI, which are associated with tasting and not tasting bitter compounds, respectively. In adults with established smoking behaviors, the association between PAV and AVI haplotypes and smoking behaviors is inconsistent. We tested whether *TAS2R38* variation influences early smoking behaviors in adolescence, a critical period of acquisition when taste may influence the early course of tobacco use.

Methods: We leveraged data from European-ancestry participants from the Nicotine Dependence in Teens cohort, which is a longitudinal study involving 1,294 students recruited in 1998-1999 (starting age ~12 years). In adolescents who initiated smoking during follow-up (i.e., incident smokers; n=219), Cox proportional hazards models estimated the rate of attaining early smoking milestones, namely first whole cigarette, monthly smoking, and tobacco dependence, for tasters {PAV diplotypes (i.e., PAV/PAV or PAV/AVI)} versus non-tasters {AVI diplotype (i.e., AVI/AVI)}. In current smokers at age 24 (n=148), associations between tasting status and a biomarker of nicotine intake (cotinine + 3'hydroxycotinine) and past-week cigarette consumption were assessed.

Results: While there were no diplotype-based differences in smoking first whole cigarette (hazards ratio (HR)=1.05; 95% CI 0.72-1.38), incident smokers with the PAV (versus AVI) diplotypes attained monthly smoking at a faster rate (HR=1.55, 95% CI 1.13-1.96). Furthermore, adolescents with the PAV (versus AVI) diplotypes converted to three different measures of tobacco dependence at a faster rate (ICD-10: HR=2.29, 95% CI 1.43-2.42; HONC: HR=1.87, 95% CI 1.22-2.52; mFTQ: HR=3.02, 95% CI 1.94-4.10).

In exploratory analyses, there were no significant associations between diplotype and initial smoking experiences ($p>0.13$) or cigarette taste enjoyment at smoking initiation ($p=0.75$). At age 24, those with PAV (versus AVI) diplotypes had nominally higher mean cotinine + 3'hydroxycotinine (197 versus 143 ng/mL; $p=0.05$), but there were no diplotype-based differences in mean number of cigarettes smoked per day between diplotype groups (8 versus 9 cigarettes per day; 0.90).

Conclusions: Tasters (i.e., PAV diplotypes) had a faster escalation to monthly smoking and tobacco dependence during adolescence, consistent with having higher nicotine intake in young adulthood, versus non-tasters (i.e., AVI diplotype). We speculate the variation in taste is related to a learned association between bitter taste and the pharmacologic actions of nicotine. This suggests that the taste of tobacco may be a modifiable target to reduce usage, especially early in smoking history.

A04

Composite CYP3A phenotypes influence tacrolimus dose adjusted concentration in lung transplant recipients

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Abstract:
Interpatient variability in tacrolimus pharmacokinetics is attributed to metabolism by cytochrome P-450 3A4/5 isoenzymes (encoded by *CYP3A4* and *CYP3A5*). *CYP3A5* testing and dosing guidelines for tacrolimus exist, but the clinical role of genotyping is uncertain. *CYP3A4* variants also contribute to the variability in pharmacokinetics, but the effects of composite phenotypes incorporating *CYP3A5* and *CYP3A4* increased and decreased function variants have not been evaluated. We performed a retrospective cohort study of lung transplant recipients to evaluate the dose adjusted tacrolimus concentrations (C0/D) by composite *CYP3A* phenotypes. *CYP3A4* and *CYP3A5* alleles were used to classify patients as CYP3A rapid, normal, intermediate or poor expressers. The cohort included 92 individuals with majority of patients self-identified as Caucasian (94.5%) and had double LTx (79.4%). The median inpatient stay was 9 days. The median tacrolimus C0/D differed significantly between CYP3A phenotypes (p=0.0001). The CYP3A poor expresser median tacrolimus C0/D was 373.47 [interquartile range (149.16-490.27) (ng/mL)/(mg/kg/d)], more than threefold higher compared to intermediate [81.39 (62.56-184.09)] and rapid expressers [107.88 (90.37-116.08)] and nearly twofold higher than normal expressers [190.51 (147.59-267.51)]. For rapid expressers, the tacrolimus C0/D was almost half compared to normal expressers. Pairwise post hoc analysis showed significant differences between CYP3A poor and rapid expressers, poor and intermediate expressers, and rapid and normal expressers (p<0.00001, p=0.0032, p=0.0001, respectively). A composite *CYP3A* phenotype significantly influences tacrolimus C0/D during the early postoperative period. Additional prospective studies are necessary to inform how *CYP3A* phenotypes can be used for the clinical management of LTx recipients.

A05

Implementation of pre-emptive pharmacogenetic screening in pediatric oncology.

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Introduction: Pre-emptive pharmacogenetic (PGx) screening enhances drug efficacy and safety used in pediatric oncology based on an individual genetic profile. However, PGx screening is not yet part of standard clinical pediatric oncology care. Various initiatives have facilitated the integration of pre-emptive PGx screening. These include open access evidenced-based guidelines of the Clinical Pharmacogenetic Implementation Consortium (CPIC) and recommendations of the Dutch Pharmacogenetic Working Group(DPWG), which are supported by Pharmacogenomics Knowledgebase (PharmGKB) and Pharmacogene Variation Consortium (PharmVar). The initiatives currently comprehend recommendations for approximately 90 gene-drug pairs. Motivated by the growing evidence in gene-drug interactions and the high need for precision medicine in pediatric oncology, we aim to implement pre-emptive PGx screening into clinical practice of the Princess Máxima Center for Pediatric Oncology. Using the frameworks of existing PGx guidelines, we developed pediatric oncology PGx guidelines. Furthermore, we created a PGxtest panel to screen using diagnostic Whole Exome Sequencing (WES). Here, we describe our practices and challenges we encountered during the implementation of pre-emptive PGx screening in pediatric oncology.

***Methods*:** From existing pharmacogenetic gene-drug guidelines, we selected those that discuss supportive caredrugs and chemotherapy used in pediatric oncology. Subsequently, we designed a PGx test panel that includes the genetic variants present in the CPIC Allele Definition Table (supplementary material of CPIC guidelines). The PharmGKB/PharmCAT Pharmacogenomic Clinical Annotation Tool is used for the extraction of genetic variants from WES data. Lastly, we reviewed the clinical relevance of the selected gene-drug pairs and genetic variants in a multidisciplinary team of pharmacists and pediatric oncologists.

Results: In total, we developed 12 pediatric pharmacogenetic guidelines based on CPIC and DPWG guidelines. We created two novel PGx guidelines for *CYP3A4* and *CYP3A5* to monitor the role of these genes in vinca alkaloid-induced toxicity. Clinical consensus on test result interpretation was obtained without difficulty, but some technical issues formed barriers for the implementation of PGx screening. The main challenge was to correctly extract the desired genetic variants from WES data in a reliable and reproduciblemanner.

We encountered problems with testing and determining suitable clinical annotation tools, *CYP2D6* sequencing and indecisive genotypes. Furthermore, as PGx research is expanding, we want to add newly discovered genetic variants that are associated with chemotherapy-induced toxicity to our PGx test panel. However, the results are conflicting and most studies do not provide suggestions for clinical interpretation of newly discovered genetic variants.

Conclusion: We showed that CPIC guidelines and DPWG recommendations can be used as a backbone for PGx screening in pediatric oncology. We will expand our PGx screening with newly discovered genetic variants involved in efficacy and toxicity of chemotherapies used in pediatric oncology.

***Keywords*:** cancer, genetic testing, pharmacogenomics, precision medicine, exome sequencing

A06

Selection and Prioritization of Clinically Actionable Drug-Gene Pairs for a Pre-emptive Pharmacogenomics Service in Hospitals and Primary Care Setting s in Singapore.

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6. Department of Pharmacology, National University of Singapore.
7. Clinical Pharmacogenomics Implementation Work Group (CPIWG): a strategic partnership between National University Health System (NUHS), Tan Tock Seng Hospital (TTSH) and SingHealth (SGH) to improve the Safety and Efficacy of Medications and HealthCare Outcomes in Singapore. It is a multidisciplinary team of scientist, clinicians, clinical pharmacologists, clinical pharmacists, clinical geneticist, and laboratory medicine specialists working to bring Clinical Pharmacogenomics Implementation to Hospitals and Primary Care Setting in Singapore as Routine and Standard of Care.

Background/Rationale

One of the first steps for a pre-emptive pharmacogenomics implementation program involves determining which drug-gene pairs are clinically actionable and are most suited for translation into practice within their respective health care systems. This process optimizes efficient planning and allocation of resources and avoids early termination of the service.

Aims/Objectives.

The Clinical Pharmacogenomics Implementation Work Group (CPIWG) has proposed a robust consensus on the selection and prioritization of “Clinically Actionable Drug-Gene Pairs” for the implementation of Pre-emptive Pharmacogenomics Services, which involves the generation of a consensus quantitative metric score that highlights the multitude of factors that need to be taken into consideration in clinical implementation efforts across hospitals and primary care settings.

Methodology

The following criteria were used to characterize and select clinical actionable drug-gene pairs: 1. Drug-gene pairs currently included in clinical pharmacogenomics and clinical practice guidelines; 2. Drug-gene pairs included on drug labels and recommended/required by drug regulators for therapeutic management and clinical decisions; 3. Drug-gene pairs with strong and consistent scientific evidence linking them to defined drug responses and for which the benefits clearly outweigh the risks. The prioritization of clinically actionable drug gene pairs was performed via ranking with a quantitative metric scoring system comprising the following criteria: *1. Feasibility* (availability of clinical champion, clinical

genotyping platforms and alternative treatments/interventions); *2. Clinical robustness* (drug-gene pairs in clinical pharmacogenomics and clinical practice guidelines, drug-gene pairs on drug labels and recommended/required by drug regulators, frequency of predicted drug response phenotype, indicated diseases and associated burden and severity, drug prescription frequency and trends); *3. Scientific Criteria* (pharmacogenomics/functional characterization, allele frequency and one gene-multiple drug interaction phenomenon).

Results

Hundred-twenty-eight clinically actionable drug-gene pairs were identified. The clinical implementation quantitative metrics was independently applied to each clinically actionable drug-gene pair to generate its composite priority score and ranking, with a higher score indicating greater priority. We identified the top 25 clinically actionable drug-gene pairs for a Pre-emptive Pharmacogenomics Service at NUHS and classified them into 6 groups as follows: **Group 1 - Hypersensitivity Reactions** (Allopurinol-*HLA-B*5801*, Carbamazepine-*HLA-B*5802/HLA-A*3101*, Phenytoin-*HLA-B*1502/CYP2C9*, Abacavir-*HLA-B*5701*); **Group 2 - Pain Management and Supportive Care** (Codeine-*CYP2D6*, Tramadol-*CYP2D6*); **Group 3 - Chemotherapy Treatments/ Immunosuppressants** (Azathioprine-*TMPT/NUDT15,* Mercaptopurine-*TMPT/NUDT15,* Thioguanine-*TMPT/NUDT15,* Capecitabine-*DPYD,* Fluorouracil-*DPYD,* Tegafur-*DPYD,* Irinotecan-*UGT1A1*, Tamoxifen-*CYP2D6);* **Group 4 - Cardiovascular Drugs** (Warfarin-*CYP2C9/VKORC1/CYP4F2,* Clopidogrel-*CY2C19,* Simvastatin-*SLCO1B1*, Atorvastatin-*SLCO1B1)* **Group 5 – Psychiatric / Mental Health Medications** (Amitriptyline-*CYP2D6/CYP2C19,* Nortriptyline-*CYP2D6*, Paroxetine-*CYP2D6,* Fluvoxamine-*CYP2D6,* Escitalopram-*CYP2C19*) and **Group 6 - Others** (Ondansetron-*CYP2D6,* Voriconazole-*CYP2C19).*

Discussion/Conclusions

We presented a systematic process for identification and prioritization of clinically actionable drug-gene pairs via a quantitative metric scoring for a pre-emptive pharmacogenomics program. This approach may be extended to other clinical environments and healthcare systems that are interested in implementing pre-emptive pharmacogenomics services through optimizing resource allocation for a successful program implementation. It is vital to emphasize that the key focus of the Clinical Implementation quantitative metric scoring should not be on the absolute scores assigned to the clinically actionable drug-gene pairs. Rather, it is the prioritization of the clinically actionable drug-gene pair relative to each other that provides the most valuable information.

A07

A multidisciplinary precision medicine approach for integrating routine germline genetictesting into the care for pancreatic cancer patients.

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Summary:
Germline genetic testing has tremendous potential to improve treatment outcomes for patients with pancreatic cancer by guiding therapeutic decision-making. A growing number of germline variants have implications for both therapeutic decision-making [e.g., *BRCA1/BRCA2* – poly(ADP-ribose) polymerase (PARP) inhibitors, *MLH1/MSH2/MSH6/PMS2* – immune checkpoint inhibitors] and increased risk for inherited disease that can impact family members. We developed and implemented a multidisciplinary workflow inclusive of pharmacogenomicists, genetic counselors, physicians, and molecular pathologists that facilitates efficient germline testing to identify opportunities for targeted therapy.

We leveraged informatic tools inclusive of the electronic health record (EHR) to create a streamlined multidisciplinary approach for implementing germline testing into the care of pancreatic cancer patients. As part of this workflow, patients are referred by their physician to Moffitt's Genetic Risk Assessment Clinic via the EHR with the option to see a genetic counselor in-person or virtually. A genetic counselor provides genetic education and, if the patient elects to undergo germline testing, performs the informed consent along with test ordering. A test kit is shipped to patients who consent virtually. Genetic testing is performed by the reference laboratory Invitae (San Francisco, CA) with patients having options of an 84-gene panel (Detect

Hereditary Pancreatic Cancer) and/or a custom 50-gene panel (Moffitt Therapeutic Panel). The Moffitt Therapeutic Panel omits genes that are not currently eligible for targeted therapy in pancreatic cancer patients (e.g., *MEN1*, *MUTYH*, *SDHB*) and includes additional DNA damage response genes (e.g., Fanconi anemia genes) that may provide opportunities for clinical trials enrolling patients harboring variants predictive of homologous recombination deficiency. A genetic counselor reviews the results with the patient and when applicable offers cascade testing to family members. For results amenable to targeted therapy, a pharmacogenomicist enters a consultation note in the EHR identifying on-label pharmacotherapy options along with clinical trial opportunities. The consultation note can trigger clinical decision support, with the note also sent to the primary oncologist via an EHR message system.

Of the pancreatic cancer patients referred to a genetic counselor between 1/1/2019-2/16/2021, 11 (3.5%) declined genetic testing and 307 (96.5%) had germline testing performed. The patient population predominately self-declared race as white (79.5%) with majority being male (60.3%). A total of 51 (16.6%) patients had an actionable test result identifying either therapeutic options or inherited disease risk. Twenty-six (8.5%) patients harbored genetic variants eligible for on-label targeted therapy or a clinical trial, with these variants also having implications for inherited disease risk. Fourteen patients to date have received targeted therapy (13 received a PARP inhibitor and 1 received an immune checkpoint inhibitor). Three patients were identified by the Moffitt Therapeutic Panel harboring deleterious variants in Fanconi anemia genes that would not have been identified by the Detect gene panel. An additional 25 (8.1%) patients carried variants associated with inherited disease risk. Overall, the vast majority of patients elected to undergo genetic testing with almost 17% having an actionable variant. Next steps include testing for additional pharmacogenes (e.g., *DPYD* and *UGT1A1*) to further guide drug selection and mitigate drug-induced toxicities.

A08

Metastatic Colorectal Cancer Treatment in the Medicare Population: An analysis to inform preemptive germline pharmacogenomic testing

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Summary:

Purpose

Chemotherapy used to treat metastatic colorectal cancer (mCRC) presents risk for life-threatening toxicity with pharmacogenomic (Pgx) associations that are related to drug metabolizing enzyme gene variants. We evaluated patterns of exposure to chemotherapy agents with Pgx associations, including fluoropyrimidines and irinotecan, among patients with newly diagnosed metastatic colorectal cancer (mCRC) to identify patient, treatment, and community characteristics that impact exposure to these agents.

Methods

We included individuals diagnosed with stage IV CRC f rom 2004-2015 in the Surveillance, Epidemiology, and End Results-Medicare linked data who were continuously enrolled in fee-for-service Medicare for 12 months prior to at least 3 months post diagnosis. We used multivariate logistic regression to examine patient, tumor, non-chemotherapy treatment, and community characteristics associated with receipt of any chemotherapy as well as Pgx associated agents (fluoropyrimidines, irinotecan) with risk for life- threatening toxicity- defined here as PGx at-risk chemotherapy. This analysis was focused on initial treatment patterns, and considered only treatments received in the three months following the month of diagnosis.

Results

We identified 16,882 patients with mCRC; of these 6,977 (41.3%) received any chemotherapy within 3 months of their first mCRC diagnosis. Increasing age (85+ vs 65-69; Odds Ratio [OR] 0.11, 95% Confidence Interval [0.09-0.12]) and Charlson Comorbidity Index score (One vs Zero: OR 0.81, [0.75- 0.87]), and marital status (OR 0.45, [0.40-0.50] for previously but not currently partnered vs currently partnered) reduced the likelihood of chemotherapy receipt. No consistent patterns were observed based on community-level factors including poverty, education, and urban/rural status. In the subgroup of patients who received chemotherapy, 5,907 (84.5%) received a fluoropyrimidine or irinotecan. Male patients (OR 1.14, [1.00-1.30]) were more likely to receive treatment with a PGx at-risk agent compared to females, while older patients were less likely to receive treatment with a PGx at-risk agent (85+ vs 65-69: OR 0.35, [0.27-0.46]). No

treatment or tumor characteristics impacted treatment with a PGx at risk agent.

Conclusion:

We demonstrate that most patients with mCRC who are treated with chemotherapy are exposed to medications with known Pgx risk for toxicities and establish that older age is the only factor that reducesthe likelihood of exposure. Pre-emptive testing would impact a significant portion of this population, potentially reducing toxicities and mortality.

A09

NUDT15 polymorphism influences the metabolism and therapeutic effects of acyclovir andganciclovir

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Nucleobase and nucleoside analogs (NNA) represent an important class of therapeutic agents, particularly in anti-cancer and anti-viral treatment. Phosphorylation of NNAs is essential for their pharmacologic activities, and genes involved in this process have been linked to NNA drug toxicity and/or resistance. Currently, there are 41 NNA drugs approved by FDA, however pharmacogenetic factors associated with therapeutic effects remain unclear for the vast majorityof them. Recently, we and others identified that nucleotide diphosphatase NUDT15 is animportant enzyme for NNA drug thiopurine and genetic polymorphism in this gene can directly inform thiopurine toxicity in patients (*Nature Genetics* 2016, *PNAS* 2020). Considering structural similarities between a number of NNA drugs and thiopurines, and also a similar process for intracellular activation, we hypothesized that NUDT15 may dephosphorylate other NNA drug metabolites and therefore modulate their pharmacologic effects. To explore this, we first screened

the diphosphatase activity of NUDT15 with a panel of commonly used anti-cancer and anti-viral NNA drugs. Among 12 NNA drugs, triphosphate metabolite of anti-viral drugs acyclovir (ACV) andganciclovir (GCV) were efficiently dephosphorylated by NUDT15 in *in vitro* enzymatic assay. Interestingly, the R139C variant protein showed a dramatic lower activity against acyclovir triphosphate (ACV-TP) and ganciclovir triphosphate (GCV-TP), in a manner similar to thiopurine metabolite. Crystal structure of NUDT15 in complex with ACV metabolite was determined with notable differences compared to the NUDT15-thiopurine metabolite complex. Because NUDT15 inactivates ACV-TP and GCV-TP and these triphosphate metabolites are directly responsible for anti-viral effects, we further examined how NUDT15 regulates drug efficacy *in vitro*. Using CRISPR-Cas9 editing, we engineered isogenic mouse M2-10B4 cell lines with wildtype or knockout of *Nudt15*. Cells were infected with murine cytomegalovirus (MCMV) followed by exposure to increasing concentrations of ACV or GCV for 96 hours. Anti-viral efficacy was measured as the change of MCMV viral load in cells quantified by IE1 expression using the high-content imaging system. Across a broad range of drug concentrations, anti-viral effects of ACV and GCV were consistently greater in *Nudt15* deficient cells than the wildtype control. Finally, we evaluated *NUDT15* genotype association with ACV efficacy in 248 patients who received this drug as anti-viral prophylaxis enrolled in the national registry of Japan Society for Hematopoietic Cell Transplantation. Patients were classified into three NUDT15 activity groups based on the numberof impaired alleles; normal, intermediate and low. CMV viremia was detected after transplant in 49.2% of patients but the frequency varied significantly depending on the combination of *NUDT15* activity in the donor and the recipient. Patients with low NUDT15 activity in either donor or recipient cells exhibited the greatest protection against CMV by ACV treatment, only 20% had viremia, a 2.4-fold reduction compared to patients with normal activity in donor and recipient cells (P=0.015). Furthermore, donor NUDT15 deficiency was linked to graft failure in patients receiving CMV-seropositive stem cells (P=0.047). In conclusion, NUDT15 is an important metabolizing enzyme for ACV and GCV, and *NUDT15* variation contributes to inter-patient variability in their therapeutic effects.

A10

Life-Threatening Docetaxel-Induced Pneumonitis in a Patient with Loss-of-Function Variants in the Pharmacokinetic Genes *CYP3A4* and *CYP3A5*

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Docetaxel is an antimicrotubular taxane derivative used for treatment of a variety of solid tumors. Due to its cytotoxicity, docetaxel occasionally causes severe and life-threatening toxicities. Both the incidence and severity of docetaxel-induced adverse events are related to exposure, and significant inter-individual variability in exposure has been described based on genetic variation and drug-drug interactions that impact docetaxel clearance.

Within hepatocytes, cytochrome P450 3A4 (CYP3A4) and CYP3A5 metabolize docetaxel into inactive metabolites, and this is the primary mode of docetaxel clearance. To avoid toxicities, the drug label recommends 50% reduction in dose when co-administered with strong CYP3A inhibitors. Similarly, pharmacogenetic evaluations have demonstrated increased docetaxel toxicities in patients with reduced- or loss-of-function variants in *CYP3A4* and *CYP3A5*. Here we present a case report of a life-threatening pneumonitis resulting from a single dose of docetaxel in a patient with loss-of-function variants in *CYP3A4* and *CYP3A5*. This patient was homozygous for the reduced-function *CYP3A4*22* variant and the loss-of-function *CYP3A5*3*, and also heterozygous for the rare reduced-function *CYP3A4*3*, all of which likely contributed to the exceptional toxicity.

Pharmacogenetic guidelines for docetaxel have not been published yet, in part, due to the need for additional evidence, therefore these results further highlight the clinical need for studying these variants further.

A11

ASSOCIATION BETWEEN VARIANTS IN ABC FAMILY GENES AND ORAL MUCOSITIS SEVERITY IN PEDIATRIC PATIENTS WITH OSTEOSARCOMA

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Osteosarcoma is the most common primary bone malignancy of childhood and adolescence, accounting for around 2% of cancers in children and less than 1% in adults. The current management of neoadjuvant chemotherapy comprises of 3 to 4 cytotoxic agents (cisplatin, doxorubicin (DOXO), methotrexate (MTX), cyclophosphamide), followed by surgical resection and additional cycles of postoperative therapy. Despite the great improvement in the prognosis of patients with osteosarcoma after the inclusion of chemotherapy in the 1970s, in recent years the cure rate has remained stagnant, with no changes in the medications used since then. Therefore, there is a need for new approaches that consider the biological characteristicsof each patient, which can improve clinical results, whether in response to treatment or in the prevention of adverse effects. Previous studies have suggested the association between *ABC* gene family variants and osteosarcoma treatment response. The aim of this study was to investigate the relationship between variants in *ABCA3*, *ABCB1*, *ABCC1*, *ABCC2*, *ABCC3*, *ABCC4*, *ABCG2*, and *ABCC6* genes with the severity of oral mucositis in pediatric patients with osteosarcoma undergoing chemotherapy with methotrexate, doxurubicin and cisplatin. The study was based on a retrospective cross- sectional observational study with data collection from medical records and molecular analysis of samples from 14 pediatric patients with osteosarcoma treated at a Brazilian hospital from 2015 to 2018, totalizing 165 chemotherapy cycles analyzed. The exonic regions of the genes were analyzed through Next Generation Sequencing (NGS) using a customized gene panel in the Ion Torrent Personal Genome Machine (PGM) equipment.Patients' cycles of chemotherapy were grouped according to the mucositis grade, as follows: no mucositis (grade 0) + mild mucositis (grade 1) (70.5%) versus severe mucositis (grade 3 and 4) (29.5%). Chi-square test was used to evaluate the association between the variants and the mucositis severity. Patients mean age was 12 and 50% were male. Regarding the chemotherapy protocol, 66.7% of patients used MTX in high doses, 30.9% DOXO predominant and 1.8% MTX + DOXO + cyclophosphamide. Patients who used high-dose MTX or

predominant DOXO had totaling 29.3% in grade3 oral mucositis, according to the WHO classification. Nine variants of the *ABC* family genes showed an association with the severity of oral mucositis. Two variants in the *ABCC4* were associated according to recessive model, rs2274405 (p=0.008) and rs2274406 (p=0.001). Regarding *ABCC6* gene, rs2856585 (p=0.014) variant was associated with the oral mucositis severity according to recessive model. Variant rs2273697 (p=0.018) in the *ABCC2* and variants rs12931472 (p=0.004), rs8058694 (p=0.002), rs8058696 (p=0.002), rs9930886 (p=0.033), and rs9940825 (p=0.033) in the *ABCC6* gene were related to the mucositis severity using the additive model. No associations were found in the *ABCA3*, *ABCB1*, *ABCC1*, *ABCC3*, *ABCG2* genes. Studies that correlate the genetic data of patients with clinical outcomes are important for the implementation of personalized medicine, in order to reduce toxicity events of antineoplastic treatment and improve the clinical response. The results found in this study should be validated in other populations and in prospective cohorts to evaluate theclinical benefits of testing these variants.

Key words: osteosarcome, pharmacogenomics, ABC family genes, methotrexate, doxorubicin

A12

Inclusion of *GSTA1* polymorphisms in busulfan first dose personalization for pediatric hematopoietic stem cell transplantation recipients.

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Abstract:

Busulfan (Bu) is among the pillars of chemotherapy based conditioning regimens in pediatric hematopoietic stem cell transplantation (HSCT). Bu is known for

unexplained inter-patient pharmacokinetic (PK) variability despite the widespread use of intravenous (IV) formulations. This variability makes *a priori* PK predictions unreliable, compelling the implementation of therapeutic drug monitoring guided dose adjustments especially in children. The main Bu metabolic pathway involves glutathione conjugation catalyzed by glutathione-s-transferases (GSTs), principally by A1 isoform. The association between *GSTA1* promoter polymorphisms with Bu PK has been reported by several research groups, despite other published conflicting results. Moreover, the drug-drug interaction (DDI) of co-administered fludarabine (Flu) with Bu, affecting its clearance (CL), and the impact of *GSTA1* polymorphism on this DDI is not well known. The influence *GSTA1* polymorphisms on Bu's PK in patients receiving Flu is also unknown.

A population pharmacokinetic (PopPK) study for IV Bu in pediatric HSCT recipients was conducted to study the influence of *GSTA1* polymorphisms on Bu's PK in a large multicentric pediatric population including patients receiving Flu. The *a priori* model predictions would be used for individualized first- dose recommendation for IV Bu in pediatric patients.

This study included genetic, clinical and Bu PK data from 402 pediatric HSCT recipients (0–20 years old) from 5 transplantation centers, that participated in prospective observational cohort studies (NCT01257854; ACTRN12612000544875). An external validation cohort, comprising 100 patients, was extracted from this data set by stratified random sampling. The influence of *GSTA1* polymorphisms was tested using two different classifications. A first classification was only based on *-69 (rs3957357)* single nucleotide polymorphism: **A* homozygous, **B* homozygous and heterozygous. A second classification used our previously our previously published GSTA1 metabolic grouping of haplotypes based on 5 Loci in *GSTA1* promoter *(-69(rs3957357); -513(rs11964968); -567(rs4715332); -631 (rs4715333); -1142(rs58912740))* into three metabolic capacity groups: rapid, normal and poor metabolizers.

An age-dependent allometric scaling of bodyweight equation was used as base model for Bu CL. A stepwise covariate analysis identified the first day of Bu conditioning, GSTA1 metabolic groups, and Flu co-administration as significant covariates influencing Bu CL. GSTA1 metabolic groups resulted in improved model predictions compared to **A* and **B* haplotype grouping. This supports the importance Considering haplotypes based on 5 loci of GST promoter for Bu PK prediction. Poor metabolizers were estimated to have 12% lower CL than normal metabolizers, while rapid metabolizers exhibited 10 % higher CL. *A priori* Bu CL predicted with the final model were accurate (mean prediction error: -0.5%; 95%CI -3.1% – 2.0%) with acceptable precision (mean absolute prediction error % = 18.7%; 95%CI 17.0% – 20.5%). An 81% of model predicted first doses resulted in predicted exposures (area under the curve) within the therapeutic window.

This multicentric PopPK study confirmed the influence of GSTA1 metabolic groups in a large cohort of pediatric patients including those receiving Flu. The model predictions for Bu CL and first doses in the external cohort were reliable. the feasibility of routine *GSTA1* genotyping for Bu dose recommendation will be assessed in an ongoing multicentric, prospective randomized trial (NCT04822532).

The results of this study have been accepted for publication in *CPT: Pharmacometrics and Systems Pharmacology* Journal. (DOI: 10.1002/psp4.12683)

Conflicts of interest:

None to declare related to the submitted work. Dr. Nastya Kassir was employed by Certara in the past. She is now employed by Genentech/Roche.

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A13

Pharmacogenomics of Clopidogrel Response in African Americans: An ACCOuNT Study

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BACKGROUND: Clopidogrel is a platelet P2Y12 receptor antagonist, which works as an antiplatelet agent in combination with aspirin for the prevention of thrombotic conditions in patients with acute coronary syndrome (ACS) or for those undergoing percutaneous coronary intervention (PCI). Non-response to clopidogrel results in recurrent ischemic events while on treatment, with African Americans suffering disproportionally from these adverse events. The goal of this study is to identify pharmacogenomic biomarkers of clopidogrel resistance in African American patients.

METHODS: Samples were collected by African American Cardiovascular Pharmacogenomics Consortium (ACCOuNT). All patients were treated with 75mg/day of Clopidogrel. P2Y12 reaction units (PRU) values were obtained after 15 days treatment. Cases were defined as patients with PRU > 230. In total, 36 cases and 89 controls were selected. The case-control Genome-wide association studies (GWAS) was performed to identify variants associated with High PRU adjusting for age, sex, hypertension and principal components. RNAseq analysis was performed to identify the gene expression difference between cases and controls. Additional *in silico* analyses were conducted on significantly associated genes and SNPs which consisting of eQTL mapping in GTEx, FUMA GWAS for functional annotation, Phenome Wide Association Studies (PheWAS) for identifying phenotypes to be associated with top SNPs, RegulomeDB score for SNP prioritization, GeneHancer Regulatory Elements and Gene Interactions analyses todetermine associated pathways.

Results: SNP rs1731982 on chromosome 7 was associated with high PRU ($p=1.37*10^{-7}$, OR=3.65, 95% confidence interval (2.01-6.64)) with the RegulomeDB rank 4. This SNP is located at the intronic region of *ELMO1* with the allele C was associated with higher expressionof *ELMO1*. PheWAS analysis showed C allele of rs1731982 to be associated with lower mean platelet volume. RNAseq differential gene expression identified the association of lower expression of *ALG14* and *LAIR1* gene to high PRU.

Conclusion: Our data suggest that *ELMO1, LAIR1* and *ALG14* may be important factors in clopidogrel response in African Americans. *ELMO1, ALG14* and *LAIR1* genes were previouslyreported to be associated with platelet count. Further functional investigations of this locus are needed to provide insight on the pathogenesis of clopidogrel response as well as improving thepersonalized treatment of African Americans on antiplatelet therapy.

A14

Exploring the association of *CYP2A6* with the nicotine metabolite ratio among African Americans to identify putative causal variants

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Background: Tobacco use is the leading cause of preventable death and disease in North America. Nicotine is the primary addictive agent in tobacco, and individual differences in nicotine metabolism by the liver enzyme CYP2A6 predict smoking behaviours as well as cessation and health outcomes. The nicotine metabolite ratio (NMR; 3'hydroxycotinine/cotinine) is a stable biomarker for nicotine clearance, with demonstrated clinical utility in personalizing cessation treatment. We previously found that common genetic variation in the *CYP2A6* region was strongly associated with the NMR in African Americans, with a unique pattern of association compared to that observed in European populations. In this study, we investigated this association in more detail to identify putative causal variants.

Methods: We completed a genome-wide association study (GWAS) of the NMR among African American smokers from two clinical trials, Pharmacogenetics of Nicotine Addiction Treatment (PNAT)-2 (n=504) and Kick-it-at-Swope (KIS)-3 (n=450). Next, we performed stepwise conditional analyses to identify independent associations in regions reaching genome-wide significance.

Results: Our GWAS confirmed genome-wide association of the *CYP2A6* region of chromosome 19 with the NMR among African Americans. Stepwise conditional analyses identified four independent associations in the *CYP2A6* region. The strongest association was observed for a single-nucleotide polymorphism (SNP) ~16kb 3' of *CYP2A6* ($p=1.11x10^{-47}$). After conditioning on this variant, a second association was observed for a SNP ~5.5kb 5' of *CYP2A6* ($p=6.30x10^{-11}$). This SNP was in linkage disequilibrium with the *CYP2A6*17* allele ($r^2=0.71$), a known functional allele conferring decreased CYP2A6 activity. Conditioning on both the first and second hits, a third association was observed for a SNP ~9kb 3' of *CYP2A6* ($p=3.98x10^{-9}$). Conditioning on these three hits revealed a final association ~85kb upstream of *CYP2A6* within a long non-coding RNA

transcript ($p=6.93x10^{-9}$). Bayesian fine-mapping is currently underway to identify the most likely causal variants driving these associations.

Conclusions: Our study highlights multiple independent genetic associations with the NMR in the *CYP2A6* region in African Americans. These variants may causally influence nicotine clearance, smoking behaviours, and tobacco-related disease risk. Identification of causal variants influencing the NMR holds the potential to streamline current trial-and-error approaches to smoking cessation treatment, targeting the right intervention to the right patient based on their CYP2A6 activity, and target prevention and cessation efforts towards those at highest risk of smoking-related morbidity and mortality. To date, the vast majority of genetic studies have excluded African ancestry individuals. While precision medicine is poised to improve clinical outcomes, findings from genetic studies in European populations are not necessarily translatable to other ancestral populations. To prevent further exacerbation of existing tobacco-related health disparities, there is a critical need for ongoing research elucidating the genetic architecture of smoking related phenotypes in African Americans.

A15

Identification of cytochrome P450 oxidoreductase alleles in African populations using publicly available whole genome sequence data.

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Genetic variation among individuals can affect how drugs are absorbed, distributed, metabolized and transported in the body by various proteins. The study of these genetic differences is known as pharmacogenomics. Variation in many relevant pharmacogenes has been well characterised in Europe and Asia. However, much fewer studies on these genes have been done in Africa. In particular, cytochrome P450 oxidoreductase (POR) has been understudied, despite it being essential for the functioning of all cytochrome P450 enzymes. In this study we investigate the genetic variation in the POR gene in five African populations represented in the 1000 Genomes dataset. POR gene haplotypes and diplotypes were called using the StellarPGx pipeline, and all variants were annotated using Ensemble's variant effect predictor (VEP).

The most commonly alleles were POR *1 and *28 (A503V), for which we observed non-significant frequency differences among the 1000 Genomes African populations. We also inferred 5 putative novel haplotypes defined by deleterious variants that have not yet been characterised by the Pharmacogene Variation Consortium (PharmVar). 15 important high impact variants that are predicted to affect drug metabolism were also analysed using an ADME optimized model.

This study demonstrates the significance of studying variation in key pharmacogenes, such as POR, in an African context as we take further steps in implementing personalised medicine. Future work entails studying how these deleterious POR variants might affect CYP-mediated drug metabolism.

A16

Population genetic polymorphisms of pharmacogenes in Zimbabwe, a potential guide for the safe and efficacious use of medicines.

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ABSTRACT

Background

Pharmacogenomics (PGx) is a clinically significant factor in the safe and efficacious use of medicines. Whilst PGx knowledge is abundant for other populations, there is scarce PGx data on African populations and little knowledge on drug-gene interactions for medicines used to treat diseases common in Africa. In response to this PGx gap in Africa, we have worked on genetic polymorphisms of pharmacogenes and exploring drug-gene interactions potentially important for Africans. This work has culminated in the design and technical validation of a pharmacogenetic testing open array

Objective

The aim of this study was to use a custom designed open array to genotype clinically actionable drug-gene interaction variants in a Zimbabwean population. This study also compiled some of the commonly used drugs in Zimbabwe and the genes involved in their metabolism.

Methodology

A custom designed open array was used to genotype for 120 gene variants in 118 black Zimbabwean healthy volunteers using TaqMan based SNP genotyping. Data was also accessed from Essential Drugs' List in Zimbabwe (EDLIZ) and the medicines were grouped into the associated biomarker groups based on their metabolism. We have also estimated the national drug procurement levels for medicines that could benefit from PGx-guided use based on the data obtained from the national authorities in Zimbabwe.

Results

The results demonstrate the applicability of an open array chip in simultaneously determining 120 genetic variants in 39 pharmacogenes in an individual, thus significantly reducing cost and time to generate PGx data. The data obtained showed that the Zimbabwean population exhibits PGx variations in genes important for the safe and efficacious use of drugs approved by the Essential Drugs' List in Zimbabwe (EDLIZ) and are procured at significantly large amounts annually. The results also show that some of the genetic variation could be important for the design and conduct of studies and the safe use of drugs being trialled for the treatment of COVID-19 in our population.

Conclusion

Our study demonstrated the potential benefit of integrating Pharmacogenomics in Zimbabwe for the safe and efficacious use of drugs that are commonly used.

Keywords: pharmacogenomics, pharmacogenes, COVID 19, open array, precision medicine

A17

A systematic analysis of the use of polygenic risk scores in pharmacogenomics

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Background: Polygenic risk scores (PRS), which are weighted sums of the alleles associated witha phenotype, have emerged as promising tools for complex trait risk prediction. The applicationof PRS in the context of pharmacogenomics provides unique opportunities to improve the accuracy of current methods aimed at predicting treatment outcomes. There exists limited information relating to the use of PRS in pharmacogenomics. Therefore, we performed a systematic review and analysis of data obtained from current pharmacogenomic PRS research.

Methods: In this systematic analysis; title, abstract and full text screening was performed by two independent authors. Data extraction was performed by three authors, with each article examined by at least two authors. Through this review, we extracted information relating to thedisease areas and drugs under investigation, as well as detailed information on PRS analysis methods. We also extracted information relating to the characteristics of base and target cohorts included in these analyses (e.g. phenotypes, ancestry and sample sizes). Finally, we examined whether current reporting of PRS results is sufficient to allow for the independent replication of findings. To do this, we assessed whether the articles adhered to recently developed reporting standards (e.g. the level of detail provided for the study background, methods, limitations and clinical implications). After extraction of the relevant data, the main findings were summarized. Results were analysed using R, and figures were plotted using the ggplot2 and ggmap R packages.

Results: Our systematic analysis uncovered 51 papers focusing on pharmacogenomic PRS, withthe majority of these papers examining the use of PRS in the context of psychiatric disorders (*n*=30). In the development of pharmacogenomics PRS, there are difficulties in obtaining large cohorts of uniformly treated samples. For this reason, the majority of PRS were derived from large-scale genome-wide association studies of disease phenotypes that were related to the pharmacogenomic phenotypes under investigation. These methods uncovered significant findings relating to schizophrenia-derived PRS and antipsychotic response, as well as coronaryartery disease-derived PRS and response to low-density lipoprotein lowering medications.

Examination of genetic ancestries revealed that the overwhelming majority of cohort participants were completely of European descent (*n*=40 studies, 78.4%). These biases were also reflected in the institutes where the research was performed, which were heavily weightedtowards institutes located in Europe, North America and Australia. Finally, we observed large inconsistencies in reporting of PRS analyses and results, particularly in terms of risk model development and application, coupled with a lack of data transparency and availability.

Conclusion: This systematic review and subsequent analysis of the extracted data has providedan overview of innovative PRS analyses that are currently being applied in the field of pharmacogenomics, including which areas show promise for clinical implementation. Current limitations include Eurocentric biases, poor adherence to PRS reporting guidelines and gaps in data transparency and availability. These findings highlight key areas and current gaps in pharmacogenomic PRS research.

A18

An Investigation of the Knowledge Overlap between Pharmacogenomics and DiseaseGenetics

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Precision medicine faces many challenges, including the gap of knowledge between disease genetics and pharmacogenomics (PGx). Disease genetics interprets the pathogenicity of genetic variants for diagnostic purposes, while PGx investigates the genetic influences on drug responses. Ideally, the quality of health care would be improved from the point of disease diagnosis to drug prescribing if PGx is integrated with disease genetics in clinical care. However, PGx genes or variants are usually not reported as a secondary finding even if they are included in a clinical genetic test for diagnostic purposes. This happens even though the detection of PGx variants can provide valuable drug prescribing recommendations. One underlying reason is the lack of systematic classification of the knowledge overlap between PGx and disease genetics.

Here, we address this issue by analyzing gene and genetic variant annotations from multiple expert-curated knowledge databases, including PharmGKB, CPIC, and ClinGen/ClinVar. Genes were classified based onthe strength of evidence supporting a gene's role in target phenotypes and the level of clinical actionability. Twenty-six genes were found to have pathogenic variation associated with germline diseases as well as strong evidence for a PGx association. These genes were classified into three sub-categories based on the distinct connection between the gene's pathogenic role and PGx effect.

Moreover, two *RYR1* genetic variants of uncertain pathogenic significance based on ClinGen/ClinVar had PGx significance according to CPIC drug prescribing recommendations for malignant hyperthermia and are considered 'diagnostic mutations' by the European Malignant Hypothermia Group.

Overall, we identified a nontrivial number of gene and genetic variant overlaps between disease genetics and PGx, which laid out a foundation for combining PGx and disease genetics to improve clinical care from disease diagnoses to drug prescribing and adherence.

A19

The Use and Potential Misuse of Medications with Genotype-InformedPrescribing Guidelines in a Nationally Representative Cohort of US Children

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Pharmacogenetics is the study of how genes and drugs interact. When robust gene-drug interactions are discovered, prescribing guidelines are developed to inform medication selection and dosing based on a patient's genotype. The Clinical Pharmacogenomics Implementation Consortium (CPIC) and other expert groups have developed genotype-guided prescribing recommendations for a variety of medications used in paediatrics to improve patient outcomes and reduce incidence of adverse effects. However, despite the existence of a growing number of guidelines, there are still questions regarding the applicability of pharmacogenetic guidelines in paediatrics and what proportion of children may benefit from pharmacogenetic testing. To address these questions, this study examined ten pharmacogenes with prescribing guidelines (CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP4F2, DYPD, IFNL3 SLCO1B1, TPMT, VKORC1) among 11,875 American children aged 9-10 participating in the Adolescent Brain Cognitive Development study using the PGxPOP pipeline. We found 99.0% of participating children had at least one actionable genotype, with an average of 3.24 actionable genotypes per child. Among children taking at least one medication with an existing guideline (N = 257), 10.9% were taking at least one medication that was not recommended for them based on their genotype. After adjusting for the concurrent use of inhibitors or inducers of the selected pharmacogenes, this increased marginally to 13.6%. The most common actionable gene-drug pair was CYP2D6 and atomoxetine.

Our findings suggest most children have actionable genotypes in pharmacogenes with prescribing guidelines and among those currently taking a medication with a

guideline about one in every eight children could benefit from pharmacogenetic testing.

A20

Computational Drug Discovery in Advanced Prostate Cancer through in vitro Drug Response Modeling

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Prostate cancer is among the most frequent malignancies and one of the leading causes of cancer deaths among men in the United States. Despite initial effectiveness of androgen-deprivation therapy (ADT) in primary prostate cancer, nearly all cases will develop resistance to these treatments, a stage known as castration-resistant prostate cancer (CRPC). CRPC accounts for virtually all deaths in prostate cancer, with a median survival between 15 and 36 months. Therefore, there is an urgent need to develop effective therapeutic strategies for patients affected with CRPC.

Traditional drug discovery pipelines, however, are often time-consuming and financially costly, providing motivation for translational researchers to develop novel methods that can quickly discover repositioning potentials from existing compounds. In this study, we used a machine learning framework to construct models based on in vitro drug screens, which we then applied to clinical transcriptomic data to impute CRPC patients' sensitivities to thousands of compounds. Furthermore, we investigated the associations between imputed drug response and patient clinical features such as disease progression, overall survival, and resistance against standard-of-care therapies. We applied this method on multiple CRPC patient cohorts and obtained robust results identifying candidate compounds by overlapping independent nominations of drugs from each data set.

As a proof of concept, our analyses identified a number of compounds that are currently undergoing clinical trials for prostate cancers. Furthermore, we identified a tetracycline derivative, COL3, which shows higher imputed sensitivity in CRPC patients who were resistant to ADT. This prediction was validated in a pair of genetically engineered prostate cancer cell lines (R1-AD1 and R1-D567). Higher efficacy of COL3 was observed in R1-D567, a CRPC cell line driven by a truncated AR-variant protein, when compared to AR-wild type cell line R1-AD1.

Overall, we have proposed a computational workflow that quickly translates CRPC patient gene expression data into actionable information regarding drug response. Our results showed consistency with traditional methods and, more importantly, inform treatment discovery through identifying a robust wide range of candidate drugs for therapy-resistant CRPC.

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A21

Pharmacogenetic Testing Knowledge and Attitudes among Pediatric Psychiatrists andPediatricians in Alberta, Canada

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Objective: To assess knowledge, attitudes, and barriers as well as ethical, legal and social concerns towards pharmacogenetic (PGx) testing among pediatric psychiatrists and pediatricians in Alberta,Canada.

Method: An anonymous electronic survey was sent to pediatric psychiatrists (n = 49) and pediatricians (n = 93) in Alberta.

Results: A total of 20 surveys were completed (response rate = 14%). Respondents agreed that PGx testing is clinically useful and a majority believed testing had the potential to aid in medication selection, dosing, switching, augmentation, and deprescribing, particularly among children with treatment-resistant conditions. However, most respondents could not identify an appropriate lab toperform testing, did not have the necessary training to interpret PGx results, and did not have access to experts that could assist them in in interpreting results.

Conclusion: The findings suggest additional PGx education and training is required to boostself-efficacy and uptake of PGx testing among pediatric psychiatrists and pediatricians in Alberta, Canada. In addition, local and global efforts to develop clinical practice guidelines, provide clear legal guidance, and ensure equitable access to testing will further facilitate the implementation of PGx-informed prescribing.

Keywords: pharmacogenetics, children, survey

A22
Pharmacogenetics in pediatric psychiatry: considerations for implementation in rural communities

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Background:

Compared to their urban counterparts, children and adolescents living in rural areas across the United States face higher incidence of mental health challenges and higher rates of suicide, yet they have limited access to specialty psychiatric care. Pharmacogenetics offers providers a tool to optimize prescribing and dosing of medications for these vulnerable patients, however, implementation efforts have overwhelmingly focused on patients living in urban areas. Rural health systems struggle to implement pharmacogenetics due to inadequate resources, lack of local expertise, and geographic remoteness. We partnered with Shodair Children's Hospital (Shodair)—the only facility in Montana that provides inpatient and outpatient pediatric psychiatric services—to develop a pharmacogenetic testing program utilizing telehealth consultation services. Innovative solutions are required to create an implementation model that may ensure equitable access to pharmacogenetics for patients living throughout Montana as well as other rural areas.

Methods:

We conducted semi-structured interviews (n = 21) with providers, staff, and administrators at Shodair to identify barriers and facilitators for pharmacogenetic implementation. Interviews were analyzed for common themes using a qualitative analysis software. Researchers independently reviewed transcripts, developed a codebook, revised, and evaluated common themes among participants. Based on the results of the thematic analysis, pre-implementation efforts focused on the development of a centralized service with telehealth capabilities to provide pharmacogenetic expertise and consultations, virtual patient education tools, and

key stakeholder engagement with hospital administrators, payers, and employment benefit plans.

Results:

Interview participants shared clear goals for a pharmacogenetic implementation effort and common needs included education and resources for providers and patients, protocols for utilizing the services, and performing testing in outpatient settings. Overall, Shodair participants were enthusiastic regarding the utility of pharmacist-led telehealth consultations through a centralized service at the University of Montana. Participants described the opportunity to lead in pharmacogenetic implementation as a positive outcome for patients across the state, and felt it aligned with the mission of the institution. As the importance of pharmacogenetic resources was a major theme among participants, we prioritized the development of online tools to share information with providers and patients. To this end, we developed an educational video describing the utility of pharmacogenetics and the process of undergoing testing and consultation. The video was well received among viewers, including healthcare professionals and those without a medical background. We have also engaged several key payers, employee benefit managers, and hospital administrators to provide information on the appropriate use of pharmacogenetics, availability of testing, clinical utility data, and potential cost-savings.

Conclusion:

We learned critical insight into the needs of a psychiatric children's hospital serving rural patients, and the interviews have guided pre-implementation efforts, including the need for a pharmacist champion, development of telehealth protocols and virtual access to pharmacogenetics expertise, and educational resources. Our research will continue to inform implementation efforts including development of metrics to capture clinical and health economic outcomes of pharmacogenetics and satisfaction among patients and providers concerning the consultation service. Our research provides a framework for implementing a pharmacogenetics delivery model for early adopter sites throughout Montana.

A23

Medication prescribing predicts utility of panel pharmacogenomic testing in breast cancer patients.

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Background: Patients diagnosed with breast cancer experience polypharmacy which leads to anincreased risk for negative outcomes such as therapy failure or adverse drug reactions.

Pharmacogenomic (PGx) testing could mitigate risks for relevant medications including breast cancer treatment as well as supportive care drugs. Understanding PGx medication exposures in this populationcould direct testing (e.g. which genes) and implementation strategies (medication-based patient targeting or testing in all patients). We aimed to evaluate the opportunity for benefit of preemptive pharmacogenomic panel testing in UPMC breast cancer patients.

Methods: Electronic health records of patients diagnosed with breast cancer from January 1, 2016 to December 31, 2018 were analyzed for medication orders involving a drug that is part of Clinical Pharmacogenetics Implementation Consortium (CPIC) level A gene-drug pair and with a CPIC PGx guideline ("PGx medication"). Incidence of new PGx medication order and the associated genes were calculated over a one year period following diagnosis. A sub-analysis was also performed to focus on orders downstream of an initial order for a PGx medication that is indicated for the treatment of breast cancer (i.e. tamoxifen/*CYP2D6*, fluorouracil or capecitabine/*DPYD*).

Results: In a large cohort of 30,968 breast cancer patients, 12,948 patients (41.8%), and 5,247 patients (16.9%) that had 1, or 2+ orders for PGx medications within the first year after diagnosis, respectively. Atotal of 4,499 patients (14.5%) had received at least 2 different PGx medications associated with 2+ different PGx genes. The sub-analysis showed 1,572 patients had a prescription of a PGx medication indicated for the treatment of breast cancer. The population was enriched for subsequent exposures with 531 patients (33.8%) having at least a second order for a PGx medication. Of these, 440 patients (28.0%) had multiple PGx medication orders associated with 2+ PGx genes suggesting additive benefits of panel testing. The most common associated PGx genes were *CYP2D6, CYP2C19, CYP2C9, DPYD,* and *SLCO1B1*.

Conclusion: The incidence of multiple PGx medication orders in patients with breast cancer was high and further enriched in patients first receiving a PGx medication indicated for breast cancer. Significantproportions of the breast cancer population also had multiple PGx medication orders involving two or more PGx genes. Together, this suggests an additive benefit of utilizing panel-based PGx testing in the breast cancer patient population.

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A24

IDENTIFICATION OF ADVERSE DRUG REACTIONS THAT MAY BE RELATED TO PHARMACOGENOMICS IN A PUBLIC HOSPITAL FROM THESOUTHERN BRAZIL

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Adverse drug reactions (ADRs) are major causes of hospitalization and mortalitywith an important impact on public health worldwide. The prevalence of severe ADRs in Brazil reaches 3.33%. Many of these reactions may be related to genesinvolved in drug pharmacokinetics/pharmacodynamics. The aim of this study wasto identify the prevalence of moderate and severe ADRs related to drugs that have pharmacogenetic guidelines in a public hospital in Southern Brazil and estimate the population of patients that present higher risk of developing these reactions based on pharmacogenes allele frequencies. Information on ADRs wascollected from 2017 to 2019.

Drugs that have a pharmacogenetic guideline plus moderate/severe ADRs were selected. The *REFARGEN* (National Pharmacogenetics Network) and *ABraOM* (Brazilian genomic variants) databases were used to identify the frequency of phenotypes in Southern Brazil or, when not available, in Brazilian population. During the period, 156 ADRs werereported, in which approximately 65% of reactions were classified as moderate and 29% as severe. The drugs with the highest prevalence of moderate/severe ADRs and that have pharmacogenetic guidelines were phenytoin, carbamazepine, tacrolimus, and tramadol. Phenytoin ADRs represented 16.67%of the total and, of these reactions, 57.14% were caused by allergic reactions and42.86% were idiosyncratic. *CYP2C9* and *HLA-B* genes are related to its responseand, according to CPIC and DPWG guidelines, patients who are *CYP2C9* Poor and Intermediate Metabolizers present higher risk of toxicity and should reduce phenytoin dose. In Southern Brazil, the estimated frequency for these phenotypes were 3.5% and 30.6%, respectively. Additionally, patients who have*HLA-B*15:02* allele have an increased risk of developing important allergic reactions and the frequency of this variant in Brazilian population is 3.5% (rs2395148). Carbamazepine ADRs represented 14.28% of the reactions and 33.33% of them were caused by allergic and 66.67% by idiosyncratic causes. It is known that the *HLA-B*15:02* and *HLA-A*31:01* alleles are involved in the development of severe allergic reactions and, according to CPIC guideline, patients who present at least one of these alleles should avoid carbamazepine.

The frequency of these variants in Brazilian population is 3.5% (rs2395148) and7.4% (rs1061235), respectively. ADRs for tacrolimus represented 19.04% and allof them were idiosyncratic. CYP3A5 is involved in its metabolism and Poor Metabolizers may present higher drug concentration when compared to Normal or Intermediate Metabolizers. The frequency of 64.3% was estimated for *CYP3A5*Poor Metabolizers in the Southern Brazil.

ADRs related to tramadol represented21.43% and, of these reactions, 22.22% were caused by allergic reaction and 77.78% by idiosyncratic causes. The *CYP2D6* gene is involved in its metabolism and the CPIC guideline recommends that Ultrarapid metabolizers should use analternate analgesic due to the high risk of toxicity. The *CYP2D6* Ultrarapid Metabolizers estimated frequency in the Southern Brazilians is 2.9%. In conclusion, moderate/severe ADRs occur in a relevant frequency and, part of them, could be avoided if pharmacogenomics tests were implemented in the clinical practice.

Keywords: Adverse drug reactions, pharmacogenetics, pharmacogenomics.

A25

Functional Genomics of Rare Coding Region Variants in the GABA Transporter, GAT-1 (SLC6A1)

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Background and Goals: *SLC6A1* encodes the GAT-1 protein, which is a GABA transporter expressed in inhibitory neurons in the brain. Genetic variants in *SLC6A1* are strongly associated with myoclonic atonic epilepsy, generalized epilepsy, intellectual disability, and autism spectrum disorder. Currently, the mechanisms by which these variants contribute to CNS disorders and their effects on GAT-1 function are poorly understood. Using compiled data, including literature and large-scale genomic datasets, a total of 154 case-associated *SLC6A1* variants have been identified. About 80% of these variants are missense and the effect on function is unknown. Moreover, the majority of these variants are germline *de novo* mutations and recurrent. The goal of this study was to characterize a large subset of GAT-1 variants to clarify the genotype-phenotype relationship and learn how these mutations affect GAT-1 protein function. This is the first large functional study of genetic variants in *SLC6A1* that are associated with CNS disorders.

Methods: From a complied list of clinically reported case variants and common variants from gnomAD, 100 variants were selected to study in uptake transport assays. Selected variants were evenly spread across the GAT-1 protein domains and preference was given to variants that had recurrent cases as well as a reported clinical phenotype. To determine functional activity of GAT-1 using radiolabeled uptake assays, wildtype *SLC6A1* cDNA (WT) was engineered into the pcDNA5/FRT expression vector, and the variants were introduced via site-directed mutagenesis. HEK293 cells, which lack GABA transporters, were transiently transfected with either vector only (pcDNA5/FRT), vector with WT *SLC6A1*, or vector with its respective mutation. Following transfection, the cells were incubated with radiolabeled GABA (^{3}H-GABA) and uptake was measured using standard scintillation counting methods.

Results: Uptake of ^{3}H-GABA transport in cells expressing the WT transporter resulted in a ~10x fold uptake over cells expressing vector only. Out of 100 variants screened, 75 of the missense variants showed complete to partial loss-of-function with a large range of activity (0%-80% of WT). Seven of 25 variants that retained or surpassed function of the reference allele, surpassed 150% of WT activity, suggesting a potential gain of function effect. Preliminary analysis did not show any correlation between the function of GAT-1 protein and the severity of the reported phenotype.

Conclusions and Future Studies:
These results show that mutations across the GAT-1 protein are associated with varied clinical phenotypes and a large range of protein function. It remains unclear how mutations in *SLC6A1* lead to more or less severe phenotypes. Given the presence of excess missense variants that demonstrate a loss-of-function effect and are recurrent mutations, we hypothesize that these missense mutations act in a dominant negative

manner, disrupting the function of GAT-1 protein from both the variant and wildtype alleles and reducing GABA transport. Future studies aim to evaluate potential dominant negative contribution of a subset of these missense variants and follow-up studies on variants demonstrating gain-of-function effect. Ultimately, understanding the mechanisms by which *SLC6A1* variants alter the function of GAT-1 will advance the development of therapies that target seizures and neuropsychiatric disorders.

A26

Combining single-cell transcriptomics with a novel microfluidic assay to investigate ethnic differences in tumor heterogeneity and plasticity at the sub-clonal level

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ABSTRACT

Prostate cancer/PCa is the 2nd-most common cancer in men in the USA with striking inter-ethnic disparity in incidence and mortality rates. African American/AA men bear a disproportionate burden of PCa, are more likelyto be diagnosed, have higher incidence rate and nearly 2-times higher mortality rate compared to Non-HispanicWhite/NHW men. Moreover, AA men have earlier mean age-of-diagnosis, higher risk of developing aggressive forms of PCa. and shorter overall survival than men of other races. Further, cancer metastasis is characterized by dissociation of cancer cells from the primary tumor site and colonization in a distant organ by traveling throughinterstitial tissues, intravasation into the blood or lymphatic vessels, and extravasation into a new site followed by proliferation and plasticity (dynamic shift between differentiated state with limited tumorigenic potential and undifferentiated or cancer stem-like cell states) or Epithelial to Mesenchymal Transition/EMT. PCa tumor cell plasticity leads to drug resistance and progression to more aggressive stages of PCa called castrate-resistant prostate cancer/CRPC and neuroendocrine prostate cancer/NEPC. Differences at the molecular and subclonallevel between patients are of paramount importance in addition to the known risk factors.

In this study, we performed single-cell RNA sequencing/scRNAseq analysis on PCa cell lines LNCaP (androgen-sensitive) and DU145 (androgen-independent) of NHW origin, and RC-77T/E (androgen-sensitive) and MDA- PCa-2b (androgen-independent/aggressive) of AA origin using droplet-sequencing chemistry (10X Genomics Chromium platform) to compare the ethnic differences in intra-tumor heterogeneity at the subclonal levels. Seuratand Partek Flow software packages were used for single-cell transcriptomics analysis and the highly variable genes were used to perform t-distributed stochastic neighbour embedding (t-SNE) analysis to visualize distinct single-cell clusters. Our scRNAseq data revealed ethnic differences in the expression of genes that play major roles in cancer progression, development, and maintenance of cancer stemness (CD44, HES1), and resistanceto standard-of-care Taxane/TX-based chemotherapy (CXCL8, CDK1, CDH1, SOX9), highlighting the distinct biology underlying ethnic disparities in PCa risk, morbidity, and mortality at the single-cell level. In addition, we predicted several genes in drug-resistant sub-populations as novel targets for PCa therapy.

Among the genes associated with EMT transition, we observed downregulation of E-Cadherin, and upregulationof Vimentin and ZEB1/2. Moreover, cell motility through confining pores plays a pivotal role in the process of metastatic dissemination during which cells undergo EMT. Therefore, we used a highly sophisticated microfluidicchip-based assay called μ-Fluidic confined cell migration assay which served as a physiologically relevant modelfor *in vitro* metastasis by recapitulating diverse microenvironmental cues encountered by cancer cells during locomotion, including the dimensionality of pores and 3D-longitudinal, channel-like tracks, to evaluate cancer cell invasion and motility. We observed striking differences in cell motility through microenvironments of differentdimensions (representing metastasis) between AA and NHW men.

Together, our scRNAseq profiling and microfluidic assays provide a novel approach to understand the basis of racial disparities in tumor heterogeneity, plasticity, and treatment response in prostate cancer. Currently, we areevaluating the racial differences in chemotherapy-induced responses at the single-cell transcriptome level using*in vitro* and *in vivo* (mouse xenograft) models of advanced-state/aggressive prostate cancer.

A27

Regulation of the CYP3A genes by a distal enhancer and regulatory variants

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Cytochrome P450 4 (CYP3A4) is the most abundant drug-metabolizing enzyme in the liver, metabolizing nearly half of all commonly prescribed medications. Expression of CYP3A4 is highly variable with unknown causes, and cis-acting regulatory elements controlling CYP3A4 transcription remain uncertain. We hypothesized that chromatin interactions within the CYP3A gene locus may regulate the four CYP3A genes (CYP3A4, CYP3A5, CYP3A7 and CYP3A43). We previously identified a distal regulatory region (named R1) that interacted with the CYP3A4 promoter. In this study, we further characterize the function of the region surrounding R1. Based on the presence of transcription factor binding and DNAse hypersensitivity sites, we extended the R1 to include two subregions, (R1α and R1β). CRISPR-mediated deletion of R1α in Huh7 cells has no effects on CYP3A4 expression, while CYP3A5 and CYP3A5 show reduced expression. In contrast, deletion of R1β reduced the expression of CYP3A4, 3A5 and 3A7, indicating that R1 may serve as a master enhancer for these three CYP genes. Publicly-available hepatocyte ChIP-Seq data showed enrichment of enhancer marks in the R1 and ATAC-Seq results generated from hepatocytes showed that the R1 region is accessible, both indicating that it is a regulatory region. Using chromatin conformation capture (4C-Seq) in hepatocytes, we confirmed that the CYP3A4 and CYP3A5 promoters interact with the R1 region and using the R1 region as the bait we identified a reciprocal interaction near the CYP3A4 and CYP3A5 promoters. Haplotypes formed by seven common SNPs in the R1 showed differential transcriptional activities in reporter gene assays. In particular, the predominate European haplotype had five-fold higher transcriptional activity than the predominate African haplotype. Interestingly, one SNP (SNP6) unique to the African population increased expression of the African haplotype to levels similar to the predominate European haplotype. Testing in a liver cohort (n=246), we found association between SNP6 and expression of CYP3A4, with each variant allele associating with a 1.8-fold increase in CYP3A4 mRNA expression (P=0.039) and a 1.6- fold

increase in CYP3A4 protein level (P=0.009). Also, we identified a different SNP (SNP4) occurring in the SRα region that was associated with 1.39-fold increased CYP3A5 expression (P=0.012) after adjusting for CYP3A5*3, *6 and *7 genotypes. The clinical association studies of these two variants are ongoing.

Our results elucidate the complex regulatory network controlling expression of the CYP3As, highlighting the necessity of studying the entirety of the CYP3A cluster instead of each individual gene.

A28

Mir-192 is a Novel Regulator of GLP1R and Insulin Secretion and Contributes to Statin-inducedDiabetes

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Statins are a commonly prescribed cardiovascular disease drug class that can increase risk of type 2 diabetes (T2D). To investigate molecular mechanisms underlying this effect, we created induced pluripotent stem cells (iPSCs) from normoglycemic patients identified from electronic health records of Kaiser Permanente of Northern California who developed T2D after statin initiation or controls who maintained normal fasting glucose on statin treatment (24 per group). RNAseq analysis of iPSCs incubated with 250nM atorvastatin, 500nM simvastatin or mock buffer for 24h identified *MIR194-2HG* as one of the transcripts exhibiting the greatest differential change between cases vs. controls. Both statins increased *MIR194-2HG* in the controls but decreased levels in cases. This long non-coding RNA encompasses two microRNAs: *MIR-192* and *MIR-194*. MIR192-5p is predicted to bind the 3' UTR of the glucagon like peptide 1 receptor gene (*GLP1R*), and we found that transfection of a rat insulinoma cell line (INS-1) with a MIR192-5p mimic caused increased *Glp1r* transcript (1.4-fold) and protein (2.1-fold) levels compared to a scrambled control. Using a luciferase reporter containing human *GLP1R* 3' UTR, weobserved similar increases in signal; however, this elevation remained even after disruption of the predicted MIR192-5p binding site demonstrating that MIR192-5p upregulates GLP1R through an indirect mechanism. GLP1R augments glucose-stimulated insulin secretion, and we found that the MIR192-5p mimic increased insulin levels in media of glucose-stimulated INS-1 cells (1.4-fold). Reduction in media insulin in INS-1 cells treated with 1 μM simvastatin was rescued by MIR192-5p overexpression, while a MIR192-5p antisense inhibitor abolished this effect.

These findings implicate MIR192-5p in statin- induced impairment of glucose stimulated insulin secretion through the regulation of GLP1R, an effect that may contribute to the increased risk of T2D in statin users.

A29

High-content imaging of 150 OCTN2 transporter variants reveals subcellular localization tobe predictive of function

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Background: OCTN2, encoded by SLC22A5, is the major membrane transporter for carnitine, anamino acid derivative essential for beta-oxidation of long-chain fatty acids. Genetic variation inSLC22A5 can cause the recessive Mendelian disease known as Carnitine Transporter Deficiency(CTD), with severe metabolic and cardiac consequences. In previous work, we functionally characterized 150 missense OCTN2 variants in a radiolabeled carnitine uptake assay, including both CTD-associated and non-CTD-associated variants. Here, we sought to investigate the association between variant function and subcellular localization using high-content imaging toinform therapeutic strategies for loss-of-function CTD variants.

Methods: Plasmids encoding OCTN2 variants with a C-terminal monomeric superfolder green fluorescent protein (msfGFP) were transiently transfected into HEK293T cells in 96 well plates.After 48 hours, the membrane was stained with Wheat Germ Agglutinin Alexa Fluor 647 Conjugate, cells were fixed with formaldehyde, and nuclei were stained with Hoechst 33342 dye. Plates were imaged with the InCell Analyzer 6500 confocal high-content imaging system, with nine images per well taken of each variant and results replicated on two independent days.

Results: Imaging revealed that 60 OCTN2 variants localized to the plasma membrane of the cell,comparable to the localization of the wild-type OCTN2 transporter. 37 variants exhibited intracellular retention with minimal or no signal on the membrane, whereas 51 variants displayed a mixed phenotype with partial membrane localization in combination with increasedintracellular signal compared to the wild-type OCTN2. Two variants were undetectable by imaging. In general, localization of OCTN2 variants was associated with degree of function: variants on the membrane had the highest median function (73.7% of wild-type OCTN2 function), variants with mixed subcellular localization had a median function of 53.0%, whereasvariants retained intracellularly had the lowest median function at 21.6%. A subset of variants exhibited complete loss-of-function despite proper membrane localization, suggesting additional mechanisms for loss-of-function.

Conclusions: High-content imaging of 150 GFP-tagged OCTN2 variants demonstrated that membrane localization is largely associated with transporter function. For the first time to our knowledge, we show that loss-of-function for many OCTN2 variants can be attributed to failureto traffic to the plasma membrane, revealing a disease-causing mechanism with the potential to be leveraged in therapeutic strategies for the treatment of CTD. Further studies are ongoingto determine the mechanisms for improper sorting of variants to the plasma membrane.

A30

Functional interpretation of human *G6PD* variants using multiplexed analyses in *S. cerevisiae*

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency affects over 400 million people. It leads to adverse reactions to drugs that produce oxidative stress, including antimalarials, antibiotics, and treatments for tumor lysis syndrome. Diagnosis by activity assay gives frequent false negatives, leading to an interest in improvinggenetic tests. We have compiled a catalog of 691 variants in the *G6PD* coding sequence identified in patientsfrom clinical genetics databases and individual publications. 479/691 (69%) are non-synonymous, yet only 172/479 (36%) of these variants have activity measurements, and only 62/479 (13%) from more than one study. A greater knowledge of the function of each variant could allow preemptive diagnosis of G6PD deficiency from gene sequencing, which could be used to assist in drug prescription and also inform female carriers to avoid triggers during pregnancy.

To address the need for systematic understanding of *G6PD* variant function, we are in the process of conducting deep mutational scans (DMS) to characterize the function of thousands of *G6PD* variants. We areperforming these scans in *S. cerevisiae*, baker's yeast, since we and others have shown that human *G6PD* functionally complements *ZWF1*, the yeast homolog. We established a system to measure the function of *G6PD* variants by their ability to rescue the growth of yeast lacking *ZWF1* in oxidative stress. Strains expressing *G6PD* variants with lower activity grew more slowly under stress; direct competition assays in continuous culture systems also showed that strains expressing high-activity variants out-competed strains with low-activity variants. We are using this system to conduct a DMS of *G6PD* variants: we transform yeast lacking *ZWF1* with a library of *G6PD* variants, compete them under oxidative stress, and determine variant frequency by next-generation sequencing.

Additionally, we are investigating the effects of multiple genetic backgrounds since *G6PD* variants have historically been studied on a Caucasian background even though that does not represent the populations most affected by G6PD deficiency. Published data on variant activity in patients, and our preliminary data, show that background haplotype can alter the effect of SNPs. The effects on variant activity are not always as would be predicted by additive effects of each individual SNP, indicating the need for our systematic characterization of the effect of SNPs on variant activity across diverse haplotypes. We plan to coordinate withclinical databases to make our findings available for use by clinicians to interpret *G6PD* variants, so that this research can be used to improve drug prescription and dosing for individuals with diverse *G6PD* variants.

A31

"Evaluating the role of pharmacogenetics in attention-deficit/hyperactivity disorder using human hepatocytes and enzyme inhibition in human liver microsomes"

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Summary

Most medications approved to treat attention-deficit/hyperactivity disorder (ADHD) were approved over 30 years ago. As a result, few studies have characterized the metabolism of these medications to current standards. The present study aimed to characterize the metabolism of racemic amphetamine, dextroamphetamine, racemic methylphenidate, dexmethylphenidate, atomoxetine, clonidine, and guanfacine using modern *in vitro* methods.

First, the rates of parent loss and metabolite formation were measured in cryopreserved primary human hepatocytes (cPHHs) and in human liver microsomes (HLMs). Test compounds were incubated for 48 hours in cPHHs and for 2 hours in HLMs. cPHHs allow for estimation of the fraction metabolized to various metabolic pathways in a system where all liver enzymes and cofactors are present. Second, the fraction metabolized by drug metabolizing enzymes was measured in HLMs using chemical inhibition, heat inactivation, and with the absence of mixed cofactors. For both test systems, positive and negative controls were included in duplicate. Experimental conditions were tested in triplicate.

The proportion of atomoxetine, racemic methylphenidate, dexmethylphenidate, and guanfacine converted to various metabolites largely matched *in vivo* reports. While many previously reported pathways were observed in cPPHs, there were some major pathways that were not observed. First, neither oxidative deamination nor beta-hydroxylation occurred in the racemic amphetamine and dextroamphetamine incubations, the former of which accounts for 20-40% of an amphetamine dose in the urine *in vivo.* This may suggest the breakdown of amphetamine is non-hepatic and related to urinary pH as previous reports have stated. Second, only about 10% of clonidine was lost over 48 hours and ≤20pmol 4-OH-clonidine was formed, possibly demonstrating the previously reported minimal role of nonrenal clearance. Longer incubations times or additional *in vivo* research are required to better understand the role of hepatic clearance and pharmacogenetics of these medications.

Well-characterized and robust gene-drug interactions (GDIs), such as atomoxetine with CYP2D6 and guanfacine with CYP3A4, were replicated in the present study. In addition, potentially novel GDIs were observed and previously reported GDIs were not replicated. First, for example, atomoxetine is extensively metabolized to 4-OH-atomoxetine by CYP2D6. Recent reports propose CYP2C19 forms N-desmethylatomoxetine and effects the clearance of atomoxetine. In the present study, CYP2C19 inhibition did not significantly reduce the formation of N-desmethylatomoxetine and its formation was minimal in cPHHS and HLMs. Together, this suggests that neither the pathway nor enzyme plays a meaningful role.

Second, after administration of methylphenidate, CYP2B6 and CYP2D6 inhibition primarily reduced the area ratio of oxo-methylphenidate and p-hydroxy-methylphenidate, suggesting a novel role for these enzymes. Given the technical limitations of non-enzymatic degradation of methylphenidate and a lack of reference standards, further research is needed to determine the clinical impact of these GDIs.

The present study represents the first modern characterization of the intrinsic clearance of many ADHD medications. Additionally, the current findings highlight the need for further research to fully understand the impact of GDIs and pharmacogenetics, particularly for medications that were approved prior to the development of more advanced *in vitro* techniques.

A32

Pharmacogenetic analysis, clinical response and adverse effects of thalidomide in pediatric patients with inflammatory bowel disease: a multicenter study

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Objectives and study: Thalidomide is an effective therapy in children with inflammatory bowel disease (IBD) refractory to standard treatment. However, thalidomide use is limitedby its safety profile. Very few pharmacogenetic studies have been conducted on IBD pediatric patients to investigate the determinants for predicting clinical response and the development of adverse effects (AEs) during thalidomide therapy. This study aimed to evaluate the correlation of candidate single nucleotide polymorphisms (SNPs) involved in thalidomide pharmacodynamics with the clinical response and the risk of AEs.

Methods: This retrospective cohort study involved six tertiary pediatric gastroenterology centers in Italy: patients with a diagnosis of pediatric IBD, treated with thalidomide, were enrolled from January 1998 to February 2019. Therapy efficacy and development of AEs during thalidomide treatment and the presence of genetic variants in 4 candidate genes: tumor necrosis factor alpha (*TNFα*; rs1800629, rs1799964, rs361525), vascular endothelial growth factor A (*VEGFA*; rs3025039, rs699947, rs2010963), interleukin-8 (*IL8*; rs1126647, rs4073) and interferon-gamma (*INFG*; rs2069707, rs2430561) were evaluated. Efficacy wasassessed after 12 weeks of therapy; clinical remission was defined as Pediatric Ulcerative Colitis Activity Index or Pediatric Crohn's disease Activity Index ≤10. DNA was extracted from peripheral blood and genotyping of variants was performed by KASP assays. Univariate associations, odds ratio (OR) and confidence intervals (CI) of variants with efficacy and AEs of thalidomide, were evaluated by logistic regression models.

Results: Seventy-seven patients were enrolled, 27 (35.1%) with ulcerative colitis, 49 (63.6%) with Crohn's disease and one patient (1.3%) with undifferentiated IBD. Median age at diagnosis of IBD was 13 (range 2-20) years. Fifty-eight patients (75.3%) achieved

clinical remission. AEs were reported in 56 patients (72.7%): electromyographic anomalies (46.7%), symptomatic neuropathy (32.4%) and dermatitis (20.7%) were the most frequent. Logistic regression analysis showed that the variant rs2010963 of the *VEGFA* gene was associated with the risk for developing dermatitis (OR 4.63, CI 1.28-16.68, p-value 0.02) and the variant rs4073 of the *IL8* gene was related to a lower probability of symptomatic neuropathy (OR 0.49, CI 0.25-0.98, p-value 0.04).

Conclusion: Our findings show an association between the variant rs2010963 in *VEGFA* gene and an increased risk of dermatitis, which could be related to the presence of higher levels of *VEGF* that may promote skin inflammation. The variant rs4073 of the *IL8* gene was found to be protective for the development of symptomatic neuropathy, probably because it reduces the production of *IL8* involved in several inflammatory diseases. None of the candidate variants were associated with thalidomide clinical efficacy or with isolated electromyographic anomalies.
If our results will be confirmed by further studies, the analysis of these polymorphisms could be used to predict the risk of developing neuropathy and other AEs during thalidomide therapy in pediatric IBD patients.

A33

Species-specific functional differences in the ABC transporter P-glycoprotein

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Summary: The ATP-binding cassette transporter P-glycoprotein (P-gp/ ABCB1/ MDR1) is an integral membrane glycoprotein involved in the efflux transport of a wide range of structurally diverse molecules, including endogenous and xenobiotic substances. Highly expressed on the surface of intestinal cells as well as other tissues including endothelial cells of the blood-brain barrier, P-gp serves as a mechanism to protect organisms from foreign and potentially toxic chemicals, including prescription drugs. Divergence in P-gp function may arise over evolutionary time since each species is exposed to different substances within their environment or diet. With the exception of the human and mouse orthologs of P-gp, little is known about the substrates and inhibitors of other species orthologs of P-gp.

Goal: The goal of this study was to characterize and compare the P-gp from Homo sapiens (human) and domestic animals including Ovis aries (sheep), Sus scrofa (pig), Canis lupus familiaris (dog), and Felis catus (cat). We hypothesized that the varied diets of these animals expose them to different environmental chemicals that may induce changes in P-gp function overtime, leading to species-dependent substrate-specificities.

Methods: HEK293 cells transfected with pcDNA3.1+C-eGFP vector (GenScript) containing the full-length human, ovine, porcine, canine, or feline ABCB1 cDNA, or with empty vector (EV), were established using Lipofectamine LTX (ThermoFisher) per manufacturer's instructions. The stable clones were selected with 1000μg/mL geneticin. P-gp Isotopic efflux studieswere performed in cells expressing each ortholog with common P-gp substrates including digoxin, fexofenadine, and quinidine.

Results: HEK293 cell lines stably expressing functional copies of domestic animal P-gps were established. Microscopy with GFP chimeras show that P-gp orthologs are expressed on the plasma membrane. Preliminary results show digoxin efflux was comparable among the species orthologs ofP-gp with over 2-fold less digoxin in cells compared to EV cells except for sheep P-gp; sheep P-gp appeared to transport digoxin out of cells to a lesser extent than other species, having a 1.7-fold decrease in digoxin levels. Further, human P-gp was more efficient in removing fexofenadine (1.4-fold decrease) and quinidine (1.9-fold decrease) from cells than the other four P-gp orthologs, averaging 1.2-fold and 1.4-fold decreases,respectively. **Conclusions:** Our results indicate that species differences in the kinetics of substrates for domestic animal orthologs of P-gp exist. These results suggest that evolution and environmental factors may affect P-gp function across species.

A34

Genetically faster *CYP2B6* activity is associated with smoking cessation success on bupropion: mediating effects of hydroxybupropion on long-lasting cessation

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Background and Aims: CYP2B6 is a highly genetically variable enzyme and converts bupropionto its active metabolite hydroxybupropion; hydroxybupropion concentration is associated with smoking cessation success. CYP2B6 activity and bupropion-aided cessation differ between women and men. The aim of this study was to determine whether genetically normal, relative to reduced, *CYP2B6* activity increases bupropion-aided cessation via higher hydroxybupropionconcentration, the duration of this mediating effect on cessation, and whether this differs betweenwomen and men.

Methods: African American light-smokers (≥10 cigarettes/day) (NCT00666978) with detectable bupropion and/or hydroxybupropion concentrations were dividedinto normal (n=64) and reduced (n=109) *CYP2B6* activity groups based on the presence of reduced function *CYP2B6*6* and *CYP2B6*18* alleles. Using mediation path analysis, we tested whether genetically normal (vs. reduced) *CYP2B6* activity increases cessation via higher hydroxybupropion. A sex-based analysis was also performed. **Results:** Normal (vs. reduced) *CYP2B6* activity was associated with increased cessation success at end-of-treatment (week 7), which was mediated by higher hydroxybupropion concentration (OR=1.25, 95% CI=1.03, 1.78). The mediating effect of hydroxybupropion was also

significant at week 26 (OR=1.23, 95% CI=1.02, 1.70), indicating long-lasting effects that persist many weeks beyond the active treatmentphase. The mediation effect was similar in women (n=116; OR=1.33, 95% CI=1.01, 2.30) and men(n=57; OR=1.33, 95% CI=0.92, 3.87). Further, a moderated mediation analysis found no effect ofsex.

Conclusions: Genetically normal *CYP2B6* led to higher hydroxybupropion concentrations which in turn increased cessation. This genotype effect occurred during bupropion treatment (week7) but importantly persisted through long term follow-up (week 26), and did not differ by sex. Ourfindings could be used to optimize cessation outcomes on bupropion. For example, genetically reduced *CYP2B6* metabolizers, who form less hydroxybupropion compared to normal metabolizers, may benefit from a higher bupropion dose or an alternative treatment. As bupropionis also an anti-depressant, our findings may have additional application in the treatment of major depressive disorder.

A35

Pilot Analysis of medication associations with CYP2C9, 2C19 and 2D6 haplotypes inpediatric surgery and burn patients

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Critical care patients, particularly surgery and burn patients, often receive dozens of medications during hospitalization. These patient populations may also have increased co- morbidities and higher risk of drug-drug interactions, which may result in decreased drug efficacy or increased risk of adverse drug reactions. Children requiring surgery are of concern as they may be even more sensitive to adverse drug reactions than adult patients. Precision medicine aims to better understand a person's genetic profile and adjust therapeutic dosing for an individual based on variants identified. Unfortunately, pharmacogenetic testing is not widely implemented and has faced many challenges integrating into the clinical workflow. The lack of implementation is multifactorial: 1. lack of knowledge on translating genetic information into clinical action, 2. interpretation of genotype testing results and 3. lack of uniform recommendations for selecting gene/drug pair implementation. To further advance implementation, clinical research to evaluate the frequency of clinically significant genetic variants and assess how often they influence the efficacy of prescribed medications is importantto improve outcomes. Research leading to a better understanding of functional impact of the plethora of genetic variants of unknown significance will be a key driver in improving the medicalcare of the future.

We have ongoing studies in pediatric burn and surgery patients to study opioid pharmacogenetics. A preliminary analysis in 30 patients was conducted to identified clinically significant haplotypes in several cytochrome P450 drug metabolizing enzymes and evaluate thepatients' medication lists to identify potential drugs affected. Whole-exome and whole-genome sequences were analyzed with the Aldy allelic decomposition framework to identify CYP haplotypes. Seventeen patients were identified with predicted altered function in one or more ofthe following genes: *CYP2C9, CYP2C19* and *CYP2D6*. The majority had decreased function, except for some patients with *CYP2C19* variants resulting in rapid and ultrarapid haplotypes.

Several patients have haplotypes with unknown functional significance in *CYP2D6*. Examples of medications patients received during their hospitalization that rely on these pathways for their primary metabolism include, but are not limited to, celebrex, diphenhydramine, diazepam, famotidine, glycopyrrolate, ibuprofen, ketorolac, methadone, and oxycodone. Many other medications utilize these pathways as minor routes of metabolism. This research demonstrates the high frequency of CYP haplotypes in patient populations and the need for future clinical research to further evaluate the clinical relevance of these haplotypes and the additional impact of drug-drug interactions and other clinical confounders that may be inducing or inhibiting these pathways.

A36

Aripiprazole exposure, cytochrome P450 2D6, Autism Spectrum Disorder, andadverse events in children and adolescents

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Background: The Food and Drug Administration (FDA) aripiprazole label includes pharmacogenomic guidance based on cytochrome P450 2D6 (CYP2D6) metabolizer status. However, there are limited data on the impact of CYP2D6 genetic variation regarding adverse events (AEs) in children. Our objective was to test the hypothesis that children with decreased or no CYP2D6 activity experience more AEs in children and adolescents. Further, children with autism spectrum disorder (ASD) are often treated with aripiprazole and have been noted to have more AEs than children without ASD, but whether this is due to ASD itself or other risk factors is unknown. Thus, we also examined the associations of ASD to aripiprazole AEs.

Methods: This retrospective cohort study used BioVU, a de-identified biobank linked to electronic health record data. The cohort included children ≤18 years of age with ≥1 day of aripiprazole exposure. The primary outcome was AEs, defined as any untoward event or laboratory abnormality attributed to aripiprazole resulting in a clinical action (e.g. decreasing the aripiprazole dose) and identified by manual review. DNA from individuals was tested for 10 *CYP2D6* allelic variants and copy number status, activity scores (AS) were assigned to each genotype, and CYP2D6 metabolizer status was determined based on the current CPIC recommendation definitions via manual review. Associations among AEs, metabolizer status, and clinical variables were determined using Cox regression analysis comparing poor and intermediate metabolizers (PM/IMs) to normal and ultrarapid metabolizers (NM/UMs) and those with ASD to those without an ASD diagnosis.

Results: The cohort included 206 individuals, with a median age of 14.0 (IQR 9.8-16.2) years. 105 (50%) were male, 52 (25%) had ASD, and 62 (30%) experienced an AE. The most commonAEs were sedation, abnormal movements, and weight gain. There was no difference in time to adverse event between PM/IMs and NM/UMs (hazard ratio (HR) 0.86, 95% confidence interval (CI) 0.6-1.6) in univariate Cox regression analysis. When adjusting for age, gender, race, starting dose (in mg/kg), and use of concomitant CYP2D6 inhibitors, there was no association ofbetween CYP2D6 phenotype and AEs (HR 1.0, 95% CI 0.6-1.8) and no other variables were predictors of AEs. In univariate analysis, children with ASD experienced an earlier time to adverse event (HR 1.7, 95% CI 1.1-2.9), but there was no association of ASD status to AEs (HR1.7, 95% CI 0.9-3.0) when adjusting for age, gender, race, starting dose and concomitant CYP2D6 inhibitor use; no other variables were significant predictors of AE.

Conclusion: In this pediatric cohort, CYP2D6 metabolizer status is not associated with aripiprazole AEs. ASD diagnosis was associated with AEs in univariate analysis, but not in multivariate analysis, indicating that other factors (e.g. age, sex, dose) rather than ASD itself underlie the increased risk for AEs in this population of patients. Further study is needed to identify predictors of pediatric aripiprazole response.

A37

Prioritisation of Clinically Actionable Gene-Drug Pairs for Cost-effectiveness Analyses in Singapore

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Purpose: Pharmacogenetic (PGx) test involves identification of presence or absence of gene variant(s) toguide individualised therapy. An analysis of the UK Biobank reported that 99.5% of participants have at least one actionable pharmacogenetic variant, with an average of 3.7 actionable pharmacogenetic variants involving an average of 12.2 drugs. As such, PGx panel testing, which allows analysis of multiple genes inone assay, can be potentially more cost-effective than conducting PGx tests individually. Moreover, ancestry differences in the prevalence of PGx allele frequencies mean that non-European health systems should curate a panel tailored to local needs. This study details the process of incorporating both clinical and economic considerations to prioritise gene-drug pairs for cost-effectiveness analyses (CEA). This is done as an initial step in curating a pre-emptive PGx panel test for adults in Singapore, a multi-ethnic Asiancity state.

Methods: Clinically important gene-drug pairs were initially determined through literature review and were confirmed by the Clinical Pharmacogenomics Implementation Working Group (CPIWG) at the National University Hospital, comprising an oncologist,

endocrinologist, endocrine surgeon, pharmacists and pathologist. These gene-drug pairs were then assessed against a selection framework which involves both clinical and economic criteria. Clinical criteria were (1) high evidence for pharmacogenetic testing, (e.g., consistent recommendations across PGx guidelines) (2) high frequency of gene variant; (3) clinical utility (i.e., will result in a change of patient management), while economic criteria were (4) frequency of drug usage; (5) availability of local and international data to conduct CEA (e.g., odds ratio of gene-drug association in specific ancestry group and local cost of managing adverse drug reactions).

Results: The CPIWG shortlisted 25 clinically actionable gene-drug pairs based on literature review and local clinical practice. Of those, ten gene-drug pairs (in no particular order) were prioritised for cost- effectiveness evaluation: (1) HLA-B*58:01–allopurinol, (2) TPMT/NUDT15–azathioprine, (3&4) DPYD– capecitabine/ fluorouracil, (5) HLA-B*15:02–carbamazepine, (6) CYP2C19–clopidogrel, (7&8) CYP2D6– codeine/ tramadol, (9) SLCO1B1–simvastatin; (10) CYP2C9/VKORC1–warfarin. To elaborate on reasons in de-prioritising other gene-drug pairs: NUDT15/TPMT–mercaptopurine since it is used in paediatric conditions; UGT1A1–irinotecan dosing recommendations require further discussion and planning; CYP2D6 antidepressants because it is difficult to attribute therapeutic failure as a consequence of carrying the variant allele, since factors such as non-adherence to treatment or drug-drug interactions may influence therapeutic outcomes. Lastly, HLA-B*57:01–abacavir, DPYD–tegafur, NUDT15/TPMT–thioguanine and CYP2C19–voriconazole owing to a low volume of prescriptions.

Conclusion: The selection framework highlights issues faced in curating PGx panels for implementation. This challenge can be addressed through a systematic approach in prioritising gene-drug pairs for CEA, the results of which will be used to determine possible inclusion in a pre-emptive PGx panel test.

A38

Medicare coverage of pharmacogenomic testing: An overview of the current landscape.

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Summary:

Background Genetic variability can influence the effectiveness and safety of drug therapy.[1] Variable insurance coverage of genetic testing and discrepancies among clinical guidelines create confusion for providers and patients in regard to access and clinical actionability of testing.[2] Patients often bear the financial burden of pharmacogenomic (PGx) testing when coverage is denied. Medicare is a major insurer in the United States, insuring 18.4% of the Americans.[3] This population is more likely to experience polypharmacy, including medications that have established gene-drug interactions for which PGx testing would allow for safer and/or more effective prescribing. We compared existing PGx test coverage policies across the 12 Part A/B Medicare Administrative Contractor (MAC) jurisdictions to identify national trends.

Methods The Medicare Coverage Database on the Centers for Medicare and Medicaid Services (CMS) website was used to identify policies pertaining to PGx testing for the twelve MACs. Two reviewers compared the identified policies for common themes including covered indications, exclusions, provider qualifications, coverage of panels and combinatorial tests, technical requirements, documentation requirements, and coverage of additional and repeat testing.

Results Five approved and two proposed local coverage determinations (LCDs) were identified with 2 LCDs covering more than one jurisdiction. Ten of the twelve jurisdictions had approved (n = 7 jurisdictions) or proposed (n = 3 jurisdictions) policies for coverage of PGx testing, insuring 50.29% and 31.18% of Medicare Fee-For-Service (FFS) beneficiaries as of 09/30/2020, respectively. The first of these LCDs (n = 2) were initially approved in July 2020 and represent 7 states in the Southeastern United States. The remaining approved LCDs (n = 3) were approved shortly thereafter in August 2020 and represent 6 states in the Midwest. Once approved, the 2 proposed LCDs will extend coverage of PGx testing to an additional 12 states. This will leave 2 jurisdictions--representing 10 states--without policies addressing PGx testing. When comparing the policies, the 5 approved LCDs were identical to one another and the 2 proposed LCDs were also identical to one another. The proposed LCDs differed in language from the approved LCDs,

however, the overall intent regarding covered indications, exclusions, repeat testing, and documentation requirements was consistent. Differences between the approved vs. proposed LCDs included provider qualifications and coverage of panel-based and combinatorial testing.

Conclusion Eighty-three percent of MACs have a proposed or approved LCD in place for coverage of PGx testing. While the intent of all LCDs is largely consistent, proposed LCDs differ from the approved LCDs in provider qualifications, coverage of combinatorial and panel tests, additional testing, and technical requirements. This is a promising step towards a broader accessibility of PGx testing for a key patient population.

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A39

Pharmacogenetic interventions to improve outcomes in patients with multimorbidity or prescribed polypharmacy: a systematic review.

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Background:

The delivery of healthcare is primarily built around singular diseases, yetageing populations and increased prevalence of chronic diseases globallymeans greater combined burden of multimorbidity and polypharmacy.Traditional interventions are not widely effective; a more holistic and integrated approach to healthcare delivery is required for these patients. Pharmacogenetic or pharmacogenomic analysis has potential as a component of medicines optimisation. However, the adoption of pharmacogenetics in this respect may require more evidence.

Objective:

To investigate the effect of pharmacogenetic interventions on outcomesin adults with multimorbidity or prescribed polypharmacy in all healthcare settings.

Methods:

PubMed, Embase, CENTRAL, CINAHL, AMED, PsycInfo and several clinicaltrials registers were searched for studies involving multi-medicine pharmacogenetics in adults with multimorbidity or polypharmacy. Studies were included without restrictions on methodological design ifthey reported on at least two outcomes derived from consensus-based core outcome sets for multimorbidity and polypharmacy. Risk of bias assessment was performed per Cochrane guidelines. Narrative synthesiswas undertaken to summarise the data; meta-analysis was inappropriate due to the heterogeneity of included studies.

Results:

The search yielded 10,725 citations, of which fifteen studies of diverse design and variable quality met the inclusion criteria. Six non- comparative studies, three observational studies, three randomised controlled studies, and three ongoing studies in

primary care, mostly involving pharmacist-led medication management, were included. The studies reported effects on health service utilisation, estimated improvements in healthcare costs, enhanced drug interaction identification, and reinforced clinical decision-making. One small randomised study demonstrated encouraging impacts of pharmacogenetics on hospitalisation rates using a multi-gene, multi- drug, multi-disease, pharmacist-led medicines optimisation intervention.

Conclusion:
The incorporation of pharmacogenetic screening into medication optimisation for adults with multimorbidity and polypharmacy could have significant benefits for patients and health systems. However, dueto study design heterogeneity and the quality of the included studies, itis difficult to draw generalisable conclusions. Further pragmatic, robuststudies looking at pharmacogenetics in diverse, real-world patient populations, are required to establish the benefit of multi-medicine pharmacogenetic screening on patient outcomes.

A40

Clinical pharmacogenomic test reporting preferences among adult and pediatric providers

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Abstract:

The increasing availability of practice guidelines has resulted in growing interest in implementing clinical pharmacogenomic testing; however, there is ongoing regulatory debate whether clinical pharmacogenomic test reports should include therapeutic recommendations. Previous studies have concluded that most providers do not feel equipped to independently interpret pharmacogenomic results. Therefore, we aimed to assess provider preferences on clinical pharmacogenomic report content. Adult (36%) and pediatric (60%) providers participated in an anonymous online national survey, including physicians (54%), pharmacists (22%), nurse practitioners (12%), and other providers (22%), which were diverse in age and gender, largely white (75%) or Asian (16%), and concentrated in urban and suburban medical centers in the Eastern U.S (n=105). Half of all respondents (54%) identified as being somewhat familiar with pharmacogenomics; however, only 36.5% had previously heard of the Clinical Pharmacogenetics Implementation Consortium (CPIC). Importantly, the majority (66%) of providers agreed that clinical pharmacogenomic reports should include identified genotypes, metabolizer phenotypes, potentially impacted medications and therapeutic recommendations, compared to only 7.7% who disagreed.

Moreover, most providers agreed that all potentially impacted medications should be reported, including those with FDA label recommendations and from other sources (e.g., CPIC). Similarly, 72% of providers indicated that the therapeutic recommendations should include contraindicated medications, dosing information, and patient monitoring recommendations when

applicable. Interestingly, physicians preferred more therapeutic recommendations and dosage guidance information than pharmacists ($p=0.0007$), suggesting that pharmacists may feel more confident than clinicians to translate pharmacogenomic results into therapeutic recommendations. Regarding panel testing, the majority (72%) of providers indicated they were not worried about including panel results in patient medical records.

However, only ~30% of providers indicated they were not worried about their clinical responsibility for panel results beyond the original indication for the test or for panel results ordered by a different provider. Taken together, these data indicate that the majority of clinical providers prefer to have explicit therapeutic recommendations included in clinical pharmacogenomic test reports; however, additional resources are necessary to support the delivery of pharmacogenomic panel results to providers through electronic medical records.

A41

Clinical importance of *CYP2D6* duplicated allele testing for accurate phenotype prediction

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Keywords: pharmacogenomics, genotyping, *CYP2D6,* duplication

Background: Cytochrome P450 2D6 (*CYPD26*) is involved in the metabolism of approximately 25% of all prescription drugs. Approximately 13% of people in the United States have a *CYP2D6* copy number variation (CNV) such as a deletion or duplication that can alter *CYP2D6* enzyme activity. For individualswho have a duplicated *CYP2D6* gene, further evaluation of the duplicated allele may be necessary for accurate phenotype prediction. Yet, this testing is often not routinely performed in clinical laboratories leading to inconclusive or potentially inaccurate results. Here, we evaluated a large cohort of patients from across the United States who had been clinically tested for *CYP2D6* to determine how many individuals would benefit from further *CYP2D6* duplicated allele testing.

Methods: Genotype results from individuals who had undergone clinical *CYP2D6* genetic testing at ARUP Laboratories were evaluated. Genomic DNA from whole blood or saliva was extracted, and genetic testing was performed using a TaqMan Real-Time OpenArray® targeted genotyping panel on theQuantStudio™ 12K Flex instrument (ThermoFisher). Data was analyzed using the TaqMan Genotyper software. *CYP2D6* copy number was also assessed for exon 9 and intron 6 on the same QuantStudio™ instrument and data was analyzed using the CopyCaller software (ThermoFisher). Patients were predictedfor a metabolizer status based on the activity scores of the detected alleles and CNV calls if any.

Results: A duplicated gene was identified in 141 cases. Sixty-two patients were inconclusive for the metabolizer phenotype and thus would benefit from Sanger confirmation on the duplicated allele to accurately calculate the activity score. Of the 62 patients with a duplicated gene, 32% contained functional and nonfunctional alleles, 19% had functional and decreased function alleles, and 2% had a
**10* allele paired with a decreased function allele. For 79 patients, determining the duplicated allele wouldnot change the activity score or phenotype prediction. It still leaves 44% of ARUP patients with a duplicated *CYP2D6* allele that would benefit from *CYP2D6* duplicated allele testing.

Conclusion: *CYP2D6* duplicated allele characterization should be included as part of routine clinical testing for accurate phenotyping. A suggested testing cascade that includes the use of long-range PCRfollowed by bi-directional Sanger sequencing will be presented.

A42

Pediatric CYP2D6 Metabolizer Status and Post-Tonsillectomy Nausea and Vomiting After Ondansetron Administration

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Introduction: Ondansetron, metabolized by CYP2D6, is an anti-nausea and anti-emetic medication commonly used in the pediatric population to prevent post-operative nausea and vomiting (PONV). In adults, decreased ondansetron efficacy is observed in individuals with higher CYP2D6 activity, prompting designation of the gene/drug pair as CPIC Level A and a recommendation to use an alternative therapy in CYP2D6 ultrarapid metabolizers (UM). Despite ondansetron being the CPIC Level A drug most commonly prescribed to children, pediatric data for the pharmacogenetic association are lacking. Our goal was to determine if CYP2D6 metabolizer status within the ondansetron-treated pediatric tonsillectomy population was associated with risk of PONV in the post-anesthesia care unit (PACU).

Methods: We conducted a retrospective cohort study of pediatric patients who underwent tonsillectomy and received ondansetron on the day of the procedure. Data were obtained from BioVU, an institutional biobank that links DNA to deidentified electronic health record (EHR) data. Inclusion criteria were tonsillectomy (± adenoidectomy) at age <18 years and ondansetron administration pre- or intra-operatively. Exclusion criteria were prior gastrectomy, gastrostomy, fundoplication, cyclic vomiting syndrome, concurrent endoscopic bronchoscopic procedure, missing outcome data, or failed genotyping. PONV was defined as receiving a dose of antiemetic medication while in the PACU. Subjects were tested for 10 *CYP2D6* allelic variants and copy number status, and genotype data were translated into phenotypes as recommended by CPIC via manual review. Fisher's exact and chi square were used as appropriate for categorical variable analysis. Wilcoxon rank-sum test and multivariate logistic regression were also used for statistical analysis.

Results: The final cohort included 640 individuals (median age 6.6 years, 305 (47.6%) female), 104 (16.3%) of whom had PONV. Rates of PONV were similar across groups: UM and possible UM, 1/14 (7.1%); normal metabolizers 64/353 (18.1%); intermediate metabolizers 33/234 (14.1%); poor metabolizers 6/39 (15.4%); indeterminate 3/15 (40%). In multivariable analysis adjusting for age, sex, and time under anesthesia, CYP2D6 phenotype was not associated with PONV with an odds ratio of 1.37 (95%CI 0.9, 2.1) when comparing PM/IM vs NM/UM.

Conclusion: This study demonstrates that in this pediatric population, there were no significant differences in PONV based on CYP2D6 phenotype. The upper limit of the confidence interval indicates that if an association is present for this pediatric population, it has a smaller effect size than what has been observed in adults. Potential reasons for this observation include pediatric weight-based dosing versus fixed dosing in adults, different enzymatic contributions by CYP3A and/or CYP1A,

greater influence of clinical risk factors for PONV, other medication interactions, or limitations of the retrospective dataset. Further investigation is needed to determine mechanisms for ondansetron inefficacy in children and to determine the clinical impact of *CYP2D6* variation on pediatric ondansetron response.

A43

Characterisation of *CYP2D6* Allelic Variation in African Populations: AnIntegrative Bioinformatics Approach

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Background and goals: Cytochrome P450 2D6 (*CYP2D6*), which is responsible for the metabolism of ~25% of commonly prescribed medications, is known to harbour numerous allelic variations. These genetic variations could alter individual responsesto medicines metabolised by *CYP2D6*. However, only a small fraction of African ethnolinguistic groups has been genetically characterised for *CYP2D6* pharmacogenomic variation to date. This has been in part due to lack of large-scale genomic datasets and also due to genotyping challenges due to the complex architecture in the *CYP2D6* genomic locus. We assessed the *CYP2D6* allelic diversity,mainly across sub-Saharan Africa (SSA), based on 962 high coverage African genomes, including samples from the Human Heredity and Health in Africa (H3Africa) Consortium as well as the 1000 Genomes Project.

Methods: Consensus *CYP2D6* star alleles were inferred from the whole genome sequence (WGS) data after separateallele calling runs using Astrolabe, Aldy, Stargazer, Cyrius, and

StellarPGx (developedby the authors). Predefined activity scores were used for phenotype prediction basedon the diplotype information.

Results: (1) We inferred over 20 potential novel *CYP2D6* haplotypes – most of which were rare and African-specific. **(2)** Our results highlight the frequency distributions of key decreased function alleles across Africa e.g. *CYP2D6*17*, **29* and **10*, as well as no-function alleles including CYP2D6*4, *40and *56. **(3)** Using high coverage WGS data facilitated calling structural variant allelessuch as *CYP2D6*5* (gene deletion), **1xN*, **2xN*, **4xN*, **17x2*, **29x2* and hybrids e.g.**68+*4*. **(4)** Phenotype prediction analysis showed that the distribution of *CYP2D6* metaboliser phenotypes is non-uniform across SSA.

Conclusion: This research highlights the diverse landscape of *CYP2D6* star alleles and predicted phenotypes, mainly in SSA populations, which could inform future precision medicine strategies. Future directions include comprehensive validation of the novel CYP2D6 alleles inferred in the study and extending allele calling efforts to other key pharmacogenes.

B01

Pharmacogenetic study to elucidate putative dopaminergic mechanisms of antidepressant action

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Background:

The gap in understanding the etiology of major depressive disorder inhibits the optimization of treatment in patients. Dopaminergic neurotransmission is known to impact the therapeutic effectsof antidepressants in unipolar and bipolar depression. Investigating polymorphisms within the dopamine receptors would facilitate further understanding for the treatment in depressed patients.

Our study looked at the depression patients who were diagnosed with a depressive episode, according to the criteria of ICD-10 (F32/F33). Patients who participated in the study participated for a period of four weeks.

Methods

163 patients of the Tomsk cohort who were at least moderately ill patients with major depressive disorders were used to establish treatment responses using the Hamilton Depression Rating Scale (HAMD-17). More than half of the patients were not treated with antidepressants previously.

Patients were genotyped for 14 SNPs in *DRD1*, *DRD2*, *DRD3*, *DRD4*, *MOAB* and *SLC6A3* to determine the impact of polymorphisms in dopamine neurotransmission related proteins. Clinical status was measured before initiation, after two weeks and after four weeks of antidepressant treatment. The difference in HAMD-17 scores between the measurement periods were assessed and were definedas the outcome measure.

Multiple linear regression was conducted to determine the association between the genotypes and difference in HAMD-17 across the study period. Covariates of age, sex, antidepressant medication and depression diagnoses were included in the regression.

Over the course of four weeks, HAMD-17 scores were measured at initiation (24.1 ± 4.9), at two weeks (12.9 ± 5.0) and at four weeks (5.1 ± 3.9) for each patient. The difference in HAMD-17 scoreswas found to be 11.2 ± 4.4 between initiation and two weeks, 7.8 ± 5.3 between two week and four week, and 19.0 ± 5.3 throughout the entire study.

DRD4 rs11246226 CA heterozygous patients were found to respond less compared to homozygous C patients during 0 – 2 weeks and 0 – 4 weeks. Patients with *MAOB* rs1799836 heterozygous GA and homozygous A improved better during 2 – 4 weeks and 0 – 4 weeks. No further associations were found within the genotypes and differences in HAMD-17 across the study.

Conclusions

The findings shed some light on possible polymorphisms which may impact the dopaminergic mechanisms of antidepressant action. However, the results are preliminary due to the limited population size and the small number of variants investigated. Moving forward, further research into the involvement of habenular dopamine D4 receptors in the antidepressant response, especially to clomipramine, would build on our current findings.

B02

SLC6A4 Genetic Variation and Antidepressant Tolerability and Efficacy: A Systematic Review and Meta-Analysis

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Abstract:
Antidepressants are used to treat several different psychiatric disorders, however a large proportion of patients do not respond to antidepressant therapy and often experience at least one side effect. A common insertion-deletion polymorphism (5-HTTLPR) in the serotonin transporter (SLC6A4) gene has been frequently investigated, but its association with antidepressant efficacy has primarily been studied in patients with major depressive disorder (MDD). Here, we performed a systematic review and meta-analysis to assess the association between the 5-HTTLPR polymorphism and 1) efficacy in psychiatric disorders other than MDD and 2) adverse drug reaction (ADR) rates across all psychiatric disorders. Literature searches were performed up to December 2019 in PubMed and EMBASE, yielding 82 studies that met inclusion criteria and 12 of these studies were included in the meta-analyses. Efficacy analyses showed that Caucasian carriers of the 5-HTTLPR LL or LS genotype were more likely to be responders compared to the SS carriers (LL/LS vs. SS: OR 1.898, 95%CI 1.108-3.252, p=0.020). No associations were detected between 5-HTTLPR genotypes and ADR rates. In alignment with previous meta-analysis focussed on MDD populations, the 5-HTTLPR polymorphism may serve as a marker for the prediction of antidepressant efficacy in Caucasians with non-MDD psychiatric disorders.

B03

ABCB1 gene variants and antidepressant response in patients with depression: A systematic review and meta-analysis

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Introduction:

The p-glycoprotein efflux pump, located at the blood brain barrier (BBB) and encoded by the *ABCB1* gene, has been shown to alter plasma concentrations of various antidepressants in the brain. Previous work has suggested a role of *ABCB1* single nucleotide polymorphisms (SNPs) in modulating antidepressant treatment response. In particular, three functional SNPs (rs1045642, rs2032582 and rs1128503) were most often implicated in treatment response. While some commercial tests have begun to include ABCB1 SNPs in their testing panels, the clinical utility remains unclear. Therefore, we conducted a systematic review investigating the association between selected *ABCB1* SNPs and antidepressant treatment response in patients with major, recurrent, or chronic depression. In addition, a meta-analysis is being conducted with new datasets which will be presented.

Methods:

A systematic literature search of published articles in English using PRISMA guidelines was conducted in PubMed® between the time period of January 2000 to June 2021. Published studies were included if they: 1) were clinical trials or cohorts; 2) investigated the association of at least one *ABCB1* gene variant and antidepressant response or remission; 3) recruited outpatients or inpatients diagnosed with major depression; 4) included patients 18-65 years of age; 5) included patients treated with at least one antidepressant medication. Our meta-analysis aims to include six *ABCB1* SNPs (rs1045642, rs2032582, rs1128503, rs2032583, rs2235015, rs2235040) and their impact on

antidepressant treatment, however analyses are still ongoing.

Results:

We have identified twenty-seven studies (N = 5878 patients) for inclusion in this systematic review. The mean age of subjects in the review is 42.6 (5.8) years. Majority of studies (66.7%) included patients of European ancestry. Twenty-six studies included at least one of these

six *ABCB1* SNPs: rs2032583, rs2235015, rs2235040, rs1045642, rs2032582, rs1128503, in their analyses. The *ABCB1* rs2032582 (G2677T/A) and rs1045642 (C3435T) were the most investigated SNPs. For the *ABCB1* rs2032582, studies reported a significant association between the different genotypes and antidepressant treatment outcome, with an increased chance of treatment response or remission to paroxetine, citalopram, or fluoxetine in patients carrying at least one T-allele. For the *ABCB1* rs1045642, 19 studies examined the association between the different genotypes and antidepressant treatment outcome, with majority of included studies (79%) reporting non-significant results.

Conclusions:

Preliminary results from this systematic review suggests that the *ABCB1* rs2032582 might predict antidepressant response in a subgroup of patients taking selective serotonin reuptake inhibitors (SSRIs) that are known to be p-glycoprotein substrates. A more definite role of this SNP in antidepressant treatment outcome will be evaluated through our ongoing meta-analysis.

B04

CYP2C19 Phenotype Influences Escitalopram and Sertraline Pharmacokinetics in Pediatric Patients

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Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed for the treatment of depressive and anxiety disorders in pediatric patients. The highly polymorphic cytochrome P450 enzyme, CYP2C19, metabolizes SSRIs (e.g. escitalopram and sertraline) and influences SSRI plasma concentrations. The effect of CYP2C19 on SSRI pharmacokinetics has been examined in adults, but few studies have evaluated this in youths. Importantly, understanding variation in SSRI pharmacokinetics could enhance our ability to predict tolerability and efficacy for these medications. Therefore, our objective was to evaluate the impact of CYP2C19 phenotypes on sertraline and escitalopram pharmacokinetics in children and adolescents.

A total of 209 remnant blood samples from hospitalized children and adolescents (n=200) aged 6 to 20 years prescribed either sertraline or escitalopram were collected. Plasma concentrations for sertraline and desmethylsertraline (*n*=107, mean age: 14.5 (range: 6-17), 73% female) and escitalopram and desmethylescitalopram (*n*=104, mean age: 15.0 (range: 10-20), 69% female) were determined via LC/MS/MS. Two patients overlapped between both cohorts because they received both medications during their hospitalization. Genomic DNA was isolated from buccal swabs or whole blood samples and patients were genotyped for seven alleles with no function (*2, *3, *4, *5, *6, *7, *8) and the increased function allele (*17). A *1 genotype was inferred from the absence of the previous alleles. Using Clinical Pharmacogenetic Implementation Consortium guidelines, CYP2C19 phenotypes were determined and dose-specific pharmacokinetic models were developed using MwPharm++ (Mediware, Czech Republic) to extrapolate exposure (area under the curve [AUC]) and the maximum concentration (Cmax) for each phenotype.

Escitalopram samples consisted of poor (n=4), intermediate (n=32), normal (n=38), rapid (n=24), and ultrarapid (n=6) metabolizers, whereas sertraline samples consisted of intermediate (n=33), normal (n=39), rapid (n=31), and ultrarapid (n=4) metabolizers. Concentration-to-dose ratios were significantly decreased in patients with faster CYP2C19 metabolism relative to those with slower metabolism for both escitalopram (ANOVA test for linear trend, $p<0.001$) and sertraline (ANOVA test for linear trend, $p=0.002$). Faster CYP2C19 metabolizers also had significantly lower ratios of escitalopram to its primary metabolite desmethylescitalopram (ANOVA test for trend, $p<0.001$), but ratios of sertraline to its primary metabolite desmethylsertraline were not significantly associated with metabolizer status (ANOVA test for trend, $p=0.17$). Individual

patient-specific models revealed associations between CYP2C19 phenotype and the AUC and Cmax for both sertraline and escitalopram.

In children and adolescents, CYP2C19 metabolizer status affects escitalopram and sertraline pharmacokinetics across the dosing range. Poor metabolizers treated with sertraline will be available for future analysis as sample collection is ongoing. Future studies are needed to clarify the relationship between pharmacokinetics and response and tolerability in children and adolescents.

B05

Examining cardiovascular outcomes with the ABCD-GENE Score amongpatients receiving *CYP2C19*-guided antiplatelet therapy after PCI

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Background:

The ABCD-GENE (Age, Body mass index, Chronic kidney disease, Diabetes and *CYP2C19* Genotype) Score was developed to predict clopidogrel response after percutaneous coronary intervention (PCI). We compared major adverse cardiovascular events (MACE) between clopidogrel and alternative therapy (i.e., prasugrel or ticagrelor) within each ABCD-GENE Score strata among real-world patients who received *CYP2C19*-guided dual antiplatelet therapy (DAPT) after PCI.

Methods:

Routine *CYP2C19* testing to assist with post-PCI DAPT prescribing was conducted at four institutions. MACE (death, myocardial infarction, stroke, stent thrombosis, or hospitalization for unstable angina) within 12 months post-PCI was ascertained through electronic health record review for patients who received DAPT with aspirin plus clopidogrel, prasugrel, or ticagrelor. Multivariable Cox regression was used to assess MACE risk according to ABCD-GENE Score, using the established cutoff of 10 (score ≥10 indicative of clopidogrel non-response).

Results:

A total of 2497 patients (mean age 63 years, 33% female, 19% Black) were included. Among patients with an ABCD-GENE Score ≥10 (n=687), the risk of MACE was higher in clopidogrel-treated patients versus patients treated with alternative therapy (adjusted hazard ratio [HR], 2.02; 95% CI, 1.24-3.31; p=0.005). The risk of MACE was similar with clopidogrel and alternative therapy among those with an ABCD-GENE Score <10 (n=1810; adjusted HR, 1.20; 95% CI, 0.80-1.82; p=0.375).

Conclusion:

Prasugrel or ticagrelor should be preferred over clopidogrel among patients with an ABCD- GENE Score ≥10, while clopidogrel represents a reasonable treatment option among those withan ABCD-GENE Score <10.

B06

PON1 as a biomarker in Caribbean Hispanics resistant to clopidogrel: A multiomic-based approach.

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Background: Clopidogrel is an antiplatelet drug used to prevent heart attack in cardiovascular patients. Since admixed Caribbean Hispanics are among those often underrepresented, we want to identify predictive biomarkers of poor response to clopidogrel within this population. Preliminary, we found that a genetic polymorphism at the gene encoding for paraoxonase-1 (PON1) is a good predictor of clopidogrel responsiveness among Caribbean Hispanics. Moreover, data using Tandem Mass Tag (TMT) coupled with mass spectrometry revealed that PON1 is downregulated in patients treated with clopidogrel (-49.50) when compared to other cardiovascular patients. Our aims were to ascertain *PON1* haplotypes, validate the low abundance of PON1 by western blot analysis and determine PON1 enzymatic activity in Caribbean Hispanic patients on clopidogrel. **Methods**: Blood samples were collected from consented participants and platelet reactivity tests were performed to identify poor responders (PRU≥230) using VerifyNow® P2Y12 assays. Individual genotyping of *PON1* rs662 and rs854560 variants were run using StepOne TaqMan SNP assays. Haplotype phasing was performed using SHAPEIT. For validation, Western Blot analyses were done and PON1 protein was quantified in plasma of healthy (n=6), cardiovascular (n=6), normal (n=14) and poor responders (n=10). PON1 activity assay kit was used to determine enzymatic activity in all experimental groups (n=60). Forstatistical analysis, Shapiro-Wilks normality test and one-way ANOVA test were performed. **Results**: Minor allele frequencies at these two SNPs varies among groups, with poor responders showing the largest *PON1* rs662 prevalence (0.56; 95%CI: 0.38-0.74) but the lowest for rs854560 (0.31; 0.16-0.50); whereas, the opposite was found in the cardiovascular controls (i.e., 0.41; 0.21-0.64 and 0.55; 0.32-0.76). The most commonly found haplotypes at these two loci differed significantly between normal (GT/AT) and poor responders (AA/GT). The total PON1 intensity signal had no statistical difference among the groups ($p>0.05$). However, there were significant

differences in PON1 function when healthy (22.21 µU/ml) or cardiovascular controls (22.66 µU/ml) were compared to poor responders (10.57 µU/ml, $p<0.05$) The lower PON1 enzymatic activity could indicate that it is not only a predictor of poor response to clopidogrel but also a possible risk biomarker of disease severity within Caribbean Hispanics. **Conclusions**: Caribbean Hispanic is a minority population, identifying biomarkers of the severity of cardiovascular disease and resistance to clopidogrel will help on reducing healthcare gaps.

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B07

Association between CYP2C19 polymorphism and proton pump inhibitors effectiveness and adverse effects in real-life: retrospective cohort study

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Abstract

Objective: Only 58% of proton-pump inhibitors (PPIs) recipients report being satisfied with their treatment. PPIs are metabolized through the hepatic cytochrome system, with CYP2C19 having the dominant role. Rapid CYP2C19 metabolizers might demonstrate lower efficacy of PPIs and contribute to PPI failure. In contrast, poor metabolizers may show increased efficacy due to increased exposure to PPI in serum. Most CYP2C19 studies evaluating PPIs were conducted in Asian populations, where rapid and ultra-rapid metabolizer phenotypes are rare. In addition to reduced quality of life, non-resolution of symptoms is likely an expensive clinical problem as patients tend to repeatedly utilize health care resources. Side effects frequency might vary by metabolizers' status as well, but there is paucity of data regarding the clinical and the genetic associations.

Design: Case-control studies of cancer cases and population-matched controls in Northern Israel contributed subjects for genome-wide-association-study (GWAS). GWAS analyses were performed under the NIH Genetic Associations and Mechanisms in Oncology (GAME-ON) initiative, using the OncoArray by Illumina that included a backbone and a customized panel for

dense mapping of known susceptibility regions, including pharmacogenetic markers. We used data from the computerized database of Clalit Health Services to compose a cohort of all participants with GWAS data, that have also been dispensed PPI (1.1.2002-10.11.2020). We retrieved demographic and clinical variables at cohort entry (date of PPI initiation), and self-reported consumption of foods/beverages that are known to increase peptic-related symptoms. Composite outcome was any of abdominal pain, gastroenterology clinic visit, change to another PPI, PPI dose increase, or metoclopramide prescription, reflecting symptoms persistence/recurrence, in a 2-years follow up. We also evaluated risk for hip/wrist/spine fractures, in a long-term follow up. Cohort participants and care givers were unaware of CYP2C19 results.

Results: Of 3326 patients using PPIs, there were 66 (2.0%), 739 (22.2%), 1394 (41.9%), 947 (28.5%), and 180 (5.4%) CYP2C19 poor, Intermediate, normal, rapid, and ultra-rapid metabolizer status, respectively. Lower risk for the outcome was associated with being poor metabolizer HR 0.57 (95% CI 0.38-0.86), HR 0.58 (0.39-0.87), in univariate and multivariable cox regression analyses, respectively, most apparent in omeprazole users. In long-term follow up with 20,142 person- years of follow-up: 7.6% (5 cases) within the poor metabolizers group had a new fracture, and 11.6, 12.9, 12.8, 11.1% in normal, intermediate, rapid and ultra-rapid metabolizers groups, respectively (non-significant). There was no difference between genetic groups in PPI treatment duration. However, median PPIs treatment duration was 132.7 months (mean 125.3, SD 71.4) in patients who had fracture, and 86.8 months (mean 91.7, SD 72.9) in patients not experiencing fracture until end of follow up ($p<0.0001$).

Conclusions:

CYP2C19 poor metabolizer status is associated with higher effectiveness of PPIs, and not with higher risk for fractures. Longer treatment duration is associated with new fracture.

B08

CYP2C19 PGx Genotyping Assessment in a Large Multiethnic Population

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Introduction

Pharmacogenetic (PGx) testing for cytochrome P450 2C19 (*CYP2C19*) is increasingly common. Depending on the clinical scenario, a rapid *CYP2C19* turnaround time is preferred. Currently there are 36 described *CYP2C19* haplotypes recognized by the Clinical Pharmacogenetics Implementation Consortium (CPIC®), of which many have missing or incomplete population frequency information. The Association for Molecular Pathology (AMP) PGx Working Group haspublished a guideline on the recommended minimum set of alleles to include in clinical *CYP2C19* genotyping panels. This set of alleles represents the more common and phenotypically relevant *CYP2C19* polymorphisms. We provide data on the comprehensiveness of AMP Tier 1 and Tier 2 variant alleles in a large clinical data set with a mixed population. In addition, we assess the utility of a targeted panel for rapid results utilizing previously characterized reference materials.

Methods

Comprehensive *CYP2C19* genotyping was performed on the PharmacoScan™ array looking at 27alleles/haplotypes (*1,*2, *3, *4A, *4B, *5, *6, *7, *8, *9, *10, *12, *13, *14, *15, *16, *17, *18, *19, *22, *23, *24, *25, *26, *28, *34, *35) on 1856 clinical samples, 20 Coriell and 60 characterized genomic DNA samples extracted from blood, buccal and saliva. In addition, the

reference materials were run on a TaqMan® genotyping panel that included AMP Tier 1 (*2, *3 *17) and Tier 2 (*4A, *4B, *5, *6, *7, *8, *9, *10, *35) alleles.

Results

In the 1856 clinical samples, we observed 51 samples (2.7%) that contained a non-Tier 1 or Tier2 allele. Of those, 24 would have an impacted phenotype call.

In the validation data sets, high concordance was observed between genotypes on both platforms, and both performed at equivalent quality levels. We found in the validation materials,a total of 3.3% of samples contained a genotype not captured by the AMP focused panel.

However, all discrepant results were due to the presence of normal function allele *15. In total we observed 100% agreement in phenotype assignment between PharmacoScan™ and the targeted panel.

Conclusions

Overall, the AMP recommended alleles and targeted genotyping panel provided accurate assignment of the CYP2C19 phenotypes observed in the majority of the clinical data set and in the validation materials. These data show that targeted panels are of clinical utility, particularly where clinicians require only results with actionable guidelines in fast turnaround time that will be of benefit to a majority of patients. Comprehensive panels, particularly when done preemptively, allow for inclusion of rare haplotypes, including those in underrepresented minority and multiethnic populations. Additionally, they provide critical knowledge to further define more accurate population frequencies of alleles and identify alleles with structural/copy number variation or uncertain/unknown function for future assignment. This can in turn facilitate the development of improved or more population inclusive actionable targeted panels.

B09

ZNF335 and *CNOT3* affect cellular cholesterol metabolism and statin response in HepG2cells

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Statins are the class of drugs most used to reduce plasma levels of low-density lipoprotein cholesterol (LDL-C) and cardiovascular disease risk. However, there is considerable inter-individual variability in LDL-C response among statin users, inadequately explained by genetic variants. Our lab previously used statin-exposed immortalized lymphoblastoid cell lines (LCLs), derived from participants in the Cholesterol and Pharmacogenetics (CAP) trial (40 mg/day of simvastatin for 6 weeks), to identify novel genes affecting cholesterol metabolism and statin response from whole transcriptome sequence data. Of these genes, zinc finger protein (*ZNF335*) and CCR4-NOT transcription complex subunit 3 (*CNOT3*) showed the strongest positive correlation between statin-induced gene expression changes *in vitro* vs. statin-induced changes in plasma LDL-C *in vivo*. Previous studies from our lab using an *in vivo* mouse model carrying a hypomorphic mutation in *Zfp335* (the mouse *ZNF335* homolog) exhibit reduced plasma cholesterol levels and blunted non-HDL cholesterol statin response. Additionally, ZNF335 physically interacts with two other members of the CCR4-NOT complex, indicating that *ZNF335*and *CNOT3* may be functionally related. Our objective here was to determine the functional effects of modulated *ZNF335* and *CNOT3* expression levels on cellular cholesterol metabolism and statin response. *ZNF335* and *CNOT3* were knocked-down singly in human hepatoma HepG2cells by siRNA transfection under control (lipoprotein-deficient serum, LPDS)- and statin (2μM simvastatin + LPDS)-treated conditions. LDL-C uptake was measured with BODIPY™ FL LDL by flow cytometry. Intracellular cholesterol content was quantified using the Amplex Red Cholesterol Assay Kit (ThermoFisher). Transcript levels of *LDLR* and *HMGCR*, key mediators of cholesterol metabolism, were measured by quantitative PCR. LDL-C uptake increased in HepG2 cells

transfected with *ZNF335* (*p=0.0009*) or *CNOT3* (*p=0.0468*) siRNAs, and was further increased in both groups under statin treatment. Consistent with the effect of *ZNF335* knock-down on LDL-C uptake, LDLR transcript levels increased by ~50% (control) and ~70% (statin) ($p < 0.05$). Moreover, there were smaller statin-induced reductions in intracellular cholesterol content in HepG2 cells transfected with either *ZNF335* or *CNOT3* siRNAs ($p < 0.05$)compared to the non-targeting control (NTC). These findings suggest that ZNF335 and CNOT3 of the CCR4-NOT complex play key roles in regulating cellular cholesterol metabolism and statin response, and that variation of expression of these genes may contribute to inter-individual variability in plasma LDL-C response among statin users.

B10

ABCB1 DIPLOTYPE IS ASSOCIATED WITH BLEEDING FROM RIVAROXABAN

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Background and aim. Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide andthe leading cause of ischemic stroke, which risk is reduced by about 66% with anticoagulant drugs. Direct Oral Anticoagulants (DOACs) are the guideline-recommended first-line therapy in preventingstroke in AF patients. Although patients bleed less with DOACs than warfarin, bleeding still is the main safety concern of this long-term therapy. Rivaroxaban and apixaban are DOACs renally excretedvia p-glycoprotein (p-gp) and encoded by *ABCB1* gene. The three most common coding single nucleotide polymorphisms (SNPs) of *ABCB1* gene (1236C>T (rs1128503), 2677G>T/A (rs2032582) and 3435C>T (rs1045642)) are in high linkage disequilibrium and co-inherited as haplotypes. However, recent evidence has shown inconsistent findings of the functional role of *ABCB1* SNPs andhaplotypes on the effect of DOACs in preclinical and clinical studies. Hence, this study aims to assessthe independent association between *ABCB1* diplotypes and the risk of bleeding in AF patients on DOACs.

Methods. A single-center retrospective cohort study was carried out enrolling 115 Caucasianoutpatients diagnosed with non-valvular AF on DOACs and followed-up at Michigan Medicine. Clinical data was collected by trained investigators through Electronical Medical Records and all genotyping was performed by the Michigan Genomics Initiative (MGI) with standard quality checks and using Illumina Infinium CoreExome v12.1 bead arrays® (Illumina, San Diego, CA). The primaryendpoint was a composite of major and clinically relevant non-major (CRNM) bleeding, as defined bythe International Society on Thrombosis and Haemostasis criteria (ISTH). Cox proportional hazard regression models were used to assess the independent association of *ABCB1* haplotypes anddiplotypes with the risk of bleeding from rivaroxaban and/or apixaban. Age, gender, hypertension, and use of p-gp and/or CYP450 moderate inhibitors were selected as potential confounders and wereconsidered covariates in model adjustments. Hardy-Weinberg Equilibrium was assessed using the chi- squared (X^2) test.

Hazard ratios (HR) and respective 95% confidence intervals (CI95%) were calculatedfor unadjusted (Model 1) and adjusted models (Models 2 to 5). A two-sided p-value of <0.05 was considered statistically significant.

Results. A total of 1 major (0.9%) and 16 CRNM bleeding events(14.0%) during an average of 503±352 days of follow-up were observed. Patients were stratified according to the occurrence of bleeding, and those treated with rivaroxaban (88.2% *vs.* 46.9%, p=0.002) bled significantly more than those treated with apixaban. All genotype frequencies were in Hardy-Weinberg equilibrium with p-values >.05. Eight *ABCB1* haplotypes and eighteen *ABCB1* diplotypes were identified. Three patients (2.6%) harboring the 1236T-2677T-3435T/1236C-2677T-3435T *ABCB1* diplotype were identified, of which 2 of them bled on rivaroxaban. The single patientwho did not bleed was on apixaban. In this context, a significantly higher frequency of 1236T-2677T-3435T/1236C-2677T-3435T *ABCB1* diplotype was observed among those who had bled than their counterparts (11.8% *vs*. 1.0%; p=0.010). Also, a significant and independent association of this *ABCB1*diplotype with bleeding from DOACs was observed (HR:

4.781(1.085-21.070); p=0.039), even after model adjustment for covariates (HR: 4.200(1.017-20.156; p=0.048).

Conclusion. The *ABCB1*1236T-2677T-3435T/1236C-2677T-3435T diplotype was independently associated with bleeding events in AF patients treated with rivaroxaban.

B11

Single Nucleotide Polymorphisms In SLCO1B1 And UGT1A1 Are AssociatedWith Plasma Exposure Of Atorvastatin And Its Major Metabolites: A SAPhIRE cohort Study

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ABSTRACT

Background and Aims. High systemic exposure of statin and its active acid metabolite may increase susceptibility to statin toxicity. This study aimed to identify novel genetic variants associated with plasma levels of atorvastatin (ATV) and its acid and lactone metabolites in a real-world cohort of patients.

Methods. Plasma concentrations of ATV and its hydroxylated acid metabolites (2OH-ATV and 4OH-ATV) were assessed in a retrospective cohort of (N=874) patients received single dose of 5-80mg ATV and had their blood draw at 8hr and 12hr postdose(The Surveillance And Pharmacogenomics Initiative for Adverse Drug Reactions (SAPhIRE) cohort). Genome wide association study (GWAS) of circulating levels of ATV, open acid and lactone metabolites were performed to identify biological plausible variants.

Results. Intra- and Inter-individual variation was 40.6% and 139% across ATV concentrations in the cohort, respectively. UGT1A1 rs4148323 (c.211G>A) was associated with decreased lactonization of ATV and its hydroxylated acid metabolites, with decreased plasma levels of ATV-LAC, 2OH- ATV-LAC, and 4OH-ATV-LAC ($P<0.05$), and plasma ratios of ATV LAC/ATV ($p<0.001$), 2OH-ATV LAC/2OH-ATV ($p<0.001$), and 4OH-ATV LAC/4OH-ATV ($p<0.001$). In addition, UGT1A1 rs4148323 (c.211G>A) was associated with increased 2OH-ATV-LAC/ATV-LAC ($p<0.001$). The minor allele of SLCO1B1 rs4149056 (c.521T>C) was associated with increased ATV ($p=0.001$), 2OH-ATV ($p=0.004$), and 4OH-ATV ($p<0.001$) metabolite levels. The minor allele C was associated with significant decreased ratios of ATV-LAC/ATV ($p=0.008$), 2OH-ATVLAC/2OH-ATV ($p=0.002$) and 4OH-ATV LAC/4OH-ATV ($p<0.001$).

Conclusion. SLCO1B1rs4149056 (c.521T>C) and UGT1A1 rs4148323 (c.211G>A) were significantly associated withcirculating levels of ATV and its major metabolites. UGT1A1 rs4148323 (c.211G>A) may be protective for high plasma ATV exposure.

Key words: SLCO1B1 rs4149056, UGT1A1 rs4148323, Atorvastatin, Pharmacogenetics, Pharmacokinetics.

B12

Influence of *CYP2D6* Genetic Variation on Adverse Events with Propafenone in the Pediatric and Young Adult Population

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Background: Propafenone, metabolized primary by CYP2D6, is commonly used in pediatrics for supraventricular tachycardia. In adults, propafenone adverse events (AE) are associated with CYP2D6 poor metabolizer status; however, pediatric data are lacking.

Methods: This was a single center retrospective observational study using BioVU, the institutional biobank linking DNA to de-identified electronic health records data. All patients, <30 years of age at the time of propafenone initiation, from 1994-2018, were identified. AE within the first three years of propafenone use were included. Subjects were tested for 10 *CYP2D6* allelic variants and copy number status, and activity scores (AS) assigned to each genotype. Categorical variables were compared with Pearson's Chi square and continuous variables with Wilcoxon rank sum. Logistic regression was the test for association of AE to AS, adjusting for age.

Results: 76 individuals (median 0.31 [range 0-23] years old) were included. Propafenone AE occurred in 31 (41%), of which 23 (75%) occurred in the first 3 months; 14 (18%) required drug discontinuation due to AE. The most common AE were QRS (10) and QTc (6) prolongation. Those over one year of age were more likely to have an AE (59% vs 30%, p=0.005) and more likely to require drug discontinuation (35% vs 9%, p=0.005). CYP2D6 AS were available for 69 patients. Univariate analysis demonstrated AS had an odds ratio (OR) of 0.53[0.24 - 1.12] (p=0.09) for AE. A priori multivariable analysis of AE including AS and age had OR of 0.56[0.26 - 1.23] and 1.02[0.95-1.09] respectively.

Conclusions: Propafenone AE are more likely to occur within the first three months of propafenone initiation. Older children are more likely to have AE. CYP2D6 AS are not significantly associated with AE, although an increased frequency of AE is seen in those with lower AS.

B13

Space-optimized HLA typing using Axiom™ PGx research solutions

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Abstract:

The human leukocyte antigen (HLA) complex is the human version of the major histocompatibility complex (MHC). This complex includes genes responsible for immune function. Variations in these genes can affect adverse reactions to drugs, disease susceptibility, and immune response including transplant rejection. The highly polymorphic nature of this region and the prevalence of pseudogenes create challenges for traditional genotyping methods. Combining the use of direct genotyping with advanced imputation methods over the extended MHC region allows accurate HLA typing from SNP genotype data. Applied Biosystems™ Axiom™ microarrays are designed to measure approximately one thousand to nine hundred thousand markers as needed. Content prioritization is particularly important for space-constrained arrays. The Axiom Precision Medicine Diversity (PMD) Research Array genotypes over 850,000 markers, including around 8,000 for HLA typing. The Axiom™ PharmacoFocus™ array, designed for population-scale preemptive pharmacogenomics research, genotypes around 8,000 markers. HLA typing capability on Axiom PharmacoFocus Array was enabled by optimizing HLA module to around 1,500 markers. HLA marker selection was guided to enable efficiency while maintaining high HLA typing concordance by imputation, offering HLA typing across 11 MHC loci inclusive of: HLA-A*31:01, HLA-B*15:02, HLA-B*57:01, HLA-B*58:01. A study used three Axiom microarrays with different density of HLA markers to genotype 1000 Genomes Project samples with diverse ancestries. The evaluated arrays are the PMD Array, PharmacoScan™, and Axiom PharmacoFocus. HLA types for all 11 classical loci were imputed using the HLA*IMP:02 algorithm by Dilthey et al. (2013), as implemented in Axiom HLA Analysis software. High HLA typing concordance was maintained as the number of available markers was reduced by around 80% for the Axiom PharmacoFocus array. HLA types imputed by tested arrays also compared favorably to *in silico* HLA typing by Abi-Rached et al. (2018) of 1000 Genomes Project sequencing data.

B14

"Resolving Highly Diverse HLA and CYP2D6 Alleles using HiFi Sequencing for Long-RangeAmplicon Data with a New Clustering Algorithm"

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Abstract:

Both the HLA family of genes and the CYP2D6 locus are well known for high diversity and their importance to pharmacogenetics and immunological fields. Resolving individual alleles at these loci using short read sequencing technologies requires complicated methods and often imputation due to extensive homology, gene duplication, and the need to phase variants over kilobase-long stretches of sequence. In contrast, long-range targeted amplification and PacBio HiFi sequencing fully and directly resolve and phase a wide range of complicated and difficult genetic loci without assembly or inference. To capitalize on the accuracy of HiFi data, we designed a new amplicon analysis tool, pbAA which uses a newly developed sequence clustering algorithm to rapidly deconvolve (separate) a mixture of haplotypes, enabling precisediplotyping and *-allele classification in these complicated loci.

In our first experiment we demonstrate the accuracy of HiFi reads and the pbaa tool by benchmarking performance against a multiplexed set of 6 HLA targets. Amplicons were generated using GenDx NGSgo-MX6-1 kit, which includes HLA-A,-B,-C,-DPB1,-DQB1,and -DRB1ranging in size from 3.4kb – 5.8kb. HiFi sequence data were generated on the PacBio Sequel System, demultiplexed, and processed through pbaa. The resulting consensus sequences are concordant with expected reference sequences, with confirmation of *-allele typing by NGSengine software. Down-sampling of the data was performed to define lower coverage limits for increased multiplexing.

In a second experiment we designed a set of primers to amplify full CYP2D6 genes and flanking sequence. A multi-primer approach was used to separately amplify primary CYP2D6 genes (8kb), duplicate CY2D6 genes (8.6kb), hybrid genes (10kb), and fully deleted *5 alleles (5kb). We applied this strategy to 22 samples from Coriell with well-characterized *-allele calls, sequenced the amplicons on PacBio Sequel systems, and processed the data through pbaa. Direct typing of fully phased CYP2D6 alleles as generated by this process resulted in concordantresults as compared to orthogonal technologies. Differences between previous technology's results and PacBio HiFi sequencing with direct full-length typing calls were due to higher resolution and improved calls via our method, including better CNV calls, *5 deletion calling, and high resolution subtyping for all alleles.

B15

From cell lines to pharmacogenomics. Transferring deep learning drug response predictionmodels from cell lines to personalized therapy through molecular pathways

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ABSTRACT

Computational prediction models built on cancer cell line data create an opportunity for guiding precision medicine for cancer treatment. However, it remains challenging to tailor these models to predict drug response of specific patients.

Here, we present our 3-step framework for modelling drug response for single drugs and for drug combinations on cell lines and transfer learning the models to patient tumors. A key component of a successfully translating a computational framework to the clinic is the explainability of the models. We address this issue by introducing the association of curated molecular pathways with drugs and with cell-lines/tumors based on their genomic information. These curated pathways help bridge the gap between cell lines and personalized treatment.

Our framework maps gene expression profiles from cell lines/tumors and drug targets to curated molecular pathways and uses these pathways in a deep learning neural network model to predict

drug response. We demonstrate the utility of our framework in predicting drug response on cell lines (IC_{50}), synergy of drug combinations and transfer learning method to predict sensitivity on cells extracted from patients. We first demonstrated our framework's effectiveness on cell-lines, surpassing previous methods. On single drugs, we obtained a root mean-squared error (RMSE) of RMSE of 0.35±0.02, relative to RMSE between 0.52 and 1.43 for previously published methods, while on drug combinations, we obtained an MSE of 67.1±0.2 relative to MSE of previous methods ranging between 181.7 and 255.49.

We demonstrated both generalizability of the models between data sources and explainability, whereby we showed that the top contributing pathways are aligned with current biological knowledge and demonstrating that synergistic drug combinations tend to have their top contributing factors lie closer to each other on a protein interaction network.

Finally, we address the challenge of applying the framework to clinical setting, where we leverage the framework towards suggesting the best drug for patients via transfer learning – i.e.learning from the rich genomics data collected for cell lines to predict drug sensitivity in patients. We obtained good performance on tumor data (AUC=0.81) while our pathway-based transfer learning model has identified pathways that are highly predictive of drug response

B16

Sex differences in the expression and genetic regulation of drug metabolism and transporter genes in human liver.

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Human metabolic activity and the elimination of xenobiotics exhibit sex differences. However, little is known about the underlying biology of the sex differences. Understanding the underlying cause of these sex differences can provide critical information in improving human health. Here, we characterized the sex differences in the expression level and genetic regulation of drug metabolism and transporter (DMET) genes, which have been well recognized in transforming both endogenous and exogenous compounds and are critical for human health. We first examine the differential gene expression in human liver using transcriptome data in the Genotype-Tissue Expression (GTEx) project. Among 372 DMET genes evaluated, 20 exhibit differences between the two sexes (FDR < 0.05). Sex differences were observed for 16 of these genes in an independent dataset. Common drug metabolic genes, such as *CYP3A4*, *CYP2C19*, *CYP1A2* exhibit sex-biased expression, which cautions the use of medication between males and females. To identify sex-specific genetic effects on the human liver transcriptome, we then conducted the sex-stratified cis-expression quantitative trait loci (cis-eQTLs) analysis. We identified 71 single nucleotide variants (SNPs) that exhibit sex-specific genetic effects on these DMET genes from 1.5 million associations. To elucidate the phenotypic impact of sex-specific cis-eQTLs, we examined the sex-stratified genome-wide association studies (GWAS) in the UK Biobank. We found, for example, a SNP (rs34109652), associated with high cholesterol in male, also is a male-specific eQTL with *UGT2B17*, a gene that functionally annotated to metabolize testosterone. In contrast, these relationships were not observed in females. These findings allow us to construct a male-specific network of genetic regulation in *UGT2B17*, testosterone, and cholesterol. Lastly, we test sex heterogeneity of cis-DMET variants in 452 drug side-effect and human metabolic-related traits. We detected 40 traits that have at least one SNP shows sex heterogeneity. The most significant different loci are mapped to *ABCG2* associated with a greater risk to gout in males (β-male = 0.028, β-female = 0.0009, p-difference = 2.1e-183). Taken together, our work shows evidence of sex-specific variability in liver gene expression, genetic regulation, and phenotypic impact. Further elucidating the sex different interplay between genetic variants and gene expression of these DMET genes can enable precision medicine both in the identification of different disease etiology and in medication usage among sexes.

B17

Frequency of *DPYD* gene variants and phenotype inference in a SouthernBrazilian population

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Fluoropyrimidines are chemotherapeutic drugs that may cause severe adverse events. Their metabolization occurs by dihydropyrimidine dehydrogenase (DPD, coded by *DPYD* gene). Variants in the *DPYD* were associated to the greater risk of toxicity by generating enzymes with reduced or non-existent activity. CPIC and DPWG highlight four alleles in their fluoropyrimidines guidelines as being "of primary relevance": *DPYD*2A* - rs3918290, *DPYD*13* - rs55886062, rs67376798, and HapB3 - rs75017182.

The aim of this study was to determine the frequency of the most relevant *DPYD* alleles according to CPIC and DPWG guidelines in a sample of 800 healthy adults from SouthernBrazil, donors of the Hospital de Clínicas de Porto Alegre Blood Center. *DPYD* variantswere detected by allelic discrimination through real-time PCR using customized TaqManSNP Genotyping Assays. No *DPYD*13* allele carriers were detected in our sample. Regarding *DPYD*2A*, HapB3, and rs67376798 alleles, the frequencies obtained were 0.25%, 1.06%, and 0.38%, respectively. A total of 27 individuals were classified as Intermediate Metabolizers (IM) (3.4%), which increases their toxicity risk in case they use fluoropyrimidines. No Poor Metabolizers (PM) were identified. Variant frequencieswere compared to the frequencies described at GnomAD for other populations. Frequencies for *DPYD*2A*, *DPYD*13*, and rs67376798 were similar to those found in European non-Finnish; however, HapB3 was less frequent in our sample in the proportionof 1:2 when compared to Europeans non-Finnish, but more frequent than in Africans andEast Asians. *DPYD*2A* and rs67376798 alleles also presented higher frequency in our sample when compared to Africans. Of note, the Latino population was the only one thatdid not differ from our sample in any variant analyzed. On the other hand, the frequenciesfor all the other populations analyzed (European non-Finnish, African, South Asian, andEast Asian) presented differences from our sample in at least one variant. Due to the ethnic diversity in Brazil, most of the guidelines developed so far should be used with attention, since they are usually based on European population studies. Brazilians are ancestrally heterogeneous due to the trihybrid origin (European, African and Native- American), which is responsible for a specific genetic structure that can also influence ondifferences observed on drug response. Cost-effective studies should be performed to evaluate the implementation of these tests in the clinical practice in the South of Brazil.

Keywords: *DPYD*, pharmacogenetics, pharmacogenomics, fluoropyrimidines.

B18

Genetic and seasonal determinants of vitamin D status in Confederated Salish and KootenaiTribes (CSKT) participants

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Background: Vitamin D is a prohormone that reduces the risk of developing diseases such as rickets and osteomalacia, and has been linked to a number of other diseases. Vitamin D can be obtained through diet or exposure to ultraviolet B light from sunlight, the latter resulting in percutaneous vitamin D synthesis. Vitamin D levels vary sinusoidally throughout the year with trough concentrations observed in winter months and peak concentrations in summer months. Populations living at northern latitudes (>40^{o}N), such as the Confederated Salish and Kootenai Tribes (CSKT) (~48^{o}N), are at increased susceptibility of having lower vitamin D levels in the winter months. Additionally, genetic variation has been shown to influence vitamin D levels. It is estimated that 20-85% of the phenotypic variability in vitamin D levels can be explained by genetic variation.

Methods: We recruited 472 research participants from the CSKT (185 male and 287 female; ≥18 years old) on the Flathead Reservation in western Montana. Demographic factors were collected (e.g., age, body mass index [BMI], and gender). Genomic DNA and plasma were isolated from whole blood. We resequenced twelve candidate genes: 7-dehydrocholesterol reductase (*DHCR7*), calcium-sensing receptor (*CASR*), cubulin (*CUBN*), cytochrome P450 enzymes (*CYP2R1*, *CYP27B1*, *CYP24A1*, and *CYP3A4*), retinoid X receptors (*RXR*α, β, and γ), sulfotransferase family 2A member 1 (*SULT2A1*), UDP-glucuronosyltransferase family 1A4 (*UGT1A4*), vitamin D binding protein (*GC*), and vitamin D receptor (*VDR*). We also quantitated circulating levels of vitamin D and metabolites by liquid chromatography mass spectrometry, including the clinical marker of vitamin D status, 25-hydroxyvitamin D_3 [25(OH)D_3]. Primary analysis used linear regression to test season, demographic factors, and single nucleotide variants (SNVs) and indels for significant associations with 25(OH)D_3 concentration. Subsequently, we

used multivariate regression to quantify the variability in 25(OH)D_3 concentration explained by genetic variation, season, and demographic factors.

Results: Overall, we identified 7,393 total SNVs and indels at common and rare allele frequency in the CSKT cohort. Approximately 8% (n=607) of these variants were novel based on their absence in the National Center for Biotechnology Information (NCBI) Single Nucleotide Polymorphism Database (dbSNP). We also observed variants (n=20) that have been described as being clinically relevant in ClinVar and Pharmacogenomics Knowledge Base (PharmGKB) databases. A significant percentage of CSKT participants had vitamin D levels below sufficiency across the year (~43%), defined as 25(OH)D_3 levels below 20 ng/mL. We also observed that vitamin D and metabolite levels varied in a seasonal, sinusoidal statistical model as expected with peak concentrations observed in June – August and trough concentrations in December – February. We found that age, BMI, gender, season, 12 variants in *CUBN*, *CYP3A4*, *GC*, *RXRα*, *SULT2A1*, and *VDR* were independently associated with 25(OH)D_3 concentration. Together, genetics and environment explained ~40 % of the variability in 25(OH)D_3 concentration in CSKT participants.

Conclusions: We are the first to describe the contribution of season and genetic variability to vitamin D sufficiency in an American Indian population. Our next steps will be to understand the mechanism of these findings and use these data to develop interventional strategies for the CSKT people.

B19

Pharmacogenetic evaluations of 6-mercaptopurine mediated toxicity in pediatric acutelymphoblastic leukemia patients from South Indian population

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Background: Acute lymphoblastic leukemia is the most common type of pediatric cancer and6-mercaptopurine is an important component of the treatment regime. It is an anti-proliferativedrug used in combination chemotherapy for pediatric acute lymphoblastic leukemia patients. Treatment related adverse effects are common among these patients that can also result in interruption of therapy. There are several factors that can affect drug metabolism, and geneticsis identified to a potential element. Thiopurine S-methyltransferase (*TPMT*) genotypes are clinically accepted pharmacogenetic markers, but are rare in the Indian population. Metabolism, transport, and regulation of 6-mercaptopurine engage several proteins, in our study we evaluated few genes for possible factor in contributing to treatment related adverse effects.

Method: A total of 127 pALL patients below 18 years of age (average age of 6.6 years) on 6-mercaptopurine treatment were selected for this study from Sri Ramachandra Institute of Higher Education and Research (SRIHER) and Kanchi Kamakoti Child Trust Hospital (KKCTH), Chennai, India. Eleven variants in seven candidate genes were genotyped. A Multifactor Dimensionality Reduction (MDR) analysis and multinomial logistic regression analysis were performed to infer the association of selected genotypes with TRAEs.

Results: *TPMT* alleles were rare in our study population, which is in concordance with previous studies. Among the genotypes inspected, *SLC19A1*(c.80A>G) and *NUDT15*(c.415C>T) showed significant association with the treatment related adverse effects(OR = 4.01, p-value = 0.002 and OR = 7.78, p-value = 0.002).

Conclusions: The distribution of *TPMT* genotype is not consistent in different populations andthe selected variations in *TPMT* are rare in the Indian cohort. It is understood that several proteins like SLC19A1 and NUDT15 also contribute to the metabolism of 6-mercaptopurine. Therefore, there is a need to study other variants of the metabolic pathway and investigate thefactors that can impact 6-mercaptopurine metabolism.

B20

Pharmacogenetic risk factors for escitalopram-induce hyperarousal in youth at high-risk for developing bipolar disorder

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Escitalopram, a selective serotonin reuptake inhibitor (SSRI), is an effective and well-tolerated treatment for depressive and anxiety disorders in children and adolescents. However, SSRIs and other antidepressants can induce manic symptoms in patients with bipolar disorder, a highly heritable condition. When treated with antidepressants, youth of parents with bipolar disorder have an elevated risk for a "hyperarousal event" characterized by impulsivity, irritability, restlessness, and insomnia. The risk of such an event may be influenced by pharmacogenomic factors such as polymorphisms in genes encoding cytochrome P450 enzymes which metabolize escitalopram (including *CYP2C19*, *CYP2D6*, and *CYP1A2*), as well as polymorphisms in the serotonin receptor 2A subtype gene (*HTR2A*) and serotonin transporter gene (*SLC6A4*). We hypothesize that high-risk youth with elevated serum escitalopram concentrations due to below-average CYP enzyme activity will have an increased risk of hyperarousal events, and that polymorphisms in *SLC6A4* and *HTR2A* may also influence this risk (NCT02553161). Children and adolescents of parents with bipolar disorder aged 12 to 18 were randomly assigned to receive treatment with escitalopram (*n*=65) or placebo (*n*=40) and monitored for symptoms of a hyperarousal event. After 8 weeks of treatment or early termination due to a hyperarousal event, a cheekswab for Assurex Genesight Psychotropic pharmacogenomic testing was collected and blood drawn to determine serum escitalopram and desmethylescitalopram concentrations. We are currently awaiting analysis of these blood samples. Participants who provided samples for genetic testing (*n*=87, mean age: 14.8±1.6 years, 58.6% female) were of Caucasian non-Hispanic (*n*=53), Caucasian Hispanic (n=10), African (*n*=7), and mixed or other ancestry (*n*=17). CYP2C19 and CYP2D6 metabolizer phenotypes were determined in accord with Clinical Pharmacogenetic Implementation Consortium guidelines.

A majority of participants in the treatment group provided samples for both genetic testing and measurement of serum escitalopram (*n*=42, mean age: 14.9±1.6 years, 57.1% female). CYP2C19 metabolizer phenotypes of participants from whom genetic and serum

samples were collected include intermediate (*n*=11), normal (*n*=19), rapid (*n*=11), and ultrarapid (*n*=1) metabolizers. CYP2D6 metabolizer phenotypes included poor (*n*=2), intermediate (*n*=10), normal (*n*=28), ultrarapid (*n*=1), and uncertain (*n*=1) metabolizers. The S allele of *SLC6A4* is implicated in reduced efficacy of antidepressants compared to the L allele, and high-risk youth were found to have S/S (*n*=17), L/S (*n*=50), and L/L (*n*=20) genotypes. A SNP near the *HTR2A* gene (rs6313, -1438G>A), which may increase the risk of adverse events, was also analyzed, and high-risk youth were found to have G/G (*n*=32), G/A (*n*=44), and A/A (*n*=11) genotypes.

We are still collecting data from some participants and must wait to analyze data related to hyperarousal events because our investigators must remain blinded to the treatment condition of participants who experienced hyperarousal events. After all participants have finished the protocol, we will analyze our completed data sets to determine whether genetic factors that influence the pharmacodynamic response to escitalopram may influence the risk that high-risk youth will experience a hyperarousal event. Once we finish analysis of serum escitalopram levels, we hope to characterize more specific genetic and pharmacokinetic factors which influence the risk of hyperarousal events.

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B21

Pharmacogenetic predictors of cannabidiol (CBD) plasma levels in treatment-resistant epilepsy

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Background: Cannabidiol (CBD; Epidiolex®) is FDA approved for seizures associated with Lennox-Gastaut and Dravet syndromes, and tuberous sclerosis complex, and has also been investigated for its role in treatment-resistant epilepsy (TRE). However, high variability in dose, CBD levels, and seizure control has been observed. Higher CBD levels are associated with better seizure control, however mechanisms underlying variability in plasma concentrations have not been evaluated. We aimed to identify genetic predictors of CBD levels in patients withTRE.

Methods: Patients with TRE were enrolled in a compassionate-use open-label CBD Expanded Access Program at the University of Alabama at Birmingham. Separate consents were obtained from patients willing to have CBD levels tested and patients willing to participate in the genomic study. CBD levels were drawn approximately 4 hours after the morning dose, once patients reached stable CBD doses. A reference laboratory tested levels via high performance liquid chromatography/tandem mass spectrometry. Patient samples were genotyped using the Affymetrix Drug Metabolizing Enzymes and Transporters (DMET) plus array. The relationship between genetic variants and CBD levels (ng/mL) was evaluated under an additive genetic model.

Results: Of the 169 patients in the open-label study, 96 (52% female; 48% pediatric) also consented to have CBD levels tested and participate in the genomic study. After accounting for treatment group (adult vs. pediatric), weight (kg), CBD dose (mg/kg/day), valproic acid, CBD-associated diarrhea and sedation, and the first 5 principal components, variation in ABC transporters was significantly associated with CBD levels. The *ABCC2* rs2273697 A missense variant was associated with approximately 100 ng/mL higher CBD levels (101.3 ± 34.2 ng/mL; p=0.004). Alternatively, *ABCB11* rs473351, encoding the bile salt export pump (BSEP), was associated with approximately 80 ng/mL lower CBD levels (-80.7 ± 27.0 ng/mL; p=0.005).

Conclusion: Genetic variation in the ABC transporters *ABCC2*, involved in biliary transport, bilirubin detoxification, and bile salt transport, and *ABCB11*, encoding the bile salt export pump, was significantly associated with CBD plasma levels. *ABCC2* rs2273697 A, associated with higher CBD levels, has previously been associated with decreased risk of resistance to antiseizure drugs. Additionally, *ABCB11* rs473351, associated with lower CBD levels, has been associated with resistance to treatment with the naturally occurring bile acid, ursodeoxycholic acid. Based on these findings, genetic variation in bile acid-associated pathways may contributeto observed

variability in CBD levels. Identifying genetic variants influencing CBD levels can help identify patients who may be more likely to achieve higher CBD concentrations. Given that higher levels are associated with improved seizure control, this could help identify patients with TRE who may be more likely to respond to CBD.

B22

Development and optimization of a 43 gene pharmacogenomic panel using enrichment-based capture and long-read PacBio HiFi sequencing

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The use of pharmacogenomic testing in personalized medicine has the potential to improve patient treatment outcomes and reduce healthcare costs associated with medication efficacy and adverse drug reactions. Several technologies are commonly employed for germline pharmacogenomic testing, including targeted genotyping, short-read sequencing, hybridization arrays, qPCR, and MLPA; however, many clinically significant pharmacogenomic loci remain challenging to accurately interrogate by these methods due to low sequence complexity and/or the presence of highly homologous pseudogenes. Long-read HiFi amplicon sequencing using the Pacific Biosciences (PacBio) platform has previously been reported to accurately and precisely interrogate problematic pharmacogenomic loci, including *HLA*, *CYP2D6*, and *SLC6A4*; however, multi-gene pharmacogenomic long-read HiFi sequencing panels have not previously been described. Therefore, we developed a novel method to comprehensively interrogate a panel of 43 pharmacogenomic genes using an enrichment-based capture strategy (IDT) coupled with long-read HiFi sequencing. Gene selection was centered on incorporating content with Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines and/or FDA recommendations, including selected variants with evidence for clinical actionability, which translated to 935 kb of enrichment content. Performance of the novel panel and sequencing strategy was evaluated by subjecting reference material specimens with previously characterized variant calls and star (*) allele diplotypes to enrichment and long-read HiFi sequencing. Consensus HiFi read clustering and alignment to GRCh38 implemented pbaa and pbmm2, respectively; small variants (<50 bp) and structural variants (>50 bp) were called using DeepVariant and pbsv, respectively; and haplotypes were inferred using WhatsHap. Importantly, multiplexing 24 samples on a single Sequel IIe SMRTcell resulted in an average on-target read depth of 300X, average HiFi read lengths of 7.2 kb, and median read quality of Q41. Moreover, long-read HiFi sequencing enabled full-gene variant phasing across multiple genes, and comprehensive star (*) allele diplotyping where applicable. Benchmarking pharmacogenomic variant calling against HG002 (n=150 variants) was 100% concordant with the GIAB truth set, including *CYP2D6* variants, and enrichment-based long-read HiFi sequencing also correctly called the *CYP2D6*5* deletion allele in HG00276. These data indicate that enrichment-based capture and long-read HiFi sequencing is an effective approach for multi-gene panels, including historically challenging pharmacogenomic genes, for accurate variant discovery and full-gene haplotype phasing.

B23

Pharmacogenomics Clinical Annotation Tool (PharmCAT): Current State and Future Directions

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Integration of pharmacogenomics (PGx) into the clinical workflow is critical for achieving the goal of precision medicine. PGx implementation can be facilitated by automatic methods to translate genetic test results into clinical actions such as prescribing recommendations. The Pharmacogenomics Clinical Annotation Tool (PharmCAT, https://pharmcat.org) was developed to (1) extract a specific set of variants from a VCF file derived from sequencing or genotyping technologies; (2) infer haplotypes, diplotypes and corresponding phenotypes, and (3) generatea report with phenotype-based clinical or prescribing recommendations which are currently derived from the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline recommendations.

PharmCAT v1.0.0 packages pharmacogene allele definitions, allele function assignments, diplotype-phenotype mappings and prescribing recommendations pulled from the CPIC database. PharmCAT is designed as a series of connecting modules; the output from each module provides the input for the next one. Users can run PharmCAT throughout all modules from the VCF input to the report output with CPIC prescribing recommendations, or alternatively, users have the option to run modules of their choice. Modularization provides theuser with the ability to input external information as needed and enables intermediate output for assessment at each step. The Named Allele Matcher module predicts gene haplotypes from a user submitted VCF file using the CPIC allele definitions which are based on PharmVar information. The Phenotyper module maps resulting diplotypes to phenotypes. The Reporter connects phenotypes to CPIC prescribing recommendations. The resulting JSON file is translated into an HTML file that can be converted to a PDF report designed for readability by clinicians and patients. PharmCAT also provides a pre-processing tool to assist in formatting a user's VCF file to comply with input requirements. The pre-processor includes normalization of variant representation, conversion to the expected multi-allelic format and separating multi- sample files into single sample files.

We will test PharmCAT v1.0.0 using UK Biobank, Penn Medicine BioBank (PMBB), and the AIDSClinical Trials Group (ACTG) data sets. Future steps for PharmCAT include (1) integration of theVCF pre-processing steps into the PharmCAT workflow, (2) support to run multiple single- sample VCF files and/or multi-sample VCF files (i.e., “batch” processing), and (3) FHIR compatible report output for incorporation into electronic health record (EHR) systems.

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B24

Whole-genome sequencing analysis of clozapine-induced myocarditis

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Abstract:

One of the concerns limiting the use of clozapine in schizophrenia treatment is the risk of rare but potentially fatal myocarditis. Our previous genome-wide association study and human leucocyte antigen analyses identified putative loci associated with clozapine-induced myocarditis. However, the contribution of DNA variation in cytochrome P450 genes, copy number variants and rare deleterious variants have not been investigated. We explored these unexplored classes of DNA variation using whole-genome sequencing data from 25 cases with clozapine-induced myocarditis and 25 demographically-matched clozapine-tolerant control subjects. We identified 15 genes based on rare variant gene-burden analysis (MLLT6, CADPS, TACC2, L3MBTL4, NPY, SLC25A21, PARVB, GPR179, ACAD9, NOL8, C5orf33, FAM127A, AFDN, SLC6A11, PXDN) nominally associated ($p<0.05$) with clozapine-induced myocarditis. Of these genes, 13 wereexpressed in human myocardial tissue. Although independent replication of these findings is susceptibility to clozapine-induced myocarditis.

B25

Integration of a condition-based clinical decision support system

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Background: Population based rules are used to guide prescribing resulting in a **"**trial and error" approach for individual patients to determine who will benefit and who will be harmed by medication. Fifty percent of prescribed drugs are ineffective for the individual, 35% are inappropriate, 14% are high risk, and adverse drug reactions are the 4th leading cause of death in the USA. Pharmacogenetics as part of a personalised prescribing process has the potential for improving the effectiveness of drugs and reducing the incidence of adverse drug events. The integration of pharmacogenetics to a condition-based medication decision support system (MDSS) leads to better adoption by healthcare providers, and a significant improvement in the population's quality of care and quality of life.

Aim: To develop a condition-based MDSS that provides holistic integration based on patient clinical, biochemical and physiological characteristics, drug-drug interactions, drug adverse reaction profiles, and pharmacogenetics.

Methods: For each condition, the algorithm development process begins with identifyingpublished epidemiological evidence and guidelines for the management of the disease. Pharmacogenetic evidence is reviewed using a level of evidence approach starting with those gene-drug interactions with guidelines published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG). All information is cross-referenced with the drug monographs published by the Food and Drug Administration (FDA). An interdisciplinary team of pharmacists, geneticists, and physicians reviews the evidence and develops each condition's algorithm framework. Each aspect integrated into the MDSS is weighed based on its severity and impact. Algorithms are programmed into a web application that is rigorously tested with simulated cases and data.

Results: The MDSS TreatGx includes 45 conditions and 429 drugs, covering 85% of those commonly used in primary care. Individualized drug therapy options are provided based on high-level evidence and adjusted for renal and hepatic function, comorbidities, concomitant medications, and genetics. TreatGx integrates seamlessly into a healthcare providers' workflow, avoiding ineffective alerts. It offers an optimal user experience with ergonomically designed user-specific interfaces. The medication support system has been used by over 40 physicians and pharmacists in a year. Healthcare providers stated that clinical decision support makes it easy to incorporate genetic information into decision-making and helps reduce inappropriate prescribing.

Conclusion: The clinical adoption of pharmacogenetics has faced several challenges, but one ofthe most relevant is the healthcare provider's ability to review the implications and, therefore, trigger a clinical action based on it. Thus, blending pharmacogenetics in a condition-based medication decision support system allows a smoother adoption and enhances its clinical actionable power.

B26

Pharmacogenomics Implementation in the Arthroplasty Setting.

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Introduction:
In 2015, 56 million office visits were made to Orthopedic surgeons in the United States alone. The same year, it was estimated that about seven million Americans were living with total hip and knee replacements. Following orthopedic surgery, patients often report intense and prolonged pain even after obtaining prescriptions for tramadol and hydrocodone, both of which are metabolized by CYP2D6 to more potent opioids. CYP2D6-guided opioid prescribing may improve the attainment of safer and more effective pain control. However, the most efficient means of implementing and delivering genotype-guided therapy is unknown.

Methods:
To obtain a comprehensive account of insights about the implementation of pharmacogenomic-guided post-operative drug therapy for patients undergoing unilateral total joint arthroplasty at two UF Health Orthopedic clinics, we interviewed several key stakeholders using a semi-structured interview format. Participants were interviewed from multiple aspects of the health system and at all levels of involvement in the study. The interviewees included pharmacists, physicians, laboratory managers, IT professionals, and academic executives, as well as the President of the UF Health Academic Center and the Director of the UF Precision Medicine Program. Using questions developed from the five Consolidated Framework Implementation Research domains, we interviewed participants before the implementation to identity factors that might influence intervention implementation and effectiveness as well as assess participants' perception of barriers and facilitators for the implementation. Interviews were also conducted following the implementation to assess changes in perceptions over the course of the implementation. The qualitative coding software, NVivo12, was used to analyze interview data using the Consolidated Framework Implementation Research guide to code the data. Coded data was visualized to determine motifs and actionable insights obtained from these interviews.

Results:
Over 25% percent of those interviewed stated that there were no major barriers to the PGx implementation in the Arthroplasty setting. 27% of those interviewed stated clinical time constraint, or work-flow disruption, as the number one barrier to a seamless implementation but noted that a large component of the implementation was study-specific. Speaking with patients about informed consent and walking through study parameters was acknowledged as a function of the study and would not be required if brought into standard clinical practice. Confidence in future implementations becoming more streamlined was a sentiment echoed across multiple post-implementation interviews. Those interviewed expressed that increasing access to physician education and making the PGx clinical recommendations for each patient clear and accessible to physicians during their busy clinic day would help minimize disruption to workflow in practice.

Conclusion:
Pharmacogenomics in the arthroplasty setting holds great promise to assist orthopedic healthcare professionals in improving the safety and efficacy of their prescribing practices. With adequate and accessible physician education and understanding, this technology has the potential to resultin improved patient outcomes and achieved pain control earlier in post-operative management for total hip and knee replacement.

B27

Identifying rates of clinically actionable drug-gene interactions in a health-system biorepository to guide pharmacogenetics implementation services

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Background: Although many institutions report using the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for pharmacogenetic (PGx) recommendations, existing pharmacogenetics services have variable clinical focuses, workflows, and education strategies likely secondary to the unique needs of their respective institutions. This study was undertaken to identify rates of drug-gene interactions (DGI) within our institution and increase our understanding of what providers and clinical settings were most likely to encounter DGIs. These findings can be used to identify stakeholders and guide the development of PGx focused services.

Methods: Patients were included in this retrospective chart review if they were seen at our institution for an inpatient or outpatient visit between July 1, 2014 and December 31, 2020, were enrolled in the internal genetic research biorespository, Michigan Genomics Initiative (MGI), and had an order placed for a medication with a published CPIC guideline recommendation to modify treatment based on a PGx result. The medication, order type (inpatient vs. outpatient), and location of order entry were extracted from a de-identified internal database. The order entry location was used to infer the clinical service of ordering provider. Genotype results were extracted from the biorepository and translated into phenotypes for each gene based on CPIC definitions. A DGI was defined as a medication order that would have resulted in a recommendation to modify therapy based on the patient's phenotype per the CPIC guideline if genotype had been known at time of prescribing. We excluded recommendations for CYP2D6 ultra-rapid metabolizers in the DGI analysis secondary to the inability to detect CYP2D6 copy number within the biorepository genotyping platform. Descriptive statistics were used to evaluate rates of PGx drug prescribing and rates of DGI overall, by clinical service, and by order type.

Results: Of all patients enrolled in MGI (~77,000), 76% received at least one medication with a CPIC guideline and 54% of the PGx medication orders were placed in an outpatient setting. Surgery was the service line most likely to prescribe a CPIC medication (27.8%), follow by internal medicine (11.1%), cardiology (8.1%), and oncology (7.5%). The most commonly prescribed CPIC medication was ondansetron (21.9%), followed by ibuprofen (15.9%), omeprazole (14.6%), and tramadol (7.6%).

Genotype data was available for 43,708 patients for DGI analysis and 27.7% of patients

experienced at least one DGI. The medications most likely to have a corresponding DGI in our population were: warfarin(38.9% of DGIs), ibuprofen (21.1% of DGIs), codeine (9.3% of DGIs), citalopram (8.3% of DGIs), and simvastatin (7.7% of DGIs).

Conclusions: This study has elucidated the existing landscape of DGI in patients treated within our institution. Almost 30% of individuals experienced DGIs and the majority of PGx prescriptions were in the outpatient setting. Surgical and internal medicine services were most likely to prescribe PGx medications. Understanding the clinical services most impacted by DGI will help to inform future PGx implementation efforts, including identifying clinician champions and targeting PGx-related education,as well as determining patient populations in which to initiate PGx testing.

B28

Exposure to Non-Chemotherapy Medications With Pharmacogenomic Risk In Medicare Patients Newly Diagnosed With Metastatic Colorectal Cancer

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Purpose
Many supportive care medications used during the treatment of metastatic colorectal cancer (mCRC) have pharmacogenomic (PGx) risk implications for safety and effectiveness. We examined exposure to non-chemotherapy medications with PGx management guidelines among Medicare patients treated with chemotherapy for newly diagnosed mCRC.
Methods

We conducted a claims-based analysis using the Surveillance, Epidemiology, and End Results registry linked to Medicare claims (SEER -Medicare). We included patients diagnosed from 2008-2015 with mCRC enrolled in Medicare Parts A, B, and D with insurance coverage 12 months prior to diagnosis and 3 months post diagnosis who received chemotherapy treatment . We identified non-chemotherapy agents with PGx risk for toxicity or treatment failure, defined here as PGx at-risk medications, from guidelines published by the Clinical Pharmacogenetic Implementation Consortium. We characterized incident, prevalent, and total exposure to these medications over the three months following diagnosis and used a logistic regression model to understand the impact of pre-treatment comorbidities and medication use on exposure to PGx at-risk non-chemotherapy medications.

Results
We identified 2,238 patients diagnosed with mCRC during the study period that had complete claims data availability and received chemotherapy; of these 2,078 (92.9%) were exposed to at least one medication included on the CPIC guidelines. Patients were exposed to an average of three total PGx at-risk medications and an average of two new PGx at-risk medications after diagnosis. Each additional medication used prior to diagnosis was associated with increased incident exposure to the following Pgx-at-risk medications, citalopram (Odds Ratio 1.05, 95% confidence interval (1.01-1.09)) and meloxicam (OR 1.12, (1.03-1.21)), and to antidepressants as a class (OR 1.04, (1.01-1.07)), while each point on the Charlson Comorbidity Index increased incident exposure to allopurinol (OR 1.33, (1.01- 1.73)). Of patients with PGx at-risk exposures, patients were more likely to have incident exposure to PGx-at risk medications in the pain, anesthesia, and gastrointestinal classes, and more likely to have prevalent exposure to medications in the cardiovascular or antidepressant class.

Conclusions

This analysis establishes that patients who receive chemotherapy for mCRC are exposed to multiple non-chemotherapy PGx at-risk agents early after diagnosis, making this population ideally suited for pre-emptive PGx testing. Pre-emptive testing offers an opportunity to reduce medication-related adverse events and therapeutic failure and can be combined with chemotherapy-focused germline PGx testingto increase the impact of testing.

B29

Mitochondrial DNA copy number was a potential biomarker for treatment choice between metformin and acarbose

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Background

Metformin is globally the first-line oral hypoglycaemic drug for T2D. Acarbose has been suggestedas a viable alternative to metformin in newly diagnosed T2D patients from China. These patients are more prone to β-cell failure induced postprandial hyperglycaemia, which acarbose primarily actsto compensate. However, no clinical factor or biomarker had been established to guide the choice between these two agents.

Blood-derived mtDNA copy number (mtDNA-CN) is a convenient biomarker of mitochondrial function, which had been shown to be associated with various metabolic outcomes. Since the biological actions of both metformin and acarbose are related to mitochondria, we hypothesized thatdifferential mitochondrial function among patients, as indicated by mtDNA-CN, would be associated with interindividual variation in response to metformin and acarbose.

Methods

We conducted post hoc analyses of an existing trial that compared the efficacy of metformin (n = 215) and acarbose (n = 201) as initial oral treatment in drug naive Chinese T2D patients. To examinethe associations between mtDNA-CN and drug response outcomes, we estimated mtDNA-CN by the most recommended method of deep whole genome sequencing with adjustment of aging and batch impacts. Drug response outcomes were defined both in terms of glycaemic efficacy and druginduced change in beta cell function. For each patient, the primary

outcomes of glycaemic responsewere defined as the maximum glucose reduction achieved within 48 weeks of drug initiation, measured by HbA1c, fasting glucose, or postprandial glucose respectively. The secondary outcomesof β-cell function response were defined as the maximum increment of insulinogenic index (IGI) ordisposition index (DI) in the same period.

Findings

There was no significant association between mtDNA-CN and any of the baseline characteristics. No significant association was observed between mtDNA-CN and any of the glycaemic response outcomes in patients from either the metformin arm or the acarbose arm.

For β-cell function response, higher mtDNA-CN was associated with more DI increment (beta = 0.19, $P = 0.04$) in the metformin arm, but less DI increment (beta = -0.18, $P = 0.02$) in the acarbosearm, with a statistically significant interaction ($P = 0.001$) between mtDNA-CN and the treatment arms. Consistent associations were also observed between IGI increments and mtDNA-CN.

Interpretation

Although mtDNA-CN was not associated with the primary outcomes of glycaemic response, it could potentially be used to guide the choice between metformin and acarbose as the initial oral treatment in Chinese T2D patients based on its association with the secondary impact of β-cell protection.

B30

Differential gene expression analysis at the single-cell level identifies FK866 as a novel secondary drug against taxane resistance and cancer stemness in aggressive prostate cancers

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ABSTRACT

Prostate cancer/PCa is the second most common cancer and the second leading cause of cancer deaths inUS men. Metastatic CRPC or mCRPC is clinically the most advanced and lethal disease state of PCa, with median survival of <3 years. Drug resistance (innate and emerging/acquired) to standard-of-care Taxane/TX-based chemotherapy is a principal limiting factor to achieve long-term cure in mCRPC patients. Further, thepresence of single-cell subpopulations in drug-resistant tumors with potential cancer stem cell-like properties or 'stemness'/CSCs are believed to significantly contribute to the development of drug resistance. However,no study so far has attempted to develop drugs specifically targeting these CSCs.

We have designed a novel computational algorithm to predict secondary drugs ("secDrug") against aggressive variants (mCRPC and TX-resistant) of PCa. We hypothesize that our predicted secDrugs synergizes with taxanes by particularly targeting the highly resistant and/or aggressive subclones.

Therefore, in this study, we utilized single cell transcriptomics analysis as a novel screening tool to predict the impact of our top secDrugs on intra-tumor heterogeneity and chemo-resistant cancer stem cell/CSC sub-clones in mCRPC models. First, we performed baseline (pre-treatment) droplet-sequencing based single-cellRNA sequencing/scRNAseq on parental and clonally-derived Taxane-resistant mCRPC cell line pairs DU145(P/TXR) and PC3 (P/TXR) using 10X Genomics Chromium platform followed by t-distributed stochastic neighbour embedding/t-SNE analysis to visualize cell sub-populations/subclones. Further, we utilized our novel in-house machine learning-based prediction algorithm SCATTome (Single-Cell Analysis of Targeted Transcriptome) pipeline for biomarker-based evaluation of the erosion of single-cell subclones representing TX resistance, FK866 targets, cancer stem cells, as well as subpopulations representing PCa cell plasticity.We identified distinct subclones characterized by enrichment of genes involved in cancer progression, and maintenance of cancer stemness (CD44, HES1), and drug resistance (CXCL8, CDK1, CDH1). Interestingly,these subclusters also showed high expression of NAMPT, a key enzyme in the NAD

biosynthetic pathway and the target gene of our top secDrug FK866 (an NAMPT inhibitor), indicating that FK866 may be effective against these taxane-resistant and stem like-cell subpopulation clusters. Our *in vitro* (mCRPC cell lines) and*in vivo* (mouse xenografts) validation assays showed that FK866 was indeed effective against TX-resistant and aggressive PCa.

Next, we performed post-FK866 treatment scRNAseq profiling and showed that revealed that FK866 erodes single-cell subclones with higher expression of phosphoserine aminotransferase/PSAT1 gene. PSAT1 is involved in NAD+ dependent biosynthesis of serine from glucose which is crucial for the survival of cancer cells. PSAT1 is reported to be over-expressed in tumor cells especially in chemo-resistant or non-responders. Currently, we are performing CRISPR-based gene editing followed by single-cell transcriptomics to functionally validate the treatment-related biomarkers.

Our pre- and post- treatment single-cell transcriptome analysis introduces FK866 as a secondary drug candidate against aggressive PCa and establishes PSAT1 and NAD+ metabolism as a novel and potential therapeutic target for the treatment of mCRPC. Mostly importantly, we have created a novel pipeline that integrates *in silico* prediction algorithm with scRNAseq to identify and validate novel agents by specifically targeting and eroding subclones which are high expressers of markers of drug resistance and ‘stemness’.

B31

Identification of Gene Expression Patterns and Repurposed Therapeutic Agents Associated with Polycystic Ovary Syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder, which is accompanied by a variety of comorbidities including metabolic, reproductive, and psychiatric disorders. PCOS is significantly influenced by genetics both in terms of germline genetic variants and epigenetic influences. While there are a number of treatment options for patients with PCOS, the administration of these therapeutics is done using a trial-and-error-based approach, which fails to provide optimal treatment outcomes for many patients.

Methods: We performed a transcriptome-wide association study (TWAS), using a previously published GWAS by Day et al. (2018) and the Genome-Tissue Expression (GTEx) consortium v8, to uncover heritable gene expression profiles that are associated with PCOS. After colocalization analyses, the lead variants associated with both gene expression and PCOS were included in a Phenome-wide association study (PheWAS) using the UK Biobank (UKBB) data. Drug repurposing using the genome-wide transcriptional expression data housed in CMap were performed to identify small molecules with significant similarity and dissimilarity to the PCOS gene expression profiles identified via the TWAS. Small molecules which showcased significant dissimilarity to PCOS were further investigated to discover their possible therapeutic role of

PCOS and its related ailments.

Results: The TWAS analyses revealed that increased expression of *ARL14EP* was significantly associated with PCOS (P=1.6x10^{-6}), with increased expression of this gene shown to be of particular relevance to the female reproductive organs i.e. ovaries, uterus and vagina. Upon colocalization evaluation, rs4071559 was shown to be associated with both an increase in PCOS risk and *ARL14EP* expression. PheWAS analyses revealed that this variant was associated with a number of traits of relevance to PCOS, including increased length of menstrual cycles (P=8.5x10^{-34}), which is a key clinical feature of PCOS. The CMap analysis revealed a number of possible therapeutic candidates, including prednisone, which induces ovulation in patients withPCOS by directly reducing adrenal androgen production.

Discussion: This TWAS of PCOS, being the first of its kind, has provided evidence for the role of *ARL14EP* in PCOS disease mechanisms. The drug repurposing analyses have opened avenues for the exploration of redirecting therapeutics to target the various physiology aliments associated with PCOS. By uncovering a genetic candidate of PCOS, this study has contributed togenerating knowledge that can be used to guide strategies to improve the efficiency, accuracy, timing and safety of therapeutics used in the treatment of PCOS.

B32

Identification of distinct mRNA & microRNA expression signatures and mRNA-miRNApairs associated with ethnic differences in prostate cancer aggressiveness

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ABSTRACT

Introduction

The incidence and mortality of aggressive prostate cancer (PCa) is disproportionately higher in men of African American (AA) ancestry compared with European American (EA) ancestry. Treatment options for PCa patients that develop resistance to androgen deprivation therapy (castration resistance) include docetaxel or cabazitaxel as single-agent or in combination with bevacizumab, thalidomide, and prednisone or immunotherapy. However, these chemotherapeutics typically only improve survival slightly (3-4 months), and patients develop resistance and metastatic disease (metastatic castration-resistant PCa; mCRPC). *Therefore, an understanding of the molecular mechanisms underlying ethnic differences in PCa aggressiveness and progression is essential.*

Methods

mRNA and miRNA expression were analyzed by next-generation RNA sequencing methods (mRNAseq and miRNAseq) using Illumina's NovaSeq platform to identify differentially expressedgenes (DEGs; |Fold change|>2, False Discovery Rate/FDR<0.05) associated with tumor aggressiveness and ethnicity. PCa cell lines representing different tumor types (aggressive androgen receptor non-responsive/AR- vs. non-aggressive AR+) derived from patients of EA (PC3, PC3M, DU145, DUTXR, 22RV1, LNCaP, VCaP, LaPC4, C4, C4-2B) and AA (MDA-Pca-
2b, RC77, RC165, RC43) ancestries were used. The presence of the top DEG signatures were validated using an in-house AA vs EA patient cohort gene expression profiling (GEP) dataset

followed by *in silico* validation using GEP data on prostate adenocarcinoma (PRAD) patients from the publicly available The Cancer Genome Atlas (TCGA) database. For each miRNA, functional analysis was performed using miRBase datasets and mRNA-miRNA pairs and binding sites were predicted by TargetScan. Ingenuity Pathway Analysis (IPA) was performed to identify key regulators, and predict upstream molecules and downstream effects on biological and disease processes based on the expression patterns of top DEGs.

Results

We identified distinct mRNA and miRNA expression signatures associated with PCa aggressiveness (aggressive vs. non-aggressive) and ethnicity (EA vs. AA). The top DEGs that were associated with patient survival ($p< 0.0001$), stratified by Gleason scores, were PLAU, TGFβ1, SERPINE1, MET, TIPM1, ITGA3, SERPINB5, PLAUR, MMPs, CDKN1A, and IGF1. The
transporter genes SLC25A, SLC16A, and ABCB6 were also identified as important markers of aggressiveness. Notably, PLAU, MCAM, MET, TIMP1 were top DEGs in AA vs EA cell lines whileSERPINE1 and MCAM were DEGs in the AA vs EA patient cohort. IPA identified activation of theangiogenesis pathway as a crucial factor for cancer aggressiveness. Top predicted miRNA- mRNA pairs included SERPINE1-let7, and PLAU-mir181 which potentially influence differential gene expression in late-stage cancers. Finally, immunoblotting results confirmed protein expression changes of the top DEGs.

Conclusion

An -omics-based approach was used to identify genetic signatures that provide insights into the molecular basis of PCa aggressiveness between men of EA vs. AA ancestry. Currently, we are performing mutation analysis from RNAseq data to conduct genotype-phenotype correlation analysis between somatic mutations and emerging drug response. Future studies include the use CRISPR-based gene editing to functionally validate the molecular (mRNA/miRNA) signatures. Overall, this strategy has the potential to facilitate more effective targeted ethnicity-specific personalized treatment schedules for aggressive forms of PCa and holds promise to address a known health disparity among AA men.

B33

VARIATION OF MERCAPTOPURINE METABOLITES DURING REMISSION MAINTENANCE THERAPY IN THE AIEOP-BFM 2009 PROTOCOL FOR ACUTE LYMPHOBLASTIC LEUKEMIA: ASSOCIATION WITH DISEASE OUTCOME AND INFLUENCE OF PACSIN2 rs2413739

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ABSTRACT:
In the AIEOP-BFM 2009 protocol for acute lymphoblastic leukemia (ALL), mercaptopurine is given at the planned dose of 50 mg/m^2/day; however, dose adjustments are performed to target patients' white blood cells to the optimal range of 2.0-3.0 × 10^9/L. Mercaptopurine requires a multi-step intracellular enzymatic conversion into active thionucleotides (TGN), susceptible of intra- and inter-individual variations. This study investigated whether such mercaptopurine variability, measured by TGN concentration in erythrocytes, plays a role on patients' outcome and whether *PACSIN2* intronic expression quantitative trait loci SNP rs2413739 (-511 C>T), previously associated with mercaptopurine adverse effects, affects such variability. Pediatric patients (n=265, age: median (1st-3rd quartile): 4.82 (3.03-8.53) years; 58.5% male; 10.2% relapsed, follow-up: 1338 (1377-4162) days from maintenance beginning) were enrolled. Mercaptopurine metabolites were measured by HPLC-UV (TGN: 290.79 (180.90-500.74) pmol/8x10^8 erythrocytes, 391 samples of 209 patients). Kaplan–Meier analysis showed that 11.8% of patients with TGN mean below median value relapsed at 967.5 (870.5-1108.7) days from maintenance beginning *versus* 4.9% of the others who relapsed at 513.5 (358.2-679.2) days (p=0.043). Patients with higher TGN intra- individual variability (>75th percentile of coefficient of variation for repeated TGN measurements, 371 samples in 134 patients) showed a trend towards a worse outcome compared to those with less variability (10.3% vs 4.8%). Interestingly, patients with at least one *PACSIN2* rs2413739 variant allele showed higher intra-individual variability in thiopurine exposure (14.6% of CC carriers, 26.2% CT and 45.4% of TT, Fisher test, p-value: 0.024). These preliminary results support the need of adequate and constant mercaptopurine exposure during remission maintenance therapy for pediatric ALL.

B34

ZNF335 is a novel modulator of plasma cholesterol statin response

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Statins inhibit cholesterol synthesis, lower intracellular sterol levels, increase uptake of low-density lipoprotein (LDL) particles from the circulation, and reduce cardiovascular disease risk. Though statins are generally highly efficacious, statin efficacy varies, and there is considerable inter-individual variation in statin efficacy that remains largely unexplained by genetic factors and other clinical characteristics. To identify novel genes that may modulate statin efficacy, we used RNA-sequencing data from 426 control- and 2 µM simvastatin-treated lymphoblastoid cell lines (LCLs) derived from European and African American ancestry participants of the Cholesterol and Pharmacogenetics (CAP) 40 mg/day 6 week simvastatin clinical trial. We correlated statin-induced changes in LCL gene expression with plasma LDL-cholesterol statin response in the corresponding CAP participants. Two genes, *ZNF335* and *CNOT3*, had statin responses most significantly correlated with LDL-C statin response (False Discovery Rate-adjusted P=0.0085 for both), and an additional 145 genes were correlated at a more modest FDR of 5%. For both *ZNF335* and *CNOT3* (Spearman rho=23.7 and 23.2, respectively), greater reductions in gene expression with statin treatment *in vitro* were correlated with larger reductions in plasma LDL-cholesterol *in vivo*. Interestingly, CNOT3 is a regulatory subunit of the multi-functional CCR4-NOT complex, and ZNF335 has been shown to physically interact with two other members of the same complex (CNOT6 and CNOT9), indicating that CNOT3 and ZNF335 may be functionally related. Mouse models of *Cnot3*$^{+/-}$ male mice were previously reported to have lower serum and liver triglyceride levels than wild type mice. Here, we investigated plasma cholesterol levels and statin response in "*bloto*" mice, which carry an ENU-induced hypomorphic mutation in *Zfp335* (the mouse homolog of *ZNF335*). Male chow-fed 12-week-old *Zfp335*^{bloto} mice had lower fasting plasma total (additive p=0.01) and HDL (additive p=0.0002) cholesterol levels than their wild type littermates (N=12 *Zfp335*$^{+/+}$, 15 *Zfp335*$^{bloto/+}$, and 10 *Zfp335*$^{bloto/bloto}$), though this relationship was not observed in female mice. *Zfp335* genotype and sex were also both associated with non-HDL cholesterol levels in a sex-combined model (p<0.05 for both). We next fed mice a purified chow (AIN-76A) diet for 4 weeks and a matched purified chow+simvastatin diet for the following 4 weeks. By measuring plasma cholesterol levels at two-week intervals using non-terminal bleeds, we found that male mice with at least one *Zfp335*^{bloto} allele had a blunted plasma cholesterol statin response, with smaller statin-induced reductions in non-HDL cholesterol compared to wild type littermates (p=0.017, N=8 *Zfp335*$^{+/+}$ vs. 24 Zfp335$^{bloto/bloto}$ and *Zfp335*$^{bloto/+}$). Trends were similar but non-significant in a smaller sample of female mice, and both *Zfp335* genotype and sex were significantly associated with non-HDLC statin response in a sex-combined model (p<0.025 for both). Overall, our *in vitro* and *in vivo* studies indicate that ZNF335 is a novel modulator of plasma cholesterol statin response.

B35

Effect of CYP3A5 and CYP3A4 Genetic Variants on Fentanyl Pharmacokinetics in a Pediatric Population

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Fentanyl is an anesthetic/analgesic commonly used in surgical and recovery settings. *CYP3A4* and *CYP3A5* encode enzymes which metabolize fentanyl; genetic variants in these genes impact fentanyl pharmacokinetics in adults. Pharmacokinetic (PK) studies are difficult to replicate in children due to the burden of additional blood taken solely for research purposes. The aim of this study is to test the effect of *CYP3A5* and *CYP3A4* genetic variants on fentanyl PK in children using opportunistically collected samples. Remnant plasma specimens recovered after routine clinical testing were analyzed using high throughput tandem mass spectrometry to determine fentanyl concentrations. We performed population PK analysis using data from children recovering from cardiac surgery at Vanderbilt and assessed the impact of pharmacogenomic associations (i.e. change in dose required to achieve equivalent exposure in a simulation study). Fentanyl dosing data were extracted from electronic health records data. Variant data defining *CYP3A4*1G* and *CYP3A5*3* and **6* alleles were available from prior genotyping; alleles with no variant were defined as **1*. The study cohort included 433 individuals (median age 9 months, 48% male). A total of 1937 fentanyl concentrations were available (median 4.5/individual). A two-compartment model was selected as the base model, and the final covariate model included age, weight, and surgical severity score. Clearance was significantly associated with either *CYP3A5*3* or *CYP3A5*6* alleles. A genotype of *CYP3A5*1/*3* or *CYP3A5*1/*6* (i.e., intermediate metabolizer status) was associated with a 0.84-fold (95% confidence interval [CI]: 0.71 to 1.00) reduction in clearance vs. *CYP3A5*1/*1* (i.e. normal metabolizer status). *CYP3A5*3/*3*, *CYP3A5*3/*6*, or *CYP3A5*6/*6* (i.e., poor metabolizer status) was associated with a 0.76-fold (95% CI: 0.58 to 0.99) reduction in clearance. In the final model, expected clearance was 8.85 and 6.73 L/hr for a normal or poor metabolizer, respectively, with median population covariates (7.7 kg, low surgical severity). The *CYP3A4*1G* allele was not significantly associated with clearance. *CYP3A5* variants have a statistically significant effect on clearance, but the effect may not be large enough to be clinically meaningful for dosing decisions. The simulation study showed that poor metabolizers required dose reductions of up to 20% for typical dosing scenarios. For example, a 9 month old, 8 kg normal metabolizer patient given a 2 mcg/kg loading dose followed by continuous 2mcg/kg/hr infusion is expected to reach a blood concentration of 1.29 ng/mL after 12 hours. To achieve the same blood concentration, a poor metabolizer requires 1.64 mcg/kg/hr (with the same 2mcg/kg loading dose). Typical dosing titrations in clinical practice include 1mcg/kg/hr increments, indicating that reductions of < 0.5 mcg/kg/hr may not be clinically meaningful.

B36

Species Differences in the Function of Three Mammalian Orthologs of Breast Cancer Resistance Protein, BCRP

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Breast cancer resistance protein (BCRP) encoded by ABCG2 is an efflux transporter which is localized to the apical membrane of many epithelial/endothelial barriers including the intestine, liver, and blood-brain barrier (BBB). BCRP transports a highly diverse group of substrates including endogenous metabolites, environmental toxins and therapeutic medications. Uric acid is a strong anti-oxidant, present in all mammals, and in primates is the end product of purine metabolism. BCRP plays a key role in uric acid elimination. Notably, BCRP-Q141K, a reduced function polymorphism, has been associated with high uric acid levels in many genomewide association studies. **Hypothesis:** Since uricase, an enzyme involved in uric acid metabolism, was lost during primate evolution, leading to substantially higher uric acid levels in human, we hypothesized that BCRP co-evolved during evolution to handle the variable uric acid levels in different species and this function change is substrate specific. **Methods:** Chimpanzee, sus scrofa and human GFP-BCRP were transiently transfected in HEK293 cells. Radioactivity in cells were determined after 1hr incubation for [^{14}C] Uric Acid (1:1000 in HBSS buffer) and 30 mins incubation for [^{3}H] Prazosin(1:3000 in HBSS buffer), [^{3}H] Oxypurinol (1:3000 in HBSS buffer) and [^{3}H] Glibenclamide(1:3000 in HBSS buffer) for efflux experiments. **Results**: GFP-BCRP was detected by microscopy in HEK293 cells expressing the chimpanzee, sus scrofa and human orthologs of the transporter. Functional studies revealed that all three species orthologs of BCRP transported uric acid (human:0.34± 0.006, chimpanzee:0.27±

0.006, sus scrofa:0.27± 0.008, relative to empty vector = 1), consistent with a role for the transporter across mammalian species in uric acid disposition. Further, the chimpanzee and sus scrofa orthologs of BCRP effluxed uric acid (low micromolar) at a significantly higher rate than the human ortholog (23% less accumulation in the cells than human, p-value<0.001). Furthermore, no significant differences in the efflux rates of common prescription drug substrates of BCRP (prazosin, oxypurinol and glibencamide) were observed for the three species orthologs of BCRP. **Conclusions:** Our early studies suggest that species differences between human and other mammals exist in the kinetics of interaction of uric acid with BCRP, perhaps reflecting co-evolution of the transporter with the loss of uricase. Further studies are ongoing to determine kinetic parameters of uric acid with BCRP orthologs from species before and after the evolutionary loss of uricase in order to understand if the transporter co-evolved with uricase to accommodate the immense differences in uric acid concentrations.

B37

Genome Wide Analysis of Ondansetron Effectiveness in Patients with Nausea and Vomiting Presenting to the Emergency Department

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Background: Ondansetron is the most commonly administered antiemetic and accounts for 73% of all antiemetic administrations. Prior studies suggest the drug is only effective 50% of the time and therefore pharmacogenetics may offer insight to improve effectiveness. We sought to find novel genes associated with ondansetron effectiveness.

Methods: We interrogated the genome and eHR of patients age ≥18 years recruited to the Emergency Medicine Specimen Bank to identify novel phenotypic and genomic correlations with the effectiveness of ondansetron administered in the Emergency Department (ED). We extracted demographic data, clinical variables such as co-morbid disease, concomitant medication administration, drug-interactions, laboratory values, and diagnoses to include as covariates in our GWAS analysis. We excluded patients with cyclic vomiting syndrome, diabetic gastroparesis, bowel obstruction, drug seeking behavior, or malingering. Effectiveness was determined by chart review in conjunction with ondansetron pharmacokinetic parameters. Interrater reliability was calculated by kappa statistic. Genomic DNA was extracted on an Autogen FlexSTAR+ utilizing Qiagen Flexigene chemistry. We performed genotyping using the MEGA-EX Illumina microarray chip which characterizes 2.2 million single nucleotide variants (SNVs). Imputation of non-covered base pairs was performed using TOPMed. Independent t-tests with Bonferroni multiple comparisons correction were used to identify significant continuous clinical and demographic variables. GWAS analyses were completed using the Scalable and Accurate Implementation of GEneralized mixed model (SAIGE). Variants were excluded when there was extreme Hardy-Weinberg deviation (*p* value $<1x10^{-12}$), or missingness >1%. Variants differentially expressed at $p<5x10^{-8}$ were considered significant.

Results: We completed chart review in 1,716 patients. Ondansetron was deemed effective in n=1358 (79.1%), higher than initially expected. Our interrater reliability was excellent with a kappa=0.963. The only demographic parameter significantly different between groups was race; Caucasians were less represented within the “Effective” group than “Not Effective” (n=868 (63.5%) vs. n=251 (70.1%), *p*=0.029). There were no SNVs with a minor allele frequency (MAF) > 0.05 that reached significance. There was one significant variant identified in the exonic region of *FHAD1* (8.86E-08). There were several intronic variants in genes with MAF frequencies <0.05 that approached significance: *FHAD1 (p* range*:* 7.23E-06 - 5.30E-07)*, REG4 (p* range*:* 9.79E-05 - 7.23E-06)*, NSG2 (p* range*:* 5.41E-07 - 2.86E-07)*, MAGI2 (p* range*:* 2.94E-07)*, RAC1 (p* range*:* 3.14E-06 - 1.15E-06)*. FHAD1* is involved with DNA transcription. *REG4* is highly expressed throughout the gastrointestinal tract and is proposed to be involved in managing inflammation. *NSG2* exhibits effects within the dopamine receptor signaling pathway and endosomal transport of neurotransmitters within the brain, a pathway known to be associated with nausea regulation. *MAGI2* acts as a scaffold molecule at synaptic junctions, assembly of neurotransmitter receptors, and a cell adhesion protein. RAC1 protein is known to be involved with many physiologic processes including neurotransmitter transport.

Conclusions: The frequency of ondansetron effectiveness was higher than expected, thus the study was underpowered to detect genetic variants associated with effectiveness. Preliminary data suggests a combination of genes with alterations in inflammatory pathway responses, gastrointestinal mucosal secretions, and neurotransmitter transport or receptor binding may impact a patient's response to ondansetron therapy.

B38

Increasing Complexity in PharmGKB Pharmacogenomic Pathways

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Abstract
Since 2003 PharmGKB has been the primary publicly available online resource for drug centered candidate gene pathways for pharmacogenomics (PGx). The pathways provide elegant figures for displaying the pharmacodynamics (PD) and pharmacokinetics (PK) and linking peer- reviewed published literature as evidence to support the genes and variants that impact them. There are currently 153 PharmGKB pathways (as per 08/20/2021) available with a several more in progress. Pathways feature in more than 60 publications in peer-reviewed journals by PharmGKB and the number of pathway figures used by others in reviews, books, presentations, grant proposals, teaching and student dissertations are beyond numeration.

There are 54 unique drugs with level A relationships on the CPIC gene-drug pairs list (08/20/2021): 96% of have at least one pathway (PK, PD or both). In some cases, such as allopurinol/HLA-B or halothane/RYR1, the drug pathway does not directly depict the gene, but rather the text discusses the nature of the drug-gene relationship and the gene may be depicted in related pathways.

Originally, pathway supporting evidence was collected from published literature and made available for download as an Excel file. We have subsequently developed a system using a modified version of PathVisio software to collect and encode PGx data using GPML (Graphical Pathway Markup Language) format. This system has evolved over the years, especially in how we process and display the GPML content on the website under the pathway components tab. Creating published pathways can be time intensive and includes extensive review of the literature, several rounds of discussion with coauthors, capturing the data in GPML format and drawing the high-quality illustration. We have improved underlying systems to depict and render pathway figures to streamline the process. As PGx candidate genes are identified from newly published articles, we add them to existing pathways. We have recently focused on PK pathways, updating existing pathways to increase the level of detail in the GPML and increasingthe breadth of drug coverage, especially for drugs with clinical PGx guidelines. Drug metabolite information is often found within publication figures and not easily machine readable so making this information accessible requires expert human curation. As a result of our pathway efforts, we now have over 700 drug metabolites in the database with structural cross references (usually Pubchem) and literature references.

While the high-quality illustration and detailed text are familiar and similar to what can be found in printed resources, pathways at PharmGKB can also capture more layers of informationbeyond the linking of genes and drugs and references.

B39

Identifying Potential Otoprotectants For the Prevention of Cisplatin-induced Ototoxicity Through Drug Repurposing Analyses

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Background: Cisplatin is a major chemotherapeutic agent, which is highly effective in the treatment of many different cancers. The most common adverse drug reaction associated with cisplatin is ototoxicity (hearing loss), which occurs in up to 80% of patients. Past genomic studies of cisplatin-induced ototoxicity (CIO) have shown that 38-47% of the observed variability can be attributed to genetics. This susceptibility can be traced to multiple loci, with many of these genetic variants affecting the expression of genes. As it has been reported that there is an overlap in the genetic architecture of CIO and hearing loss, this study aims to identify heritable gene expression profiles that are associated with hearing loss traits and harness these findings to identify otoprotectants for the prevention of CIO.

Methods: A transcriptome-wide association study (TWAS) was performed to investigate the association between imputed gene expression and hearing loss. These analyses were performed using summary statistics from a multi-trait meta-analysis genome-wide association study of four heritable hearing traits from the UK Biobank, in combination with a gene expression reference panel obtained from the Genotype-Tissue Expression (GTEx) Database. Analyses were performed across all 53 tissues included in GTEx, using S-PrediXcan and S-MultiXcan. Genes with $p<0.05$

in the S-MultiXcan analyses underwent fine-mapping using FOCUS. This 90%-credible gene list was filtered to include genes which were expressed in the inner ear, as determined via single cell RNA sequencing (scRNAseq) data housed in the gEAR database. Thisgene list was further filtered to include only genes whose expression was correlated with cisplatin-induced cytotoxicity ($p<0.05$), as determined using data obtained from the Broad-Novartis Cancer Cell Line Encyclopedia and the Genomics of Drug Sensitivity in CancerProject. Finally, these filtered hearing-associated gene expression profiles were inputted intoCLUE to identify small molecules with highly dissimilar gene expression profiles to those associated with hearing loss, which may be prioritized as otoprotectants.

Results: TWAS and fine-mapping analyses identified 178 genes that were associated with hearing traits and included in the 90% credible gene set according to fine-mapping results. After filtering for correlation with gene expression and cisplatin cytotoxicity, 60 genes remained. Of these, 46 genes were found to be expressed in the inner ear cells (inner hair, outer hair, Dieter and/or Pillar cells). Drug repurposing analyses revealed that the top scoring perturbagen gene expression profiles were involved in various hearing related pathways.

Conclusions: To the best of our knowledge, this is the first study to perform a TWAS of hearing loss. To increase the biological plausibility of these findings and their relevance to CIO, novel methods were applied, including the use of murine inner ear scRNAseq data and cisplatin-induced cytotoxicity data to filter genes. Drug repurposing analyses of these genes led to the identification of candidate otoprotectants. In further experimental models, the potential of these otoprotectants will be investigated to determine the capacity of these agents to protectagainst CIO without impacting anti-tumor activity of cisplatin.

B40

Clinical and Genome-Wide Association Analysis of "Speech Recognition Threshold" Following Cisplatin-Based Chemotherapy in Survivors of Adult-Onset Cancer

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Declines in speech recognition comprise one of the most limiting consequences of hearing loss. In this study, we assessed the clinical variables that are associated with symmetric deterioration of speech recognition threshold (SRT) in testicular cancer survivors treated with cisplatin-based therapy. Factors with significant associations were age, self-reported health, BMI, and years of smoking, as well as motor neuropathy. On the other hand, we did not observe significant associations of SRT deterioration with hypertension, hypercholesterolemia, peripheral sensory neuropathy, or administered cisplatin dose. A genome wide association analysis for SRT measurements identified 23 genetic variants from 10 separate linkage disequilibrium (LD) regions with suggestive significance (threshold of $P<10^{-5}$) with SRT deterioration. Among the top variants in the LD regions, three either have eQTLs or are within the coding region of genes involved in neuronal development (*ATE1*, *ENAH*, and *ZFHX3*). These results improve our understanding of underlying clinical and genetic risk factors associated with speech recognition. This study was funded through National Institutes of Health *Genetic Susceptibility and Biomarkers of Platinum-related Toxicities* grant (R01 CA157823).

B41

Role of *MTHFR* Polymorphism on Methotrexate Response in Childhood Acute Lymphoblastic Leukemia

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Abstract

Childhood leukemia is the number one cancer in children below 14 years old in Malaysia. Methylenetetrahydrofolate reductase (MTHFR) enzyme is essential for the metabolism of MTX. Polymorphisms of the *MTHFR* gene lead to altered MTHFR activity that change the individual metabolome and could therefore exert a pharmacogenetic effect. The objective of this study was to determine the metabolite profiles of children with acute lymphoblastic leukemia (ALL) treated with MTX. Thirty-eight (38) patients received HDMTX at 5000 mg/m^2 based on the treatment protocol of AIEOP-BFM ALL 2009 at Institute of Pediatric, Hospital Kuala Lumpur, Malaysia. Genomic DNA was isolated from peripheral blood using the OmegaEZNA Blood DNA Mini Kit. Metabolomics analysis was performed using Agilent 1200 Infinity HPLC system coupled the Agilent 6520 Accurate-Mass (Q-TOF) mass spectrometer. The differential metabolites analysis was performed using MetaboAnalyst v.4.0 software to investigate the most perturbated metabolic pathways in MTX therapy. The PLSDA analysis was performed to identify metabolite profiles based on patients' *MTHFR* genotypes. Metabolites with variable importance in projection (VIP) score ≥ 1 were deemed important in the PLS-DA model. In this study, 31.5% (n=12) of the patients have heterozygote *MTHFR* C677T, and 1 patient has homozygous of *MTHFR* T677T. The remaining are homozygous of*MTHFR* C677C. Five significant metabolites with a VIP score ≥ 1, which were highly expressed in the *MTHFR* T677T patients were cholesterol sulfate, tetrahydroaldosterone-3-glucuronide, PS-5, alpha-N-phenylacetyl-L-glutamine and (R)-pantolactone. Three (3) metabolic pathways were significantly associated with *MTHFR* polymorphism; the primary bile acid biosynthesis, steroid hormone biosynthesis and pentose and glucuronate interconversion. Poor metabolisers with reduced MTHFR enzyme activity had significantly increased levels of five metabolites such as cholesterol sulfate, tetrahydroaldosterone-3-glucuronide, PS-5, alpha-N-phenylacetyl-L-glutamine and (R)-pantolactone. These metabolites are potential biomarkers which increased the vulnerability to MTX induced toxicity. However, further study is required to investigate the clinical validity of these biomarkers in monitoring the response to MTX. (309words)

Keywords: Acute Lymphoblastic Leukemia, Metabolite, Methotrexate, *MTHFR*, Polymorphism.

B42

Toward A Comprehensive Assessment of the Genetic Risk of Vincristine-Induced Peripheral Neuropathy

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Background: Vincristine-induced peripheral neuropathy (VIPN) is a common debilitating toxicity of vincristine chemotherapy among pediatric cancer patients, often requiring dose-reduction and affecting both survival and quality of life. VIPN risk is influenced by the interplay of multiple factors including demographic (e.g., sex, age), clinical (e.g., vincristine dose, concomitantly used drugs) and genetic factors. Although previous studies have demonstrated several genetic variants are associated with VIPN risk, the clinical relevance of identified variants is limited by several factors including weak associations between some variants and VIPN, small sample sizes and lack of independent population replications. We previously reported the first independent population replication of the association of a *CEP72* promoter variant with VIPN incidence, supporting its involvement in the risk of VIPN. In this study, we will comprehensively assess previously reported candidate genetic variants and potentially uncover novel variants that influence VIPN risk.

Methods: We have recruited a large cohort of 2,038 pediatric patients treated with vincristine as part of their chemotherapy from ten Canadian pediatric oncology centers from 2005 to 2018. VIPN diagnosis/severity was defined according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. All relevant clinical and demographic data including age, sex, vincristine cumulative dose, and concomitant medications have been collected. The role of previously identified variants and variants in candidate genes captured by genome-wide genotyping (Illumina Global Screening Array v2.0 with multi-disease drop-in panel) will be tested using a case-control

study design with logistic regression (PLINK v1.9). Analyses will consider the genetic model used in previous studies (e.g., additive), if available, and the relevant clinical/demographic variables will be included as covariates in the statistical analyses along with genetic ancestry.

Results: Of the 1,574 patients that were assessed for VIPN, there were 692 patients who experienced serious VIPN (cases: CTCAE grade ≥2) and 727 controls (CTCAE grade 0). Patients with mild VIPN (CTCAE grade 1; n=155) were excluded to improve case/control discrimination. Genome-wide genotyping has been conducted. Genetic analyses are currently being performed and will be completed by September 2021.

Conclusion: Further replication and identification of genetic variants strongly associated with VIPN will aid in the development of polygenic risk models to better identify patients at high risk and offer opportunities for modifying treatment plans to prevent this severe reaction, reducing the burden incurred on cancer patients and their caregivers.

B43

Survey of U.S. Medical Oncologists' Practices and Beliefs regarding *DPYD* Testing Prior toFluoropyrimidine Chemotherapy

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Background: Dihydropyrimidine dehydrogenase (DPD) is the primary enzyme responsible for metabolic elimination of the fluoropyrimidine chemotherapy agents, 5-fluorouracil (5-FU) and capecitabine. Polymorphisms in *DPYD*, the gene encoding DPD, cause reduced DPD activity and a higher risk of severe fluoropyrimidine toxicity. Fluoropyrimidine dose reduction in *DPYD* carriers reduces severe toxicity and healthcare costs. Based on these clinical benefits, pre-treatment *DPYD* testing is recommended or required throughout most of Europe. However, pre-treatment testing is not recommended in the U.S. and uptake has been limited. The objective of this survey was to describe the current practice in the U.S. regarding pre-treatment *DPYD* testing and understand the factors preventing oncologists from ordering testing.

Methods: Qualtrics survey invitations were emailed to 325 medical oncologists practicing in the United States who are members of the SWOG Cancer Research Network Gastrointestinal, Breast, or Early Therapeutic Committees. Descriptive statistics were performed to evaluate survey responses.

Results: Responses were collected from 59 (18.2%) U.S. medical oncologists between April 5-May 28, 2021. 98% of oncologists "strongly" or "somewhat agree" that patients with DPD deficiency have increased risk of toxicity. For a patient starting fluoropyrimidine chemotherapy with known DPD deficiency, 65% of medical oncologists would "decrease fluoropyrimidine dosing," with the other oncologists indicating they would "switch to non-fluoropyrimidine treatment" (13%), "consult a colleague or guideline for appropriate treatment" (13%), "increase toxicity monitoring without changing dosing" (6%); only 4% "would not change their treatment." Only 32% "strongly" or "somewhat agree" that "pre-treatment *DPYD* testing is useful to inform fluoropyrimidine treatment." 20% have ever ordered pre-treatment testing and 3% have ordered pre-treatment testing for at least 10% of patients starting a fluoropyrimidine. The factors that prevent oncologists from ordering pre-treatment *DPYD* testing, in descending order of importance, were: "low prevalence of DPD deficiency" (54% "extremely" or "very" important), "lack of clinical practice guidelines that recommend testing" (48%), "uncertain what to do with the test results" (25%), "concern that testing will increase costs for my patient" (25%),"uncertain which test to order" (24%), "concern that decreasing fluoropyrimidine dosing based on test results will decrease efficacy" (23%), "lack of evidence that decreasing fluoropyrimidine dosing based on test results decreases toxicity" (21%), "uncertain how to order test" (17%), and "concern that testing will increase overall healthcare costs" (12%).

Conclusion: *DPYD* testing is seldom used prior to fluoropyrimidine treatment, despite acknowledgment that DPD deficient patients are at increased toxicity risk and that *DPYD* genotype is actionable information. Clinical uptake would be substantially increased by recommendations for pre-treatment *DPYD* testing in oncology clinical practice guidelines, whichmay require further validation of clinical utility of pre-treatment *DPYD* testing within prospective randomized clinical trials. Further education is also needed regarding which test to order, how to order, and what to do with the results, including increased awareness of Clinical Pharmacogenetics Implementation Consortium (CPIC) dosing guidelines.